SAPTHAGIRI COLLEGE OF ENGINEERING

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SAPTHAGIRI
College of Engineering
Creating Tomorrow

In Association With

International Conference on

"Global Convergence in Technology, Entrepreneurship, Computing and Value Engineering: Principles and Practices"

(ICGCP—2021)

16th - 17th July, 2021

Conference Proceedings

Jointly Organized by
Department of
Bio-Technology & Chemistry

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Preface

Sapthagiri College of Engineering, Bengaluru was established in the year 2001 by Srinivasa Education and Charitable Trust with a vision to transform its students into competent, inspired and responsible professionals. It is one of the best Engineering Colleges in India.

It is our great honour and pleasure to publish the Proceedings of the International Conference on Global Convergence in Technology and Entrepreneurship, Computing and Value Engineering, Principles and Practices - 2021 (ICGCP – 2021). The conference was held on 16th and 17th July, 2021 in virtual mode. The conference was organized to encourage the young research minds and also to bring all researchers, academics, scientists, industry experts, on a common platform.

Present global scenario demands unprecedented actions and efforts to converge social, economic and environment issues. Science, Technology and Innovations in the area of Internet of Things, Artificial Intelligence, Bio-Technology, Nano Materials and Renewable Energy must play a key role in achieving these goals. The call for Make in India products by the central and state governments has given impetus to start-ups and entrepreneurships. The conference covered all emerging areas of Science, Engineering and Technology towards fulfilling the objectives.

The response to call for papers was excellent. More than 500 papers were received from across the country, out of which 320 papers were selected for presentation and publication in the proceedings. These papers provided a wide spectrum of research covering all the areas for which the conference was intended.

We wish to express our deepest thanks and gratitude to speakers B.R. Indushekar Head, Operations Development Volvo Construction Equipment, Bangalore and Dr.Yared Abera Ergu Dean, School of Technology Ambo University, Ethiopia for delivering keynote addresses. We would like to express our gratitude and appreciation to the authors for their contributions. Many thanks go as well to all of the reviewers who helped us maintain the quality of the research papers included in the Proceedings. Our sincere thanks go to the Management for their encouragement and support for conducting the conference. We also express our sincere thanks to the members of the organizing team for their dedication and hard work.

Conference Chair of ICGCP – 2021

Dr. Ramakrishna H

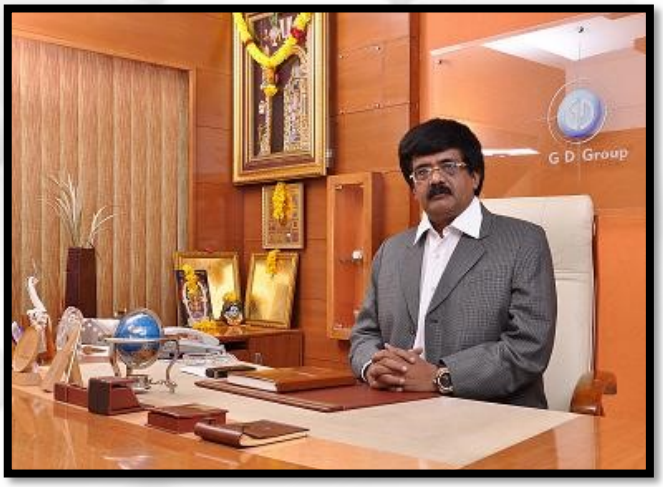
Principal.

Conference Co-Chair

Dr. Shripad Markande

Prof. & Head, Department of Mathematics.

On behalf of the ICGCP – 2021 Organizing Committee



Sri. G Dayananda
Chairman

Message from The Chairman

It gives me immense pleasure in congratulating the Chairman and team members of ICGCP- 2021, on successfully hosting the two days international conference at Sapthagiri college of Engineering. We are overwhelmed by the kind of response received by the research scholars across the country and I wish all of them a bright future and successful career. Also I would like to appreciate the contributions from the Principal, Heads of Departments, faculty and staff of the college for joining their hands in successful conduct of the international conference.

Sri. G.D Manoj
Executive Director

Message from Executive Director

On this occasion, I express my heartiest congratulations to all the participants of ICGCP-2021 for presenting and publishing their research findings in the international conference. I hope that, the two-day international conference has motivated faculty, research scholars and students to continue their research. Also on behalf of the Management, I would like to extend my appreciation to the sincere efforts of Principal, Heads of Departments, and Staff members of Sapthagiri College Engineering.

Message from Principal

Dr. H Ramakrishna
Principal

At the outset I would like to congratulate the entire team of ICGCP-2021 for successfully organizing "Global Convergence in Technology, Entrepreneurship, Computing and Value Engineering: Principles and Practices – 2021" which witnessed active participation of more than 320 research scholars from across the Karnataka and outside. On this occasion, I would like to thank our Chairman, Shri. G. Dayananda and Executive Director, Shri. G. D. Manoj for the magnanimous support extended in organizing the conference. I would also like to congratulate all the faculty, research scholars and undergraduate students for publishing their research works in the conference and I hope that the two-day interaction has motivated them to further pursue their research work and contribute to society. Also I would like to appreciate the efforts of session chairs / reviewers / heads of departments / technical support team for their contributions in adding value to all the sessions. Finally, I would like to congratulate the team ICGCP-2021 for bringing out the proceedings of the conference in a precise manner and for making it available for the researchers' community.

CONTENTS

Study on Anticancer and Antimicrobial Activity of the Green Synthesized Silver Nanoparticles Using *Solanum nigrum* Leaves Extract.

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***Abstract*— In this study, we used Solanum nigrum leaves methanolic extract (SNLME) to make silver nanoparticles (AgNPs) without the usage of any hazardous chemicals. Ultraviolet-visible spectroscopy (UV-VIS) has confirmed that biosynthesized AgNPs exists (320-370 nm). The Fourier-transform Infrared spectroscopy (FTIR) was utilised to confirm the existing functional groups of the nanoparticles and the spectroscopy and crystallisation of AgNPs in their utilisation of biomolecules as capsulating agents. The viability of silver nanoparticles has been assessed using zeta potential calculations. The morphology of AgNPs was examined using a scanning electron microscope. Synthesized AgNP showed significant antibacterial action against *Staphylococcus aureus, Escherichia coli* and *Candida albicans*. Furthermore, the green AgNPs demonstrated showed an IC50 of 29.24 µg/ml within 24h of treatment against the Human ovarian teratocarcinoma cell line (PA1 Ovarian cell line). Both antimicrobial and cytotoxicity studies exhibited a dose-dependent effect and were found to be an effective agent. According to the results obtained, silver nanoparticles is a promising therapy for cancer and pathogen annihilation.**

Keywords— *Solanum nigrum*, MTT assay, silver nanoparticles, PA1 Ovarian cancer cell line

I. INTRODUCTION

Solanum nigrum is a dicot plant belonging to the Solanaceae family. It is used to cure a number of conditions that result in mortality in humans. This plant, which may reach a height of three metres, has lance-shaped leaves with serrated edges. The plant family Solanaceae comprises about 2700 species classified into 98 genera, with a diverse range that includes many commercially significant species [1]. *Solanum nigrum*, in particular, is commonly used in ethnomedicine for its anti-oxidative, anti-proliferative, anti-inflammatory and hepatoprotective properties. Many researchers have also registered anti-cancer activity against hepatocellular carcinoma cells, human ovarian carcinoma cells, colorectal carcinoma cells, and endometrial carcinoma cells in the last few years. The leaves contain significant quantities of protein, amino acids, minerals such as calcium, iron, and phosphorus, vitamins such as A and C, fats, fibres, and methionine, rendering it a nutrient-dense green leafy vegetable [2], [3]. This plant contains several compounds that are responsible for a variety of activities. Glycoalkaloids, glycoproteins, and polysaccharides are the most active components included. Polyphenolic compounds contained in the plant include catechin, gallic acid, caffeic acid, protocatechuic acid (PCA), epicatechin, rutin, and naringenin [4]. Nanotechnology is now one of the world's fastest growing industrial sectors, requiring the creation of new nanomaterials and methods for their fabrication. It has been said that living cells operate directly at the nanoscale and perform a variety of functions ranging from energy generation to high-efficiency element extraction [5]. Nanomedicine, a merger of nanotechnology and medicine, is a comprehensive application. Various methodologies such as chemical, physical and biological processes may be utilised to generate nanoparticles.

While fast, chemical compounds utilised for synthesis and stabilisation are hazardous to the chemical technique, which lead to non-ecologically friendly by-products. For the creation of nanoparticles, biotechnological technologies are environmental and harmless. As a consequence, bacteria, fungus, plants and microorganisms are increasingly required to utilise green nanotechnology [6]-[9]. Amongst them plants are a superior platform for nanoparticles production since they are devoid of hazardous compounds and also provide natural caps. This also minimises the cost of insulation for microorganisms and enhances the cost-competitiveness of culture media as opposed to microorganism syntheses [10]. Silver nanoparticles synthesized in this manner are extensively used in chemical sensing, catalysis, photonics, biosensing, electronics, and pharmaceutical industries due to their special properties [11]. They may be utilised with a number of household goods due to their antibacterial properties, including foodstuffs, clothes, appliances, and medical equipment [12]. Silver is a well-known, low-toxicity antibacterial agent, which is why it is often used in pharmaceutical tropical ointments to prevent infection [13]. Because of their appealing physiochemical properties and good inhibitory and bactericidal effects, they play an important role in medicine. For decades, broad-spectrum antimicrobial functions have been used to deter and cure a variety of infectious diseases. Silver nanoparticles have also been confirmed to have antifungal, antiviral, anti-inflammatory, anti-angiogenesis, antiplatelet activity, and other properties [14]. The process underlying the biological formation of metal-NPs is primarily attributed to the capacity of biopolymers and microbial exopolysaccharides to serve as metal reducers or stabilizers [15]. Currently, there are essential limits to the number of bacteria which acquire resistance to regularly used antibiotics and accelerate their indiscriminate administration. As a result, with the advancement of nanotechnology in the medical sector, the discovery of several modern nano drugs centred on silver nanoparticles has shown a wide range of antimicrobial action at low concentrations that are less toxic [16]. In addition, some anti-cancer drugs have difficulty entering their target site because of insufficient dosages and thus do not efficiently show pharmacological action. Nanotechnology provides strong tools for cancer treatment by overcoming cellular limits and accessing therapeutic substances directly. Novel routes are developed for the treatment of cancer by means of the unique physicochemical properties of metal nanoparticles, including high volume-surface ratios, large optical qualities, easy production and operation [17], [18]. As a consequence, new and alternative ways of addressing the above-mentioned chaos are immediately required. In this regard, our research was designed to produce silver nanoparticles from Solanum nigrum methanol extracts and study their antibiotic activities.

II. Materials and Methods

A. *Plant Material Collection*

Solanum nigrum plant fresh leaves were collected from Bangalore and carefully washed with sterile water. In addition, 60 g of dry plant sample was weighted and soaked in 500ml of methanol before being extracted in a Soxhlet apparatus at 100°C for 6 hours. Following boiling, the solvent was cooled, filtered using Whatman filter paper, and condensed for future research.

B. *Silver Nanoparticles Synthesis*

For the synthesis of silver nanoparticles, a 0.1 M aqueous solution of silver nitrate (AgNO3) was packed. For the bio reduction technique, a 10mg/mL leaf extract of *Solanum nigrum* was mixed with 45 mL of a 20mM AgNO3 solution at room temperature. The initial colour changes, as well as the pH change, were registered. A study of UV-VIS was performed on a Systronic UV-Visible absorption spectrophotometer 117 with a resolution of 1 nm between 200 and 1000 nm and a scanning speed of 200 mm/min. The ultraviolet-visible spectra of silver nanoparticles were studied at different time intervals up to 48 hours to determine the rate of interaction between metal ions

ISBN: 979-85-27243-61-1

and leaf extract. As a control, only AgNO3 solution was used. The fluid comprising the targeted nanoparticle was purified via a 0.22-micron syringe filter and dried before being used for further characterization and biological assays.

C. Characterization of the synthesized Ag nanoparticles

The Systronic UV-Visible absorption spectrophotometer with a 1 nm resolution and a scanning rate of 200 nm / min was used for UV-Visible spectroscopy. The optimum configuration was established by striking a compromise between the most economically and experimentally feasible settings and the values that provide more absorption in the UV-Vis spectrometer measurement. The same suspension fractions (0.5 mL) may be collected and analysed at room temperature. Aqueous solution uses the UV-visible range of silver-nanoparticles to track the interaction between metal ions and the leaf extract. Silver ions and silver nanoparticles should be created within an hour. AgNO3 has been used for several processes to be regulated.

The alternative approach of infrared spectroscopy is known as Fourier Transform Infra-Red (FTIR). Infrared spectroscopy involves passing IR radiation across a sample. The numerous functional groups present in a sample were identified using FTIR. The solution of synthesised silver nanoparticles was centrifuged for 30 minutes at 10000 rpm for FTIR measurements. The pellet was washed three times with 5 mL deionized water to eliminate any free protein or enzyme not capped by the silver nanoparticles. A vacuum drier was used to dry and analyse the pellet.

The particle size distribution and zeta capacity of the Solanum nigrum AgNPs colloid were determined using a DLS technique with the Horiba between 0.2–1000 nm. For hydrodynamic diameter measurement, 1 ml of the sample has been put in the cuvette and the device has been balanced automatically for 3 minutes. For the zeta potency investigation, 1 ml of the sample were placed in the zeta cell and three times duplicated after balance.

SEM are a kind of electron microscope which generates pictures of a material by a focused beam scanning of the surface. Electrons interact with atoms and provide various signals including topographical information and surface composition. An image is created by a raster-scan pattern scan of the electron beam and its combination with the intensity of the data received. In the usual SEM mode, a secondary electron detector produced by electron-stimulated atoms is detected. Numerous elements, including the topography of the material, impact the quantity of secondary electrons detected and hence the power of the signal. Because of the resolution of some SEMs exceeding 1 nanometre, pellets should be submitted for evaluation by SEM. Thin films may develop simply by dropping a very little amount of the sample to the carbonised copper grid; an excess solution may be removed by a paper blot and the film may then dry on the SEM grid for inspection.

D. Antimicrobial Assay

The agar well diffusion approach was used to evaluate the antimicrobial activity of synthesized nanoparticles against *Staphylococcus aureus* (MCC 2408), *Escherichia coli* (MCC 2079) and *Candida albicans* (ATCC 10231). Bacteria were grown on Meuller-Hinton agar, while yeast was grown on YE Potato Dextrose agar. Fresh overnight cultures of inoculum (100 L) were spread on agar plates, and 6 mm diameter wells were cut to add 20 L of various concentrations of research sample and normal. Positive controls for bacteria and yeast were Ciprofloxacin and Clotrimazole, respectively. As a negative regulation, phosphate buffer (PBS) was used. The plates were then incubated overnight at 37°C. The diameter of the inhibition region established the next day was calculated to determine the anti-microbial behaviour of the synthesized nanoparticle.

E. Anticancer activity

The human ovarian cell line Teratocarcinoma PA-1 was utilised for cytotoxicity investigation of AgNPs. Cell acclimatisation

ISBN: 979-85-27243-61-1

occurred one week before to experimentation in a culture flask containing DMEM cultivation medium with 1% antibiotic and 10% foetal bovine serum (FBS) in an incubator with CO2 and were maintained at 37°C. Only 96 well culture platelets were tested using the MTT testing procedure when the cells achieved optimal confluence. After AgNPs treatment was carried at different doses and incubated at 37°C in the CO2 incubator for 24 hours, cell viability was evaluated. The spent media were removed after the incubation and 200 l of new media was added to each well, supplemented by 10 l of MTT (3-(4, 5-dimethyl thiazolyl-2)-2, 5-diphenyltetrazolium bromide). Following incubation, 200 l of DMSO was added to the medium, and the optical density (OD) was determined to be 540nm.

III. Results and Discussion

A mixture of *Solanum nigrum* methanolic leaf extract and silver nitrate was used to make silver nanoparticles. The browning of the reaction liquid indicated that silver nitrate had been removed using plant leaf extract. The pH was determined both before and after the nanoparticle production. The transition in pH from neutral pH 7.0 to slightly acidic pH 5.0 after nanoparticle synthesis indicated nanoparticle synthesis. Similarly, the control and treated mixtures' peaks changed during the wavelength scan, indicating nanoparticle synthesis. The formation of brown colour and, as a result, the synthesis of silver nanoparticles is depicted in Figure 1.

Solanum nigrum leaf extract in the reaction solution was tested for reduced silver nitrate using UV-VIS. At 320-370 nm, the maximum absorbance peak was detected, and the produced silver nanoparticles were employed for characterisation as well as antibacterial and anticancer studies. Figure 2 shows the UV-VIS of silver nanoparticles made with *Solanum nigrum* methanolic leaf extract. From the graph it is observed that the synthesized particles are silver nanoparticles as it absorbs UV light in range of 370 indicated by the peak.

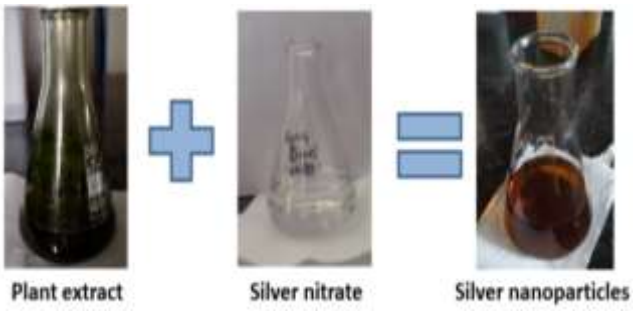

Fig. 1 Indicating the brown colour and synthesis of nanoparticles.

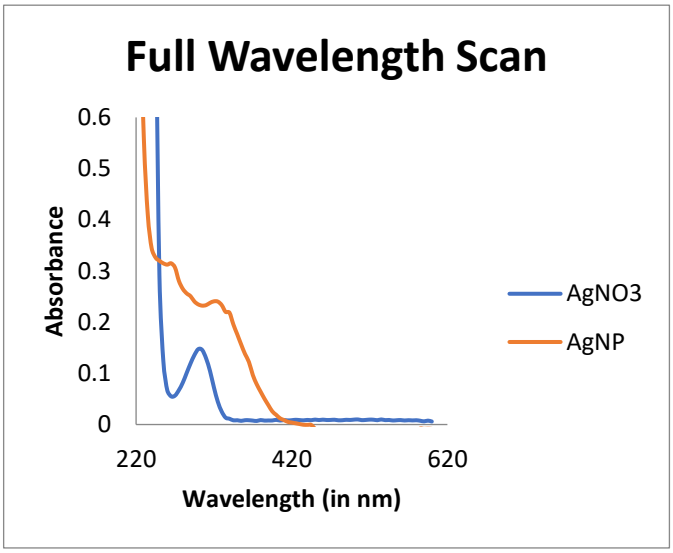

Fig. 2 UV-VIS spectroscopy of silver nanoparticles produced from methanolic *Solanum nigrum* leaf extract.

A 5mg/mL stock solution of nanoparticle sample was prepared in distilled water and sonicated for 15 minutes at 40kHz with a bath-type nicator (GT-Sonic Model: VGT-1613GTD). The nanoparticle suspension was diluted at 10:1000 with distilled water in a cuvette until the FTIR study. The Zeta potential was observed to be-33.4 mV, and the mean size distribution was 164.6nm. The zeta potential of nanoparticles determines their surface charge. Generally, nanoparticles having a Zeta Potential greater than or equal to +25 mV or less than -25 mV are relatively stable. Due to Van der Waal's inter-particle attraction, dispersions with a low zeta potential value will ultimately collide.

ISBN: 979-85-27243-61-1

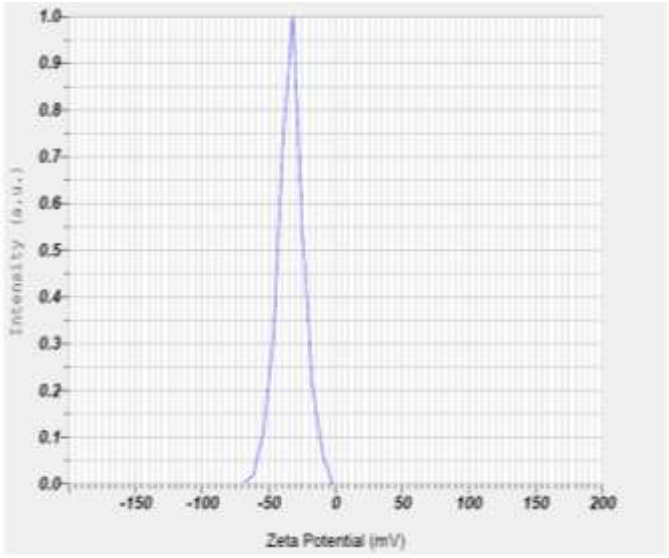

Fig. 3 Showing the Zeta Potential of synthesized silver nanoparticle.

TABLE I

SHOWING THE FUNCTIONAL GROUPS OF SILVER NANOPARTICLES AS PREDICTED BY FTIR.

Sr. No.	Standard Peak (Cm^{-1})	Samples Peaks (Cm^{-1})	Functional Group	Compound Class
1	4000-3000	3311	O-H Stretch	Alcohol
2	3000-2840	2918-2850	C-H Stretching of Methylene Group	Alkane
3	2000-1650	1733	C=O Stretch	Carboxylic acid
4	1650-1580	1567	C-N	Amide
5	1550-1500	1548-1531	N-O Stretching	Nitro Compound
6	1420-1330	1364	COO^-	Symmetric Stretch of Carboxyl anion Linked to Amide
7	1420-1330	1158	C-O Stretching	Primary Alcohol
8	1085-1050	1035	C-C Stretching	Alkane
9	1000-650	919-827	C=C Stretching	Alkene
10	1000-650	536-512	C-I	Halo Compound

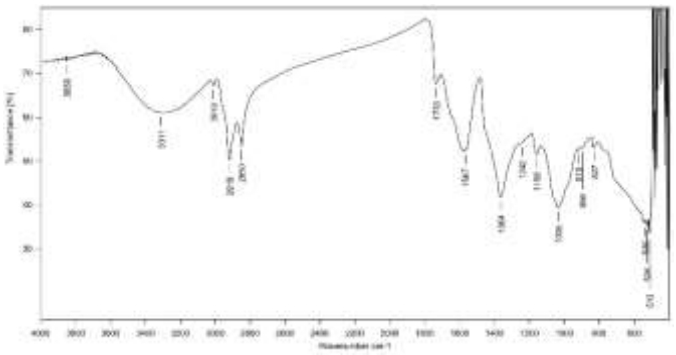

Fig. 4 FTIR graph showing the functional groups predicted in silver nanoparticle.

The FTIR graph of silver nanoparticles in Figure 4 illustrates the anticipated functional classes. The functional groups predicted by FTIR in silver nanoparticles are mentioned in Table I, and the Zeta potential values obtained are shown in Figure 3. From the SEM study, Figure 5 depicts high-density Ag-NPs synthesized by *Solanum nigrum* leaves extract. Silver nanoparticles are the white individual spots in the SEM picture, while silver nanoparticle aggregates are the larger spots. The bulk of silver nanoparticles present have a diameter of 5.2 nm and are spherical and standardized AgNPs with diameters ranging from 4 nm to 6.5 nm. The capping agent ensured that the nanoparticles remained stable and did not come into contact with each other even when aggregated. During SEM observations, larger silver nanoparticles were discovered, which may be the result of the aggregation of smaller ones.

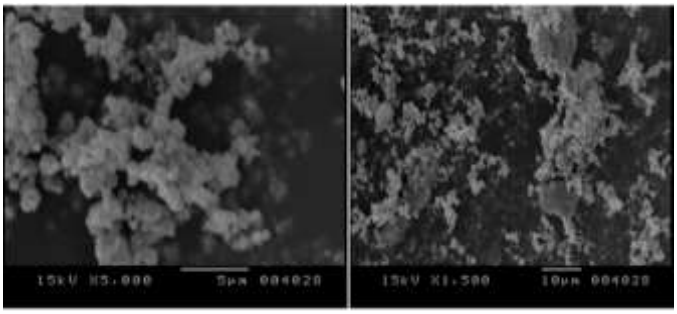

Fig. 5 Scanning electron microscopy images of the lyophilized silver nanoparticles showed mostly spherical particles of a size below 40 nm.

When talking about antimicrobial trials, it's important to remember that the results are dose-based. It showed positive inhibitory behaviour against the tested organisms as opposed to the monitor. Well 1 represents 2.5 mg/mL, well 2 represents 5 mg/mL, well 3 represents 10 mg/mL, well 4 represents harmful regulation, and well 5 contains 1 mg/mL Ciprofloxacin/Clotrimazole as a supportive monitor. Table 2 displays the inhibition zones obtained with different concentrations of silver nanoparticles to supplement the results. The synthesised silver nanoparticle has a strong inhibitory effect against Staphylococcus aureus and a moderate response against Escherichia coli, according to the results. It had a high level of inhibitory activity against the yeast Candida albicans, which was included in the study.

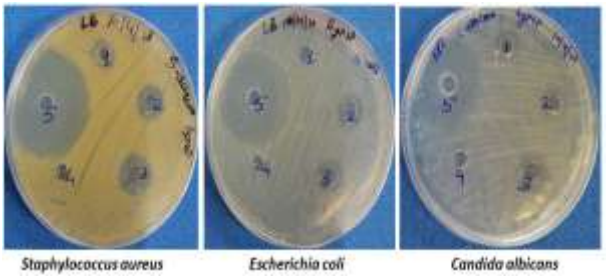

Fig. 6 Petri plates marked with inhibition zones obtained by different organisms. Well 1 – 2.5mg/mL AgNPs (20µL); Well 2 - 5mg/mL AgNPs (20µL); Well 3 - 10 mg/mL AgNPs (20µL); Well 4(-) - PBS (Negative Control) (20µL) and Well 5(+) - 1mg/mL Ciprofloxacin/Clotrimazole (Positive Control) (20µL).

TABLE II

SHOWING THE INHIBITION ZONES OBTAINED BY DIFFERENT CONCENTRATIONS OF SILVER NANOPARTICLES.

Microorganism	**Test Extract**	**Zone of Inhibition in mm**			**Positive control (+)**	**Negative control (-)**
		2.5 mg/mL (1)	**5 mg/mL (2)**	**10 mg/mL (3)**		
S.aureus	AgNPs	10	14	20	41	-
		12	18	22	40	-
E.coli	AgNPs	5	12	14	35	-
		7	11	16	36	-
C.albicans	AgNPs	11	15	18	26	-
		12	14	16	28	-

The antiproliferative activity of the synthesised nanoparticle was determined using the PA-1 ovarian cancer cell line. Tumours with a number of chromosomal defects are used to create these cell lines. The ovarian teratocarcinoma cell line PA-1 demonstrates a single chromosomal aberration: a reciprocal t(15;20)(p11.2;q11.2). These cells were sub cultured until they achieved 90-100 percent confluency, after which they were counted for cryopreservation using a Hemacytometer. The IC50 for 24h therapy against the PA1 Ovarian cancer cell line was found to be 29.24 µg/ml in a second MTT assay. Figure 7 shows a graph highlighting the silver nanoparticle concentrations that trigger cytotoxicity in the PA1 Ovarian cancer cell line. Silver nanoparticles suppress the growth of the PA1 Ovarian cancer cell line in Figure 8, allowing us to compare the texture of cells in the control and nanoparticle-treated groups. It is found that at a concentration of 29.24 µg/ml, it can destroy 50% of the cells, meaning that it has a positive cytotoxic effect on ovarian cancer.

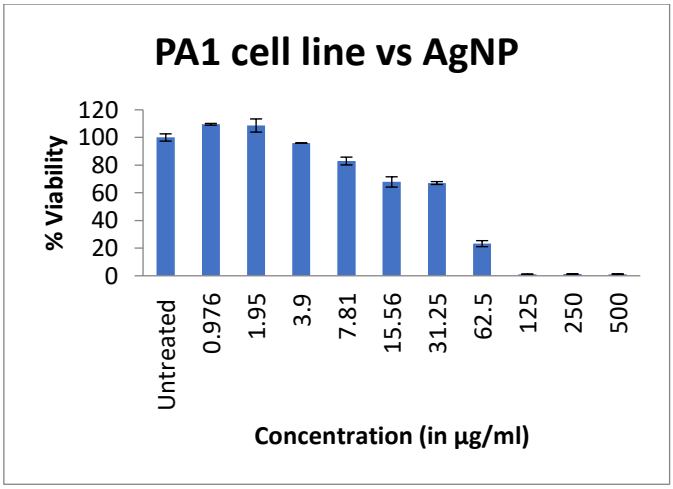

Fig. 7 Graph showing the cytotoxicity of silver nanoparticle against PA1 Ovarian cancer cell line.

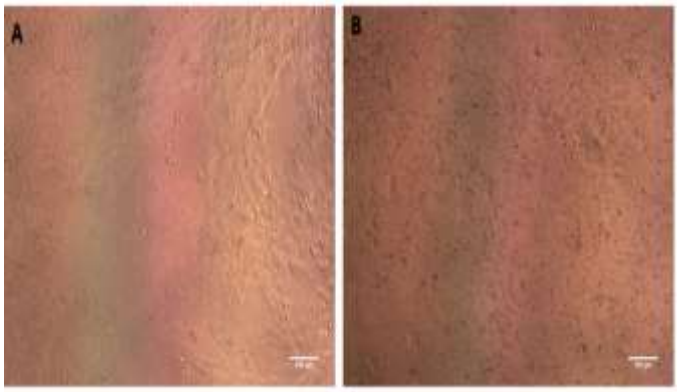

Fig 8 Anti-proliferative capacity of silver nanoparticle against PA1 Ovarian cancer cell line. A-control, B-cells treated with nanoparticle.

Green synthesis of silver nanoparticles utilizing biological microorganisms or plant extracts has developed into an easy and less expensive alternative to chemical synthesis. Since it is always environmentally safe and cost-efficient, this biological approach outperforms chemical processes. Plant extracts-mediated AgNPs synthesis has also been shown to be beneficial among biological processes. Using methods such as UV-VIS, Fourier Transform Infrared Spectroscopy, Scanning Electron, Microscopy Transmission Electron and Spectroscopy Research, Silver Nanoparticles may be identified and analysed till created. UV-VIS typically observes surface plasm resonance of nanoparticles at 320-370 nm. In addition, Fourier Transform Infrared Spectroscopy studies reveal that the plant extract functions as a reduction and capping agent for the Silver Nanoparticles. The synthetic nanoparticles are round, according to electron microscopy, and they are around 20-52 nm in size for better uses [20]. Another concept is that metal nanoparticles are adaptable agents for many uses, in particular nanomedicine for very sensitive testing, thermal ablation, by methods such as UV-VIS, FTIR, Zeta potential and SEM in terms of physical and chemistry. Biological studies, such as antimicrobial activity and anti-cancer activity, have shown encouraging results, crediting the plant to traditional medicines. While dispersion tests have shown that S. nigrum nanoparticles are a therapeutic agent, more investigation is required to verify and enhance their usage in the treatment of particular diseases. improved radiation treatment, medication and gene transfer [21].

Research has demonstrated that plant extract chemicals are used in synthesis to function AgNPs as reductants and cap agents, resulting in negatively loaded surfaces with high zeta potential in a wide variety of pH ranges, between acidic and alkaline environments. Biological trials using pathogenic cell viability strains and tests have shown that AgNPs produced are not hazardous to mammalian cells and have a high antibacterial effect. Often coupled with an interplay of silver and caping layers that include natural chemicals, the synergistic combination is an alternative to AgNPs [22]. In one sample, silver nanoparticles were green synthesized using *Solanum nigrum* leaf extract and bio-physically characterized. Light and confocal laser scanning microscopic photographs were used to examine the absorption and aggregation of it in the intestines of C. *cornuta* neonates. The ecotoxicity of nanoparticles to the freshwater protozoan ciliate, *Paramecium* sp. was 100%. As a result, they concluded that, when opposed to ionic silver nitrate, these silver nanoparticles are less harmful to both invertebrate and vertebrate models [23]. S. *nigrum* extracts and purified substances have since been used in animal models to research antitumor, antiseizure, anti-inflammatory, and hepatoprotective behaviours. The extract has been found to have beneficial results in the majority of trials [24]. The health benefits of different natural chemicals and their derivatives have therefore increased in recent years in order to achieve greater efficiency with fewer bad impacts [25]. Consequently, the final results emphasise the strongest synthesis of nano-particles, as shown

IV. CONCLUSIONS

According to the findings of this report, the Methanolic leaf extract of *S. nigrum* has good reducing and capping properties for the green synthesis of AgNPs. Due to the capping and reducing nature of the phytoconstituents in the *Solanum nigrum* extract, a cap was formed around the AgNP, making them stable. The synthesized AgNPs have a strong antimicrobial

property and can therefore be used to tackle the proliferation of multidrug-resistant strains. Furthermore, synthesized Silver Nanoparticles (AgNPs) showed an IC50 of 29.24 µg/ml within 24 hours of care against the PA1 Ovarian cancer cell line which proves that the synthesized nanoparticle is a potential agent in cancer therapy. Given that green synthesis is an environmentally sustainable, effective, and low-cost process, nanoparticles made from this plant species may be used instead of extracts to inhibit or prevent cancer cell development. This approach has many benefits, including cost-effectiveness, time saves, and the ability to synthesize small-size particles for inhibiting PA1 cancer cell proliferation and inducing apoptosis. As a result, we anticipate that this approach would be effective in therapeutics and selective drug delivery.

ACKNOWLEDGMENT

This work is supported by R.V College of Engineering and Cellkraft Pvt Ltd Bangalore India.

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ISBN: 979-85-27243-61-1

CULTIVATION OF *CHLORELLA VULGARIS* FOR NUTRACEUTICAL APPLICATION AND BIODIESEL PRODUCTION FROM WASTEWATER.

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Abstract

In the recent past, considerable interest has been seen all over the world in employing microalgae for wastewater treatment. The technology is at a maturing stage. Algae can be used to treat both municipal and industrial wastewater. Algae play a major role in the aerobic treatment of waste in the secondary treatment process. Algae-based municipal wastewater treatment systems are mainly used for nutrient removal (removal of nitrogen and phosphorus). The added benefit is the resulting biomass that can be used as a nutraceutical. and biofuel feedstock. As proper nourishment is a growing concern with increasing world populations, sustainable sources of nutritional value are needed. Due to the diverse nutritional components algae can produce and concentrate, along with their simple and rapid growth characteristics, these autotrophic organisms are exceedingly desired for use in nutraceuticals and nutritional supplements. Omega-6 fatty acid and omega -3 fatty acids are polyunsaturated fat, essential for human health because they cannot be made in the body. The lipid content of microalgae can reach up to 70%, with high concentrations of omega-3 and omega-6 fatty acids. Micro algal biomass is also an excellent meal supplement for fish species such as Carassius auratus. Many types of algae have documented health benefits from strengthening the immune system to fighting cancer and heart disease. Biofuels made from bio-products reduce the need for petroleum oil and offer considerable benefits for sustainability and reduce pollutant and greenhouse gas emissions. Of the biofuels, biodiesel is highly promising. This paper evaluates the use of *Chlorella vulgaris* as a nutraceutical and Biofuel.

Keywords: *Chlorella Vulgaris*, Nutraceutical, Omega-3, and Omega-6 fatty acid, Biofuel.

Introduction:

Chlorella vulgaris is a species of green microalga in the Chlorophyta division and one of the most extensively studied microalgae. The first pure culture of a eukaryotic microalga was described in 1890 by the Dutch microbiologist Dr. Martinus Willem Beijerinck. *Chlorella*-like organisms have been around for more than 2.5 billion years. [1]

Table 1: CLASSIFICATION OF *CHLORELLA VULGARIS [2]*

Microalgae	*Chlorella vulgaris* Beyerinck (Beijerinck)
Empire	Eukaryota
Kingdom	Plantae
Phylum	Chlorophyta
Class	Trebouxiophyceae
Order	Chlorellales
Family	Chlorellaceae
Genus	Chlorella
Type species	This is the type species (lectotype) of the genus Chlorella
General environment	This is a freshwater/terrestrial species

Chlorella vulgaris has a diameter of 2 to 10 µm and is spherical, subspherical, or ellipsoid in shape. It is found in freshwater, marine, and terrestrial environments, and it has a high photosynthetic ability as well as the ability to grow quickly under autotrophic, mixotrophic, and heterotrophic conditions. The cell wall provides mechanical and chemical protection, and its relationship to heavy metal resistance has been reported, which explains why *Chlorella vulgaris* is one of the most commonly used microorganisms for waste treatment. [1]

Carbon is taken up by *Chlorella vulgaris* cells via the enzyme carbonic anhydrase, which catalyzes the hydration of CO_2 to form HCO_3 and a proton. The rate of carbon dioxide bio fixation by *Chlorella vulgaris* is close to 250 mg L^{-1} d^{-1}.

Chlorella vulgaris, a green microalga, is the most studied eukaryotic algae species in wastewater treatment. It was discovered that *Chlorella species* have a high nitrogen and phosphorus removal efficiency. All of these characteristics have led to it being one of the first microalgae to be considered for large-scale cultivation and commercial production.

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Constituents of Chlorella vulgaris:

Proteins, lipids, carbohydrates, pigments, minerals, vitamins, and bases esterified to saturated or unsaturated fatty acids make up the majority of *Chlorella vulgaris*. Furthermore, it contains a high concentration of PUFAs, which is comparable to that found in fish oils, as well as some essential fatty acids such as omega-3 and omega-6. [3] The composition varies depending on the species and culture environment, such as light intensity, temperature, pH, salinity, and medium nutrient levels. [4]

Cultivation of Chlorella vulgaris:

To grow this microalga in the large volumes, two approaches are taken majorly:

- The outdoor ponds with sunlight as photo-supplier, and the
- Indoor photo-bioreactors (PBRs) with electric light suppliers.

As of now, 90% of total microalgae is produced commercially in ponds. [5]

Other methods include flocculation, filtration, and flotation. *Chlorella vulgaris* in a photobioreactor in a mixotrophic medium. Among the applications for the metabolites produced by *Chlorella vulgaris* is the production of lipids, as well as the synthesis of proteins and some carbohydrates.

Proteins and amino acids can be removed from the growing medium using alkaline solutions, ultrafiltration, or precipitation with trichloroacetic acid or 0.1N hydrochloric acid. [6]

Chlorella strains are considered potential biofuel sources because they contain high levels of protein and lipids even when grown in unfavorable conditions such as limited nutrients, extremely high or low temperatures, or light intensity.

Chlorella vulgaris can also be grown on some external carbon substrates as a source of energy and carbon for cell growth.

Because of its high resistance to harsh conditions, *Chlorella vulgaris* produces well. Cultivating microalgae artificially using PBRs by establishing materials such as a light source, a CO_2 supplying system, the pH of the culture media, the temperature, and the calculated amount of various nutrients.

Chlorella vulgaris has a total protein content of 43–58% of its dry weight depending on the growth conditions, produces more lipid (60–68%) when grown under mixotrophic conditions, and can reach up to 12–55% dry weight when grown in unfavourable conditions, particularly with limited nitrogen. [7]

Aeration or mixing via agitation prevents microalgae precipitation and homogenizes the culture environment.

In a study, the Cultivation process design is as follows, an algae-based simultaneous approach for treating swine wastewater (SWW) and creating biodiesel were investigated. *Chlorella vulgaris* (UTEX-265) was chosen as a model species in this study, and an SWW-based medium was made by diluting with tap water. In the SWW-based medium, *Chlorella vulgaris* grew effectively, and at optimal dilution ratios. All of these studies suggest that *Chlorella vulgaris* may be grown in nutrient-rich wastewater with proper dilution. The turbidity of SWW [Swine wastewater] limits its use in phototrophic cultivation of *Chlorella Vulgaris*; light penetration and hence photosynthetic activity are substantially hampered, resulting in little cell development [9].

A study conducted showed improved nutrients removal from wastewater and enhance lipid production, cultivation of *Chlorella vulgaris* in wastewater with waste glycerol generated from biodiesel

production using scum-derived oil as feedstock was studied. The results showed that nutrients removal was improved and lipid production of *Chlorella vulgaris* was enhanced with the addition of waste glycerol into wastewater to balance its C/N ratio. The optimal concentration of the pretreated glycerol for *Chlorella vulgaris* was 10 g L^{-1} with a biomass concentration of 2.92 g L^{-1} lipid productivity of 163 mg L^{-1} d^{-1}, and the removal of 100% ammonia and 95% of total nitrogen. Alkaline conditions prompted cell growth and lipid accumulation of *Chlorella vulgaris* while stimulating nutrients removal. The application of the integration process can lower both wastewater treatment and biofuel feedstock costs. [10]

In another study, the feasibility of using effluent water discharged from a secondary municipal wastewater treatment facility for mass culture of microalgae for biofuel generation was investigated. Bacteria and protozoa in the effluent water inhibited the growth of *Chlorella* sp. 227. Filtration or UV radiation can be used as pre-treatment treatments on the effluent water to reduce the effect. Filtration (by 0.2 lm) resulted in the highest biomass and lipid production of all pre-treatment techniques examined [11].

Parameters affecting the growth of *Chlorella vulgaris* (in vitro):

Light*:* One of the most crucial components for microalgae growth is light. Microalgae cells cultivated under low light settings assimilate carbon for the synthesis of amino acids and other important cell constituents, whereas sugars and starch are generated via the pentose phosphate-reducing pathway under saturated light conditions. *[2]*

Salinity: Phytoplankton in the ocean is particularly resistant to variations in salinity. majority of species thrive at a salinity that is slightly lower than that of their natural habitat. The optimal salinity range is 20–24 g/L. [2]

pH: Most cultured algal species have a pH range of 7 to 9, with the optimum range being 8.2–8.7, while other species prefer more acidic/basic environments. Failure to maintain an adequate pH can result in complete culture collapse due to the disruption of various cellular processes. [2]

Aeration/Mixing: Mixing is required to minimize algal sedimentation, guarantee that all cells in the population are exposed to equal amounts of light and nutrients, eliminate thermal stratification (for example, in outdoor cultures), and increase gas exchange between the culture medium and the air. [2]

Temperature: Temperatures lower than 16 °C will slow down growth, whereas those higher than 35 °C are lethal for a number of species. The growth rate increases with the increase of temperature to 30 °C and then decreases with the increase of temperature to 35 °C. Results of an experiment by using Taguchi's Experimental Approachindicate that the highest biomass yield was obtained at 30±2 °C, after which an increase in the temperature (to 35±2 °C) resulted in a drop in the biomass yield. [2]

Research conducted it was found that the maximum growth rate was obtained at pH of about 6.31 to 6.84, and the optimum temperature was 32.4 °C. *Chlorella vulgaris* had the best growth rate with urea growth medium by optimum temperatures of 30 °C [2]. With the presence of urea in wastewater, it can be utilized to its maximum potential and desired raw and efficient outputs (proteins, lipids, etc)

An experiment conducted to determine the effects of the addition of waste glycerol on nutrients removal and lipid production from synthetic wastewater by *Chlorella vulgaris*

was carried out. The results showed that waste glycerol can be metabolized by *Chlorella vulgaris* cultivated in wastewater. Glycerol concentration and initial pH had a significant effect on microalgal growth, lipid accumulation, and nutrients removal of *Chlorella vulgaris*. The respective most favorable concentrations of the crude and pretreated glycerol were 5 and 10 g L^{-1} with the optimal initial pH 7.0 for microalgal lipid production. Eight varieties of fatty acids were identified. The main fatty acids components of the microalga were C16–C18, which are suitable for the production of good-quality biodiesel. [10] [2]

In addition, a glucose concentration of 5 g/L in *Chlorella vulgaris* culture medium significantly increased the ultimately harvested biomass containing higher amounts of lipid, carbohydrate, and proteins.

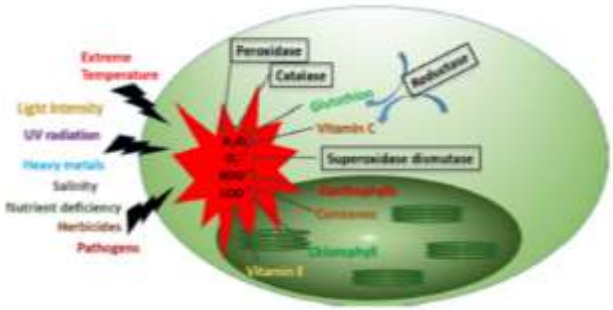

Figure 1: Critical parameters affecting the algal growth

NUTRACEUTICAL APPLICATION OF *Chlorella vulgaris:*

A nutraceutical is a nutrient or food that is thought to have curative properties and provides medical or health benefits, such as disease prevention and treatment.

Examples are mega vitamins, minerals, and hydrolysed proteins.

Around five decades ago, mass production of certain protein-rich microalgae was thought to be a viable option for closing the predicted "protein gap." Comprehensive analyses and nutritional studies have shown that these algal proteins are of high quality and comparable to traditional vegetable proteins. Around five decades ago, mass production of certain protein-rich microalgae was thought to be a viable option for closing the predicted "protein gap." Comprehensive analyses and nutritional studies have shown that these algal proteins are of high quality and comparable to traditional vegetable proteins.

Chlorella vulgaris is a single-celled alga that grows in water and contains a lot of the green pigment chlorophyll. It possesses more chlorophyll per pound of body weight than any other plant on the planet. *Chlorella vulgaris* is a nutrient-dense "superfood" that detoxifies the body and supports liver function.

These small miracle oligopeptides are rich in amino acids and contain more than 20 vitamins and minerals. Among other nutrients, *Chlorella vulgaris* contains a lot of beta-carotene, iron, lysine, zinc, and iodine. Because of its incredible nutritional profile, chlorella vulgaris has been named a "superfood" by some.

Chlorella vulgaris has a long history of use as a food source, and it offers a unique and diverse blend of useful macro-and micronutrients, including proteins. Among them are the following: Chlorella vulgaris contains 50–60% protein, which means it provides all nine essential amino acids. Vitamin B12: Some *Chlorella vulgaris* variations may contain vitamin B12, however, more research is needed.

ISBN: 979-85-27243-61-1

Chlorella vulgaris has a high iron and vitamin C content. You could get anything from 6–40% of your daily intake depending on the supplement. It also contains a lot of vitamin C, which helps with iron absorption, as well as other antioxidants: Antioxidants abundant in these microscopic green cells. Other vitamins and minerals present in *Chlorella* include: trace amounts of magnesium, zinc, copper, potassium, calcium, folic acid, and other B vitamins. Like other algae, chlorella contains omega-3 fatty acids. Only 3 grams of chlorella contain 100 mg of omega-3s.

Chlorella vulgaris can be a good source of fibre if consumed in big amounts. Most supplements, on the other hand, don't even include 1 gram of fibre per serving. Although the evidence is limited, chlorella vulgaris has been shown in animal and human experiments to enhance immune response.

In one short survey, males given *Chlorella vulgaris* produced more antibodies than those given a placebo. Antibodies aid your body's defence against foreign invaders; therefore, this discovery is highly positive. In another small eight-week study, healthy adults who received *Chlorella vulgaris* exhibited signs of increased immune activity. Nonetheless, the findings have been inconclusive, with some studies revealing little to no effect and others indicating no effect at all.

In one study, they discovered that supplementing with *Chlorella vulgaris* increased immune function in adults aged 50 to 55, but not in people over 55. So, while *Chlorella vulgaris* may have immune-boosting qualities in some cultures and age groups, it does not appear to do so in all. The more and larger-scale study is necessary.

According to numerous researches, consuming 5–10 grams of chlorella vulgaris daily lowered total and LDL cholesterol and triglycerides in those with high blood pressure and/or slightly elevated cholesterol. *Chlorella vulgaris* contains the following nutrients, which may help improve blood lipid levels: Niacin, a B vitamin, has been associated with lower cholesterol levels.

Fibre is a cholesterol-lowering agent. Carotenoids: Carotenoids have been shown to naturally lower cholesterol levels. Antioxidants help to prevent LDL cholesterol oxidation, which has been linked to heart disease. *Chlorella vulgaris* contains antioxidants such as chlorophyll, vitamin C, beta-carotene, lycopene, and lutein. Antioxidants can help with the treatment of a wide range of chronic conditions. Some of these antioxidants appear to suppress the formation of advanced glycation end products (AGEs), which are responsible for many of the issues associated with diabetes.

Chlorella vulgaris has been demonstrated to influence gene aging in both animal and laboratory studies. Furthermore, a human study discovered that supplementing with *Chlorella vulgaris* increased antioxidant levels in chain smokers, a group at higher risk of oxidative damage. Although the majority of this study appears to be promising, it is still in its early stages.

Although the effect of *Chlorella* on aerobic endurance has only been studied once, it was found to be helpful. Researchers gave a group of young individuals either six grams of *Chlorella vulgaris* or a placebo for four weeks. At the end of the study, the *Chlorella vulgaris* group showed a significantly improved ability to saturate their lungs with oxygen, which is a measure of endurance. This advantage could be attributed to *Chlorella's* branched-chain

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amino acid content, which did not affect the endurance of the placebo group.

For a long time, *Chlorella vulgaris* has been utilized as a food source because it contains a unique and diversified composition of beneficial macro-and micronutrients, including proteins. *Chlorella vulgaris* is a green unicellular microalga with important biological and pharmacological properties for human health. *Chlorella vulgaris* contains proteins, omega-3 polyunsaturated fatty acids, polysaccharides, vitamins, and minerals, and has a long history of use as a food source.

Clinical trials have shown that *Chlorella vulgaris* supplementation can improve hyperlipidemia and hyperglycemia, as well as protect against oxidative stress, cancer, and chronic obstructive pulmonary disease. Purified peptides from *Chlorella vulgaris* species have been described as having a high potential for protecting DNA from oxidative damage in cells. Peptides derived from *Chlorella vulgaris* species have the potential to be employed in the prevention of diseases such as atherosclerosis, cancer, and coronary heart disease .

Chlorella vulgaris is also used to feed fish. Fishmeal has historically been used in fish feed, and in recent years, as fish output has increased, microalgae have emerged as an economical and environmentally acceptable alternative, as well as containing nearly all nutrients required by fish .

The effect of feeding Chlorella on Gibel carp (Carassius auratus gibelio) growth performance and physiological parameters (blood parameters and digestive enzyme) was studied. In comparison to the control, the addition of 0.8-1.2 percent Chlorella resulted in improved growth, greater amounts of lysozyme (blood parameter that affects protein/lipid metabolism and immunity of gibel carp), and a higher amount of digesting protein (amylase, lipase, and protease) (without Chlorella supplementation). Furthermore, the cholesterol level in fish given Chlorella was lower than in the control. The effects of Chlorella vulgaris (supplement food) on blood and immunological parameters of Caspian salmon exposed to the Viral Nervous Necrosis virus were investigated, and it was discovered that the presence of Chlorella in fish given diet could act as a natural immunostimulant.

Another study used Chlorella meal supplemented with dietary cellulase to replace fish meal in crucian cap Carassius auratus, and after evaluating growth performance, digestive enzymatic activities, histology, and myogenic gene expression, they concluded that Chlorella meal could completely replace fish meal.

Microalgae are addressed as a source of nutraceutical antioxidants due to their rich content of carotenoids, vitamins, and phenolics. Various studies have aimed to maximize the yield of antioxidants by induction of different stress factors. Example, an experiment showed that trace amounts of selenium. [2]

BIODIESEL PRODUCTION BY *CHLORELLA VULGARIS*

Chlorella vulgaris is a type of microalgae that are unicellular photosynthetic organisms that use light energy and carbon dioxide (CO_2) to produce food . Because of its rapid growth and ease of cultivation, *Chlorella vulgaris* is one of the most appealing algae species for biofuel production. Microalgae are non-edible and can grow in a wide range of environmental conditions with no impact on the human food supply chain. These microalgae offer a viable biomass feedstock to produce biodiesel The composition of fatty acids

(FA) in biodiesel determines its qualities. The qualities of biodiesel fuel are determined by the properties and structure of individual fatty esters, such as chain length, degree of unsaturation, and chain branching. Cetane number, the heat of combustion, cold flow viscosity, and exhaust emissions are all affected by these fatty acid esters characteristics .A research team discovered that when the number of double bonds in fatty acid methyl esters (FAMEs) increases, the values of cetane numbers, viscosity, and heating values decrease.

In terms of renewability, pollution reduction, economy, and adaptability to current technology, emulsified water containing microalgae in diesel and biodiesel is viewed as a possible fuel.

However, there are certain issues with using microalgae slurry-biodiesel emulsion directly in a diesel engine, as follows:

1. The presence of huge microalgae aggregates causes injection blockage or damage.

2. High viscosity, which makes gasoline flow more difficult.

3. Because of the emulsion's low stability, the heavy phase of the emulsion settles in the fuel tubes.

Thus, a series of steps must be taken to obtain biodiesel that is suitable for diesel engines and meets the required specifications.

The following are the steps in the production of biodiesel by *Chlorella vulgaris*:

1. **Cultivation:** Microalgae and cyanobacteria can be grown in open or closed ponds using photoautotrophic methods or heterotrophic methods (where algae are grown without light and are fed a carbon source, such as sugars, to generate new biomass). Using the biology of the algae strain and integrating it with the best downstream processing options is key to designing an optimal culture system. The culture system chosen is critical to the cost, scalability, and long-term viability of algae-to-biofuel systems.

2. **Harvesting/dewatering**: Pre-processing activities such as harvesting and dewatering are required in some methods for converting algae to liquid transportation fuels. Algal cultures are typically produced in water, and before extraction and conversion, process steps to concentrate harvested algal biomass may be required. These steps can be time-consuming and energy-intensive.

3. **Extraction**: Lipids (including triglycerides and fatty acids), carbohydrates, and proteins can all be extracted from algal biomass. As mentioned previously, proteins can be used for co-products (nutraceutical products). Lipids and carbohydrates are fuel precursors (e.g., gasoline, biodiesel, and jet fuel),

4. **Conversion**: Chemical, biochemical, and thermochemical processes, as well as a combination of these, are available as conversion technologies. Depending on the conversion technology used, the end product varies. In this review, we will discuss the chemical method of conversion and how it can be used with certain catalysts. .

Increasing the lipid content in *Chlorella vulgaris*:

Chlorella vulgaris is one of the most promising biofuel algae species due to its quick growth and easy cultivation. However, its low lipid content makes it still commercially feasible. Therefore,

ISBN: 979-85-27243-61-1

increasing lipid content in this species is a crucial study area to address. Dual requirements for boosting biomass and lipid production are hard to satisfy. A study claimed that many microalgae have increased lipid storage under environmental stress. Increasing lipid content under stress may impact the productivity of biomass. Biomass productivity and *Chlorella vulgaris* lipid content productivity can be increased by applying specific cultural conditions . The lipid content of Vulgaris can rise by up to 56.6% of dry biomass weight by adding 1.2 to 1.5 $mol \cdot L^{-1}$ $FeCl_3$. *Chlorella vulgaris* lipid content is highly affected by growth circumstances. For example, a research group stated that microalgae lipid content reduced from 14.71 % to 5.90 % as the temperature rose from 25 to 30 °C . Another group observed that the lipid production from mixotrophic *Chlorella* species is more than the sum of lipids produced by photoautotrophic and heterotrophic cultures. Another study determined that when the wavelength of red light is administered during the growth stages, the dry weight of lipids contained in *Chlorella* cells is doubled.

Lipid Extraction: Bligh and Dyer Method

For lipid extraction, a *Chlorella vulgaris* culture is isolated and an algae growth test is used. A combination of 2.5 ml chloroform and 2.5 ml methanol was used to extract the lipids (1:1). To speed up the lipid extraction process, 1 ml of 0.85 percent NaCl can be added to the mixture.

The mixture is thoroughly mixed before being centrifuged for 15 minutes at 4000 rpm. The presence of methanol/water (top layer) and chloroform (base layer) was discovered. The chloroform layer was also cleansed with anhydrous sodium sulphate powder before being gathered into the weighted measuring device. Under highly sanitized nitrogen gas, the natural solvents therein were evaporated. The remaining lipids are weighed as well. This is based on the lipid content of the algal dry weight. The equation Lipid content (%) x dry biomass (mg/L) was used to get lipid efficiency (mg/L). Salts, acids, and solvents can all be used to extract lipids.

1. Acid treatment:

a) Addition of HCl:

1 mL cell suspension + 3.75 mL chloroform/ methanol/ 12N HCl (2/4/0.1 v/v/v). 1.25 ml chloroform is incorporated with vortex for 30 seconds after vigorous mixing, followed by 1.25 ml water with equal mixing. After 10 minutes of low-speed centrifugation, the lower chloroform layer is removed and put into a glass tube for dissipation.

b) Addition of acetic acid:

1 mL of water contains 3.75 mL of chloroform: methanol combination (1:2). Vortex the mixture for 10 to 15 minutes before adding 1.25ml of chloroform. After 1 minute of mixing, 1.25 ml of water was added and the mixture was centrifuged at 4000rpm for a minute. The lower layer upper layer is discarded, and the lower layer is collected with a Pasteur pipette. For the extraction of acidic phospholipids, acetic acid (0.5 percent v/v) is added to the extracted.

2. Salt treatment:

a) Addition of NaCl:

1 mL of water mixed with 3.75 mL of chloroform: methanol (1:2) mixture. After vortexed for 10-15 minutes, add 1.25ml of chloroform to the mixture. After 1 minute of mixing, 1.25 ml of water was added and the mixture was centrifuged at 4000rpm for a minute. Using a Pasteur pipette, collect the lowest layer and discard the upper layer. To remove the lipids, the separated layer is treated with 1N NaCl.

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b) Addition of KCl:

1 mL of water mixed with 3.75 mL of chloroform: methanol (1:2) mixture. After vortexed for 10-15 minutes, add 1.25ml of chloroform to the mixture. After 1 minute of mixing, 1.25 ml of water was added and the mixture was centrifuged at 4000rpm for a minute. Using a Pasteur pipette, collect the lowest layer and discard the upper layer. To remove the lipids, the separated layer is treated with 1N KCl.

3. **Solvent Extraction:**

In a Soxhlet glass sample tube, a 1g sample of pre-dried and pulverized biomass (microalgae) is weighed accurately. The sample tube was transferred to the extraction chamber. In the solvent cup, a 50ml aliquot of the extraction solvent containing hexane: isopropanol (3:2) was transferred and placed on the heating plates. The cooling water supply to the condensers was opened. The extractions spanned three hours. After the extraction process was completed, the samples were allowed to cool for at least 15 minutes before the solvent cups containing the lipid extracts were removed. Solvent extraction was used to extract the lipids.

Transesterification:

The parent oil used to make biodiesel is made up of triglycerides **Figure 2,** which are made up of three fatty acid molecules esterified with a glycerol molecule. Triglycerides are reacted with methanol to make biodiesel in a process known as **transesterification or alcoholysis.** Transesterification produces methyl esters of fatty acids, which are biodiesel, and glycerol (**Figure 2**). The reaction occurs stepwise: triglycerides are first converted to diglycerides, then to monoglycerides, and finally to glycerol.

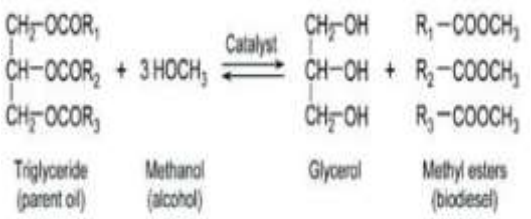

Figure 2 *Oil is converted to biodiesel by a process called transesterification. Groups R1–3 are hydrocarbons.*

To make 1 mol of glycerol and 3 mol of methyl esters, transesterification requires 3 mol of alcohol for each mole of triglyceride. **Figure 2.** The reaction has reached a state of equilibrium. For each mole of triglyceride in industrial operations, 6 mol of methanol is used. The high concentration of methanol ensures that the reaction proceeds in the direction of methyl esters, i.e., biodiesel. On a weight basis, the yield of methyl esters approaches 98 percent. Acids, alkalis, and lipase enzymes accelerate transesterification ,. Transesterification catalysed by alkali is about 4000 times faster than transesterification catalysed by acid. As a result, commercial catalysts such as sodium and potassium hydroxide are routinely utilized at a concentration of roughly 1% by weight of oil. Alkoxides, such as sodium methoxide, are more effective catalysts than sodium hydroxide and are being employed more frequently. Because methanol boils off about 65 °C at atmospheric pressure, alkali catalysed transesterification takes place at around 60 °C at atmospheric pressure. Methanol and oil do not mix; hence the reaction mixture contains two liquids.

phases. Other alcohols can be utilized, but methanol is the cheapest. To prevent yield loss due to saponification reactions (i.e., soap formation), the oil and alcohol must be dry and the oil should have a minimum of free fatty acids. Glycerol and methanol are removed from biodiesel by repeatedly washing them with water.

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FAME analysis

The determination of total lipids represented as fatty acid methyl esters (FAME) is covered by this Laboratory Analytical Procedure (LAP). The percentage of FAME content is calculated based on the dry weight of the sample. The method is based on a whole-biomass lipid transesterification to FAME, which avoids the need for extraction and allows access to all fatty acids in the biomass, providing a true reflection of biofuels potential.

Conclusion:

Considering the potentials of regulating microalgal cell culture conformation and its quick growth, several photosynthetic algal species are addressed to be a good resource for manufacturing CO_2 -free nutraceuticals and biofuels. However, at now, the commercial production of microalgal biomass mostly corresponds to a restricted high-value sector of industries like as food supplements or cosmetic additives. Up-scaling of this method demands more efforts to make the commercial microalgae production of nutraceuticals and biofuels and the requisite facilities should be financially justifiable. Then, more investigations are required to further increase the stability and productivity of outdoor microalgae production systems. The systems reviewed above, help adjustment of critical parameters impacting biomass, protein, antioxidants, and lipid (biofuel) production efficiency. Accordingly, parameters such as light availability, temperature, and nutritional composition should fully be regarded based on the main product of industrial facilities. However, optimization on out-door cultural systems is still a remarkable limitation to overcome.

Increased energy use leads to a reduction in limited non-renewable resources termed fossil fuels. Researchers look for alternative energy sources to satisfy the requirement of the current generation without compromising the ability of future generations to meet their needs., As a result, presently the production of energy from biological sources is highly practical and takes advantage of fossil fuel due to the limited impact on the environment. Due to the first generation

and second-generation issues from bioethanol, the researchers were obliged to come up with another alternative called the third-generation biofuel production. Diesel powered automobiles and light trucks are widespread in many nations. Most of the diesel fuel used in diesel engines is refined from crude oil and are called petroleum diesel. Biofuels manufactured from biomass or materials derived from biomass and include biodiesel and renewable diesel. Microalgae like *Chlorella vulgaris* possess lipid content and biomass in their cell mass. The lipid concentration in this microbe has transformed the course of the diesel business by giving an alternative to diesel. The crude lipid generated from microalgae is successfully transformed into biodiesel. The lipid component of microalgae undergoes further transesterification resulting in biodiesel. Chlorella vulgaris culture when cultivated in vitro undergoes a range of processes in order to boost the lipid content. Still research is needed to be done to perfect the method of raising the lipid content in the microalgae. Lipid separation is done using Bligh and Dyer Method which is one of the best methods for lipid separation. After separation, lipids undergo transesterification using various catalysts in order to obtain biodiesel efficiently. This biodiesel synthesized is now possessing all the properties of a diesel whose potential may now be identified by

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FAME analysis. Although Biodiesel is an environmentally friendly option it has its own limitations. It requires large number of conditions to be maintained and fertilizers to be applied. The present methods available for extraction is rather expensive. Many studies are being conducted to reduce costs and improve energy efficiency.

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Parameter	Mechanism/Strategy	Result
NO3	More nitrate utilization and more protein accumulation	Protein content increased up to 44.3%
Traumatic acid (10^{-6}-10^{-5} M)	Increase in antioxidant enzymes activity (SD, catalase, POD, GR)	The lipid peroxidation stopped by TA, SH-group proteins underwent oxidative destruction
N-phenyl-2-naphthylamine (2.5 mg/L)	Inhibited photosynthesis, triggered ROS synthesis, disrupted the subcellular structure	As an allelochemical, 2.5 mg/L of it significantly increased antioxidant enzymes activities e.g. SOD, POD, and catalase
Nonylphenol (0.1-1.0 mg/L) exposure time	Induced oxidative stress	Obvious effects on antioxidant responses in the first day High NP contents changed the SOD, catalase, POD, and GSH levels
Elevated light intensities (400 µmol photon/ms)	Potential source of zeaxanthin, So-called "molecular sunglasses" mechanism	Induced color change of microalgae from green to yellow Extra produced xanthophylls act as potential antioxidants, stabilize the membrane, and protect cells from intensive radiation
Iron-dependent oxidative stress	Triggered oxidative stress by surplus iron, decreasing the cellular growth rate of phytoplankton	>200 µM iron supply reduced the *Chlorella vulgaris* growth level and did not change the β-Carotene content >90 µM iron availability raised vitamin E , vitamin C , and total thiol content
Sodium Nitroprussiate, CTAC/Flu surfactant, Polycyclic aromatic hydrocarbons	SNP alleviated the pollution damage of surfactants and PAHs by providing external NO- for *Chlorella vulgaris* cells	Supplying 20 µM SNP Increased the biomass, the chlorophyll concentration, and the activity of SPC, SOD, POD, and catalase, Decreasing MDA and ROS amounts
Trifloxystrobin	Decreased antioxidant enzymes' activity Disturb photosynthesis in *Chlorella vulgaris* Destruct the cellular structure	255.58 µg/L of trifloxystrobin (IC50): Reduced transcription of genes associated with the photosynthesis, soluble proteins, and T-AOC Increased SOD & POD activities and ATP expression
Azoxystrobin	AZ disrupts the *Chlorella vulgaris* growth through: reducing energy/photosynthesis associated mRNA expressions Inducing ROS overproduction	510 µg/L (IC50) of AZ: Reduced the chlorophyll and soluble protein content Increased the T-AOC level Weakened SOD, POD, GSTs, and GPx activities, and GSH content

pomelo chakota leaves as a new dye for preparation of DSSC same is analysed by using the UV-Spectro Photometer

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Abstract: *In the world utilization of sun's rays is not hundred percentage, There are many researches going on to utilize the sun's rays (Both Light and heat energy) in to useful energy for generation of solar energy, There are some devices invented to utilize the sun's rays some of the devices like solar cell, solar cooker, solar trackers, solar drier etc. Silicon solar cell is a first generation solar cell, New method to develop power generation is DSSC (Dye sensitized solar cell),where we can minimize the utilization of the silicon solar cell, with less cost we can prepare the DSSC by using the natural dye.in this paper we discuss one of the natural dye that is pomelo chakotra/chakota leaves, This dye is undergone some of the analysis with the different solvents and some of the analysis like UV-Spectrophotometer,DS-500 Spectrophotometer,PH-Meter and colorimeter.Wo3 semi conductive oxide material for the preparation of DSSC.*

Keywords: GW,JNNSM,DSSC,LUMO,HOMO,CNT

1. INTRODUCTION

DSSC is the third generation solar cell where the DSSC is abbreviated Dye Sensitized Solar cell, here in DSSC we are having four main components they are working electrode (Photo anode), Dye, Counter electrode and Electrolyte shown in the figure below

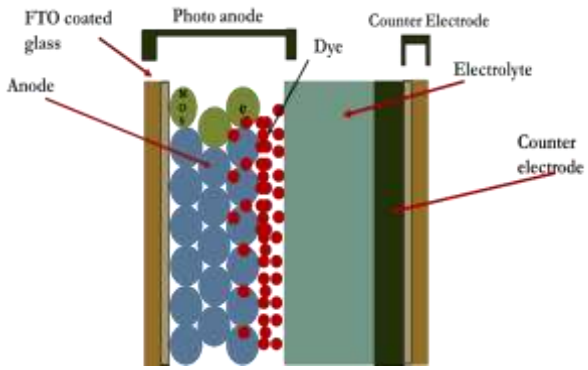

Figure no 1 : Components of the DSSC

Where in this paper we are going to discus about the chakotha leaves and this natural dye is used as a dye in the preparation of the DSSC.WO3 material is used as a semi conductive oxide (Thin film on ITO coated Glass),electrolyte (Ki+I2+acetonitrile),carbon soot as counter electrode.

2. Methodology

2.1 Dye Preparation

Figure no 2 : Chakotha leaves

Plug the leaves from the plant, Wash them to remove the dust, Dry the same in a room, Cut the leaves into small pieces ,With the help of mixer make the leaves into power ,Add the solvents as shown in below table no 1 ,Filter the Dye which are soaked in different solvents with quantity mentioned in the table no 1 with the help of what's man filter the dye is filtered and stored in a separate bottle same is wrapped with aluminium foil.

Chakotha Leaves		
Slno	Solvents Names	Quantity in ml
1	Ethanol	85
2	Methanol	120
3	Distilled water	130
4	DM water	100
5	Linced oil	150
6	Ethanol	50
	Acetic acid	10

Table no 1 : Solvents and quantity used

2.2 UV-Spectro Photometer

Take the Two cuvette as shown in the figure 3A, in one cuvette reference sample is taken as shown in figure 3B, The sample is placed in a sample holder as shown in the figure 3C,lid is closed and reference value is set as shown in the figure 3D, Plunger is pulled samples as shown in E taken one by one filled in another cuvette kept in sample older closed the lid and noted down the reading of the unknown sample by varying the lambda values and noted down the reading for every 5nm reading and tabulated In the tabular column as shown in the figures 3F,G,H,I,J,K,L and the cuvette is washed with the distilled water and repeated the steps 3F to 3O till all the samples done.

Figure no 3 : UV-Spectrophotometer

Tabular Column : UV spectrometer					
Dye Name : Choktha leafs					
Slno	Solvents	λ (nm)	%T	Abs	Con
1	Distilled water	400	40.0	0.391	545
2	Ethanol		27.7	0.555	739
3	Methanol		47.5	0.426	430
4	DM water		25.5	0.590	742
5	Linsed oil		28.1	0.532	480

Table no 2 : lambda 400nm constant

Tabular Column : UV spectrometer					
Dye Name : Choktha leafs					
Slno	Solvents	λ (nm)	%T	Abs	Con
1	Distilled water	400	40	0.391	545
2	Ethanol	400	27.7	0.555	739
3	Methanol	600	41.4	0.396	423
4	DM water	400	25.5	0.590	742
5	Linsed oil	600	51.5	0.293	266

Table no 3: lambda variations

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After taking the reading we have to find the absorption, concentration and transmittance and tabulated as shown in the tabular column separate tabular column is drawn for different wavelengths, same wave length as show in the tabular column 2 & 3.

2.2.A Energy Gap

For maximum wavelength that is 1000nm is taken as show in the table no 4 ,The Determination of the optical energy gap of dye
absorbed by the semi conductive oxides surface is considered by using formula

$$E = h\nu = \frac{hc}{\lambda}$$

h is the Plank's constant = 6.63 X 10^{-34} Js
ν is the frequency
λ is the wavelength
C is the Speed = 3.0X10^{8} m/s
1ev=1.60X10^{-19} J
E stands for photon energy or optical energy gap

Tabular Column : UV spectrometer					
Dye Name : Choktha leafs					
Slno	Solvents	λ (nm)	$Eg = h(C/\lambda)(ev)$		
			h Js	C m/s	Eg
1	Distilled water	1000	6.63E-34	300000000	2.15415E-30
2	Ethanol	1000			2.15415E-30
3	Methanol	1000			2.15415E-30
4	DM water	1000			2.15415E-30
5	Linsed oil	1000			2.15415E-30

Table no 4 : Energy band gap

2.2.B Adsorption coefficient

The absorption coefficient determines how far into a material/, light of a particular wavelength can penetrate before it is absorbed .the absorption coefficient of the respective wavelength by the division of the absorbance with the wavelength shown in below equation using Boltzmann constant

$$Absorption\ coefficiet = \frac{4\pi k}{\lambda}$$

λ is the wavelength
k Boltzmann constant
k = 1.38X10^{-23} J/K

2.2.C Dye adsorption percentage

was determined from the dye concentration difference by using formula of equation

$$dye\ adsorption\ \% = \frac{c_o - c}{c_o}\ X\ 100$$

c_o is the initial concentration
C is the concentration after certain period

2.3 PH -Meter

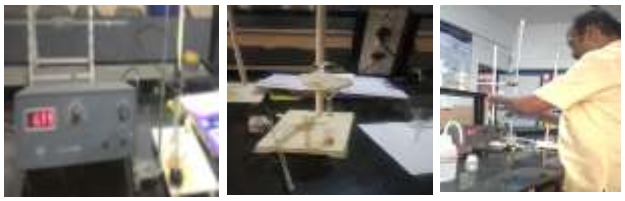
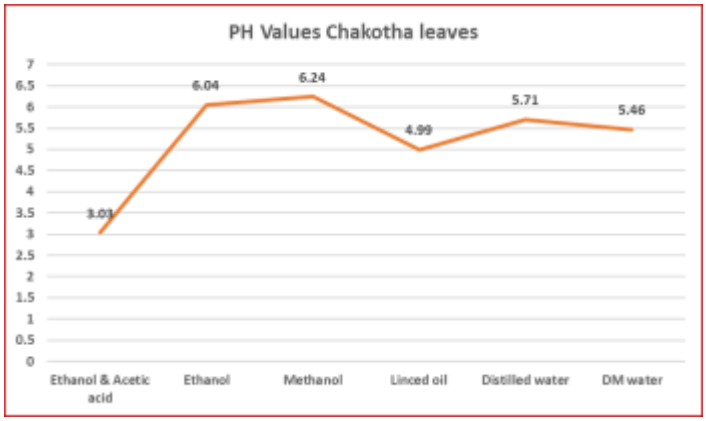


Figure no 4 : Ph-Meter

PH values of the samples Chakotha leaves		
Slno	Particulars (Solvents)	PH Values
1	Ethanol & Acetic acid	3.03
2	Ethanol	6.04
3	Methanol	6.24
4	Linced oil	4.99
5	Distilled water	5.71
6	DM water	5.46

Table no 5 : Different solvents PHMeter

Take the calibrated PH meter and find out the PH values of each samples same measured is tabulated in the tabular column no 5 and plotted a graph ash shown in the graph no 1.



Graph no 1 : PH values of Chakotha leaves

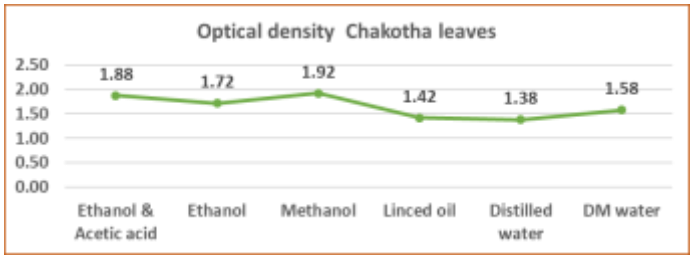
2.4 Colorimeter

Take the calibrated colori meter and find out the optical density and lambda values of each samples same measured is tabulated in the tabular column no 6 and plotted a graph ash shown in the graph no 2 & 3.

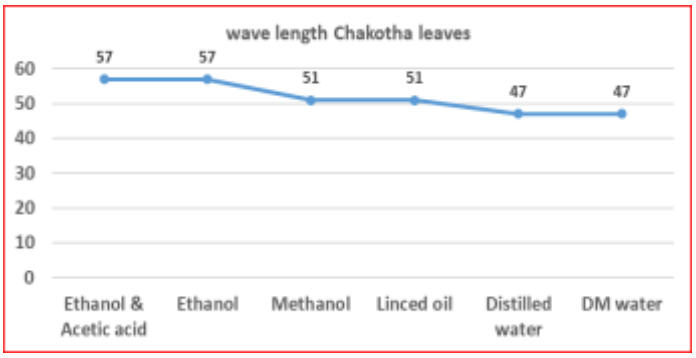
Figure no 5 : Colorimeter

Colorimeter : Filter selection for maximum optical density Chakotha leaves			
Sln o	Particulars	wave length	Optical density
1	Ethanol & Acetic acid	57	1.88
2	Ethanol	57	1.72
3	Methanol	51	1.92
4	Linced oil	51	1.42
5	Distilled water	47	1.38
6	DM water	47	1.58

Table no 6: Colorimeter used for chakotha leaves analysis (optical density)



Graph no 2 : Optical density



Graph no 2 : wavelength of Chakotha leaves

2.5 Finding the Rf valvue

Rf value is calculated for identifying the spots

$$Rf = \frac{\textbf{Distance travelled by the solute}}{\textbf{Distance travelled by the solvent front}}$$

RF value is always lies between 0 and 1, but ideal value ranges from 0.3 to 0.8,Rf value is constant for every compound in a particular and the chromatography paper is used for analysis and to know the components present as shown in the blow figure 6 and components present in the tabular column no 7 .

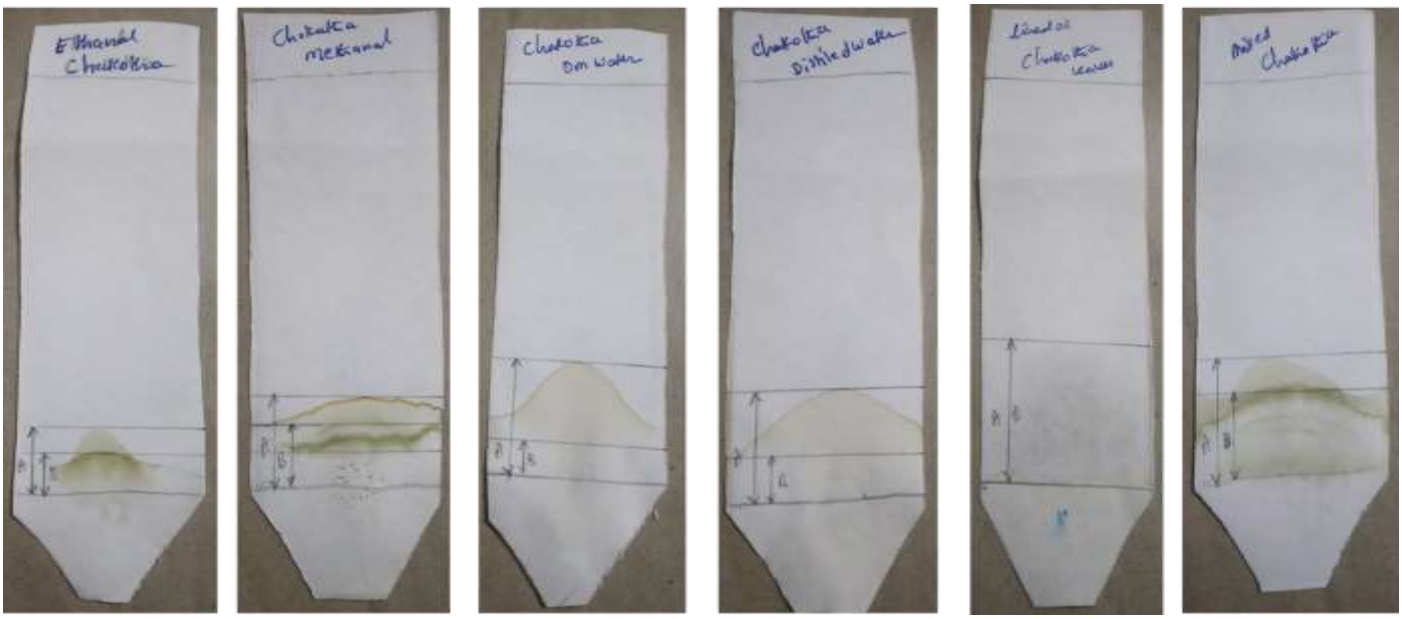


Figure no 6: Chromatography for Chakotha leaves for different solvents

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Dye Name : Chakotha leaves					
slno	Solvents	A	B	Rf Value B/A	Pigment present
1	Mixed	3	2	0.67	Chlorophyll b Caraton
2	DM water	2.7	0.4	0.15	Caraton
3	Linced oil	3.3	3.3	1.00	Chlorophyll b Caraton
4	Distled water	2.5	1	0.40	Caraton
5	Ethanol	1.6	1	0.63	Chlorophyll b Caraton
6	Methanol	2.2	1.5	0.68	Chlorophyll b Caraton

Tabular column no 7 : Pigments present

2.6 UV-Spectrophotometer D500

D500 UV spectro photometer is used for finding the absorption and finding out the lambda values, Take the cuvette in one cuvette reference solvent is taken and then the samples is taken in one cuvette which is to be measured and same steps is repeated for other samples and plotted graphs as shown in the figure no 7 below.

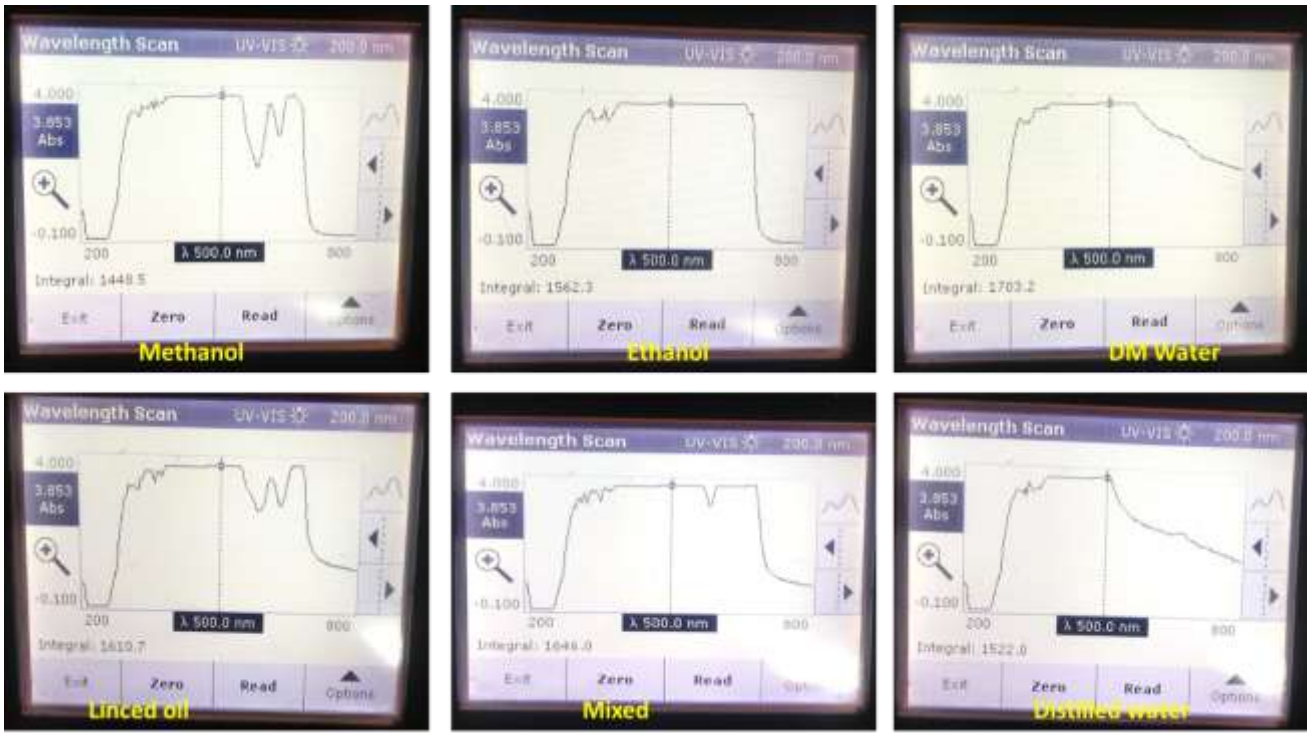

Figure no 7: Chromatography

2.7 Experimental setup

Readings Taken from the DSSC					
Slno	Description	VOC	ISC	FF	Effency
1	Chakotha Leaves (WO3)	193	0.35	0.011	0.092

Table no 8 : Study for the efficiency

Conclusion

1) From the Table no 8 we can see that Chakotha leaves used as a dye with the WO3 as a semicoductive oxide material and found the efficiency is 0.092

2) Chakotha leaves is used in the preparation of DSSC is more suitable

ISBN: 979-85-27243-61-1

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Ethnoveterinary product against animal ticks using Biodiesel Industrial byproduct

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***Abstract*— Ticks are small blood feeding ectoparasites. Ticks of domestic animals directly cause poor health and loss of production to their hosts. Ticks also transmit numerous kinds of viruses, bacteria, and protozoa between domestic animals, these microbes cause diseases which can be severely debilitating or fatal to domestic animals, and may also affect humans. The impact of ticks on livestock production and health includes tick borne disease morbidity and mortality, huge loss of milk and meat production, damage on the quality of skin. Soap is prepared with the crude glycerin which is the byproduct of biodiesel industry from honge oil. It has been experimented on animals and found to be effective in removing ticks and supports the skin health of the animals.**

Keywords*— *ticks, soap, Ethno veterinary, crude glycerin, animal soap, honge oil etc.

I. INTRODUCTION

Parasitic diseases are found to be a major impediment to the productivity and health of animals. Ticks are pathogens to domestic animals and humans so it is necessary to know the impact of tick-borne diseases and their effects. Ticks come under the ectoparasite family they are non-permanent, obligate, and bloodsucker insects. They feed on reptiles, mammals, aves, and amphibians through which they transmit pathogenic fungi, protozoa, viruses, and other bacteria. They are classified into two categories Argasidae (soft body ticks) Ixodidae (hard ticks). There are nearly 800 species of ticks found in all regions of the earth. Their distribution varies from different climatic conditions, so complete eradication of ticks in India is not feasible due to several factors. The products which are available now are mainly made of chemicals due to which they have some adverse effects on animals and humans. The effects being observed are skin rashes on animal skins, gradual loss of hairs in animal skins, in rare cases – carcinogenic traces are also found in Animals. Ticks will reduce the yield of the productive animals and also may extend the infection to the human beings who consume products of the infected animals. The conventional method of treatment is also proving harmful to the Human community as the traces of harmful chemicals are found in the human body due to the consumption of milk from cattle.

Millettia pinnata is a leguminous family it is found in tropical and temperate regions; it has excellent medicinal properties and special mention in Ayurveda. It is having a high amount of omega 9 fatty acid because of which is absorbed by the derma layer making skin healthy and it is also proved for its anti-inflammatory activity and used for many skin diseases, because of this property it is used in soap making industries. With the development in biotechnology and the scope for herbal usage growing worldwide, organic-based products are being introduced into the market for human beings. A need to develop organic assured bio-product for the well-being of domestic animals and increase their productivity was also essential. One of the safe, easy, and effective methods to remove ticks is washing the animal with the honge oil-derived soap. The developed soap has the property to eradicate the ticks found in skins of Cows, Buffalos, Sheep, and other domestic animals with no harm to either animal or human beings.

II. METHODS

A. Materials

Crude glycerin is a byproduct of the biodiesel industry. For the present study, crude glycerin is collected from Bioenergy Research, Information & Demonstration center, Department of Biotechnology, BEC, Bagalkot, Karnataka. NaOH used is obtained from Nandi traders Bagalkot, Karnataka. The Soap has experimented for Ticks study on 20 cows, 15 Buffalos. This experimented has been conducted for 3 months (application of soap is done once a week).

ISBN: 979-85-27243-61-1

B. Experimental method

Soap is prepared with the crude glycerin which is the byproduct of biodiesel industry from honge oil, that crude glycerin is collected directly and boiled till the complete evaporation of methanol (unused methanol molecule may be present during biodiesel production), once the methanol is evaporated, then NaOH solution is added. When it reaches 50ºC temperature continues stirring is to be done till it becomes the thick solution, and then prepared solution is poured into the mold, after settling soap will be ready to use for animals to make free from ticks. There is no addition of color, preservative, or fragrance to the soap.

We have selected 35 livestock that was infected by ticks, which were 20 cows and 15 buffalos. The prepared soap was applied on each animal once a week for about three months; the ticks have adhered mainly to the abdominal, upper back, neck region, and also other parts of the body.

III. Result

As we refer to the Fig 2 it is given that in March month there is maximum recovery i.e. Nearly 55% and 73% of the ticks have been released from the Cows and Buffalos, in April totally 30% and 20 % of the remaining ticks from the cows and buffaloes were detached, and lastly in the months of May 15% and 7% of ticks were released completely from the skin of the Cows and Buffalos. After this three month of application of soap on the livestock's complete removal of the ticks from the animal skin was done.

IV. Conclusion

Ticks infestation on livestock tends to be a serious problem in the livestock industries due to which the productivity in the animals is reduced and it is also a big challenge for all the workers to overcome this problem. The result obtained from the present study manifests that among the 35 infested livestock, by application of Honge based soap for about 3 months has been 100 % effective in removing all the ticks adhered to the skin of the livestock. There was no physical harm is done for the animals while applying the soap and there were no adverse reactions or allergic effects for the animals and for the applier. This study helps an easy, safe and harmless way to remove all the ticks adhered to the animal.

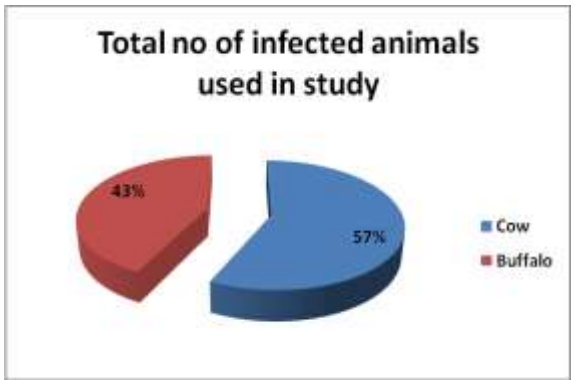

Fig 1: Total no of infected animals used in study

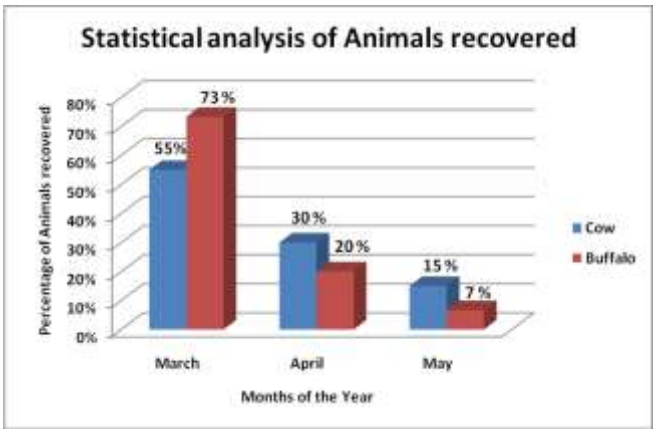

Fig 2: Statistical analysis of Animals recovered

Fig 3: Ticks attached on Neck and Ear part

Fig 4: Application of soap to cow

Fig 5: Soap to be applied on the infected animals

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Isolation of tannins from *Citrus limon* with honey mixture and its antagonistic activity against bacteria.

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Abstract

Isolate phytochemical namely tannins from *Citrus limon* with honey mixture to check antimicrobial activity against bacteria. Phytochemicals was extracted using methanol. Further well diffusion method and antibacterial activity to check the minimum inhibition concentration was evaluated by micro titer 96 wells plate method for tannins isolated from the combination and assessed with tetracycline as control against organisms *Bacillus cereus* MTCC 1272, *E-coli* MTCC 433, *Salmonella typhi* MTCC 3231, *Pseudomonas aeruginosa* MTCC 2453, *Staphylococcus aureus* MTCC 87, *and Streptococcus mutans* MTCC. Tannin showed antimicrobial activity extracted from the mixture.

Introduction

Millions of people lives have been saved because of antibiotics and have contributed to the major gains in life expectancy over the last century. Infectious diseases caused by resistant microorganisms are associated with for morbidity and mortality. To screen various medicinal plants for their potential antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant and phytochemicals. The most important of these bioactive compounds of plants are tannins, phenolic compounds, steroids, resins, fatty acids, alkaloids, flavonoids and gums which are capable of producing definite physiological action on body. Another driving factor that encouraged scientists to search for new antimicrobial substances from various sources including medicinal plants has been the rapid rate of plant species extinction. Medicinal plants are relied upon by 80% of the world's population and in India there is a rich tradition of using herbal medicine for the treatment of various infectious diseases, because of Ayurvedha and Siddha for inflammations, injuries and other chronic diseases.

Materials and Methods

1. Sample collection and preparation: *Citrus limon* was collected from Bangalore market and lemon juice was filtered. Further lyophilised and subjected to honey 1:1 ratio which was collected from Nilgiris biosphere.

2. Isolation of tannins (methanol extraction): *Citrus limon* and honey (1:1) sample extracted from methanol was grounded with methanol for10 min in 1: 5 (sample : solvent :: 1g: 5 ml solvent) at 50°C. The extracts are then added to 95% ethanol. The mixture is kept for 12 hours at room temperature and then centrifuged for 10 min at 4000rpm and then filtered to obtain tannin rich extract and dried (Kun et al. 2011). The extraction was carried out in triplicate. percentage yield of tannin rich extract was obtained by drying the extracts using rotary evaporator. Yield was calculated.

Yield (%) = (extract weight after extraction and drying)/ (dried plant weight before extraction) ×100 (Sitthichai et al. 2014).

3. Qualitative phytochemical analysis of the extracts.

Test for Tannins (Ferric chloride test): tannin extract were treated with 3 - 4 drops of ferric chloride solution. Formation of bluish or green precipitate was indication of presence of tannins.

4. Well diffusion method:

1 ml of fresh bacterial was pipetted in the centre of sterile petri dish. Upon solidification, wells were made using a sterile cork borer (6 mm in diameter) into agar plates containing inoculums. Then, 100 µl of each extract was added to respective wells. The concentration of extracts has been selected based on MIC. The plates were incubated at 37°C for 18 h. Antimicrobial activity was detected by measuring the zone of inhibition (including the wells diameter) appeared after the incubation period. DMSO at a concentration of 10% was employed as a negative control (Daoud et al., 2015).

5. MIC: methanol extact sample with *Citrus limon* + Honey (Tannins) and Control (tetracycline) were assessed for their MIC property against organisms (*Bacillus cereus* MTCC 1272, *E-coli* MTCC 433, *Salmonella typhi* MTCC 3231, *Pseudomonas aeruginosa* MTCC 2453, *Staphylococcus aureus* MTCC 87, *and Streptococcus mutans* MTCC). **Sample preparation was done using** 10mg of the sample which was dissolved in 1mL of Dimethyl sulfoxide (DMSO) respectively and 100µL of dissolved sample are used to check the Minimum Inhibition Concentration (MIC) for respective organism. **Culture was Prepared** and after 24h cultured organisms were centrifuged and 6000rpm for 10 min, supernatant was discarded, and the pellet was adjusted to absorbance 0.5 by using 1% saline (0.1g sodium chloride in 10mL of distilled water) spectrophotometrically at 600nm. Luria Bertani (LB) broth (tryptone 10g, sodium chloride 10g, yeast extract 6g and distilled water 1000mL) 100mL was prepared and autoclaved at 121°C for 15 mins. **Plate Preparation was done using** 300µL of deionized water was added in the border (surrounding) wells of the micro titer plate (A_1 to A_{12}, B_{12} to H_{12}, H_1 to H_{11}, and G_1 to B_1) to prevent the sample from drying. 100µL of sterilized LB broth was added to all the remaining testing wells. 100µL of 0.1% of

resazurin dye was added to the wells B_2 to G_2 in respective plates and named as colour blank. In wells B_3 to G_3 test organism and 100µL of 0.1% of resazurin was added in respective plates as culture control. 100µL of the sample Lemon + Honey (Tannins), was added to respective plates from wells B_4 to G_4 and serially diluted by transferring 100µL of the sample to subsequent wells upto 11^{th} well and 100µL of the excess sample was discarded from 11^{th} well, 24h cultured 100µL of organism and 100µL of 0.1% of resazurin dye was added to the diluted samples respectively. The plates were incubated at 37° C for 24h. (Sarker, Nahar & Kumarasamy; 2007).

Results

1.Sample preparation: sample *Citrus limon* was collected and lemon juice was filtered. Further lyophilised and subjected to honey 1:1 ratio.

2. Isolation of tannin (methanol extraction):

Citrus limon : Honey – lyophilized 3g methanol extract in 15 ml methanol: 15 ethanol

Samples(methanol)	Final weigh t (g)	Initial weigh t (g)	% Yiel d
Citrus limon : Honey	0.449	3.07	14.6 2

Tannin's estimation Methanol extract

Concentration of tannic acid (µg/mL)	Mean Absorbance at 720nm
0	0.00
40	0.126
80	0.240
120	0.354
160	0.439
200	0.522

Samples	Mean absorbance	Amount (mg/g of extract)
Citrus limon + Honey	0.143	453.968

3. Qualitative phytochemical analysis of the extracts:

Test	**Lemon : Honey**
Tannin methanol	Slightly +

(+) indicates present; (-) indicates absent; (Slightly +) indicates present in traces

4. Well diffusion method:

The tannin methanol extract of *Citrus limon* and honey showed maximum inhibitory zone against standard *S. aureus*, *Pseudomonas aeruginosa*.

5. Minimum inhibition concentration:

Presence of any blue colouration indicates inhibition of the organism, whereas, no blue colour indicates no inhibition of the organism.

Citrus limon + Honey (Tannins) to test against organisms:

100µL of culture was inoculated to serially diluted sample wells (*Bacillus cereus* was inoculated to wells from B_4 to B_{11}, *E-coli* was inoculated to wells from C_4 to C_{11}, *Salmonella typhi* was inoculated to wells from D_4 to D_{11}, *Pseudomonas aeruginosa* was inoculated to wells from E_4 to E_{11}, *Staphylococcus aureus* was inoculated to wells from F_4 to F_{11}, *Streptococcus mutans* was inoculated to wells from G_4 to G_{11} and 100µL of 0.1% of resazurin dye was added to all the test sample wells. The plates were incubated at 37° C for 24h. The least concentration where no turbidity was observed was determined and noted as the MIC value.

Figure: (L+H) *Citrus limon* + Honey (Tannins) (methanol extract) micro titer plate

The minimum inhibition concentration of *Citrus limon* + Honey (Tannins) for *Bacillus cereus* is 1000µg, *E-coli* 1000µg, *Salmonella typhi* 1000µg, *Pseudomonas aeruginosa* 1000µg, *Staphylococcus aureus* 500µg and *Streptococcus mutans* NIL. Few wells showed slight inhibition by slight bluish colouration, whereas *Staphylococcus aureus showed most inhibition for tannin extract.*

Conclusion:

Tannin methanolic extract was isolated from *Citrus limon* and honey and the concentration as checked using tannic acid standard. Additionally, the tannin methanol extract of *Citrus limon* and honey showed maximum inhibitory zone against standard *S. aureus*, *Pseudomonas aeruginosa*. On the other hand, the extract indicted pronounced antibacterial activity against *S. aureus*. Earlier studies have reported antibacterial properties of ethanol extracts of aloe vera against the pathogens (Agarry et al., 2005). According to the antibacterial assay done for screening purpose all extracts in general are more effective.

MIC showed that the most susceptible organism was *S. aureus* which was sensitive to tannin methanolic extract. The susceptibility of this bacterium to isolated extracts has been studied which reported strong antibacterial potential against *S. aureus* as compared to *other organism*. It was interesting to note that the tannin methanolic extracts from *Citrus limon* presented antimicrobial activity to *S. aureus* the most according to MIC studies. Previous reports also revealed the antibacterial efficacy of the investigated in plant extracts.

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Triple negative breast cancer and computational repurposing of drugs

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ABSTRACT

Triple negative breast cancer (TNBC) is one of the most heterogeneous and the most aggressive type of breast cancer. It is mainly characterized by the lack of key receptors normally found in breast cancers, such as the oestrogen receptor (ER), progesterone receptor (PR) and the human epidermal growth factor 2 (HER2). The aggressive nature of TNBC owes to their very early relapses (3-5 years), lower survival rate, poor prognosis and poor diagnosis. Hence, there is a need to find a suitable drug for the treatment of TNBC. There are a number of observations in regard to the problems in the area of TNBC research. Firstly, there is a cluster of TNBC cases in women of African descent and who are under the premenopausal stage. Further, the prognosis of TNBC is very poor in comparison to non-TNBC breast cancer types, despite good initial response to chemotherapy, and in addition there is an overlap among the BRAC-associated breast cancer type and the TNBC phenotype. Lastly, there are no effective TNBC specific targeted therapies. There are many studies that are related to characterizing the features of TNBC at their molecular level, there are also many studies in preclinical and clinical studies of therapies for TNBC. This review deals with the molecular characterization of TNBC and the various approaches of repurposing of drugs in the scientific field and the computational approaches in Repurposing of drugs are emphasised here.

INTRODUCTION

Breast cancer, a heterogenous disease is one among the most frequently diagnosed disease and is the second leading cause of death due to cancer among women, after lung cancer. Immunohistochemically, TNBC is identified by the lack of hormone receptors such as Oestrogen receptors (ER), Progesterone receptor (PR) and the Human epidermal growth factor 2(HER2) expression [1].

Even though TNBC is much more chemotherapy sensitive than any other type of breast cancer, it is characterized by having the most aggressive behaviour with a very high risk of relapse. The relapse could occur within the first 3-5 years in younger women after the adjuvant chemotherapy completion [2].

Once TNBC has metastasised, there is a high chance that it will involve the growth and spread of tumours to the critical organs such as brain lung and liver. This could lead to a shorter overall median survival rate than the other subtypes [3].

Annually about 1-1.5 million cases of breast cancers are reported; about 10-20% of the patients are diagnosed with TNBC. TNBC is considered as an orphan disease since it lacks the hormone receptors and biological markers, it is also marked by presence or absence of the tumour nodes in metastatic stage, involvement of lymph nodes, tumour size etc., are all considered for the prognosis and therapeutic management [4].

TNBCs are seen all the more regularly in more young ladies (age, <50 years), especially in those of African American plunge, and frequently present as span cancers [5]. Radiologically, TNBCs are regularly distinguished as hyperdense masses without related calcifications. In view of the molecular subtype, four unique kinds of breast cancer growth have been distinguished: (1) Luminal A (HR+/HER2−) (71%), (2) luminal B (HR+/HER2+) (12%), (3) HER2-enhanced (HR-/HER2+) (5%), (4) triple-negative (HR−/HER2−) (12%) [6]. TNBC can be sub-divided into 6 subtypes: basal-like (BL-1 and BL-2), mesenchymal (M), mesenchymal stem-like (MSL), immunomodulatory (IM), and luminal androgen receptor (LAR), and unspecific. An elective order separates TNBC into BL1 and BL2, M and LAR[7].

Roughly about 20% of the patients with TNBC react well to standard treatment (tumour resection, radiation and cytotoxic chemotherapy), however the remaining foster deadly metastatic infection. TNBC is more delicate to chemotherapy contrasted with different kinds of breast cancers. In

any case, even though a progression of treatments such as the customary radiotherapy, adjuvant chemotherapy, an efficient treatment, and a targeted therapy, the pinnacle hazard of TNBC relapses happen within 3–5 years, at last prompting passing in the vast majority of the cases. A new clinical preliminary has exhibited an empowering reaction of some TNBC patients to the immune-checkpoint blockade (ICB)inhibitor, Atezolizumab (anti to PD-L1), in mix with customary chemotherapy. However, most patients, including those with high articulation of PD-L1, succumbed to the infection. The recognizable proofs of TNBC subtype that are probably going to react to ICB treatment are likely to be of good study of interest. The propensity of TNBC to start metastasizing to a particular organis higher contrasted with other disease aggregates. When cells get disconnected from the intrusive front of TNBC, they relocate to particular tissue locales with the help of the capillary and multiply in their new microenvironment [8].

TNBC subtypes

Many efforts are being made to understand the molecular profile of TNBC, which would give us insights about the heterogeneity and biological complexity of TNBC subtypes at the molecular level. This helps us analyse the aggressive nature of TNBC which will benefit the researchers to evaluate and identify newer approaches for the TNBC treatment [10].

Based on the tumour gene expressing profile, Lehman et al first documented TNBC classification. By studying the micro-array data, molecular subtypes of TNBC were reported, these subtypes had unique gene expression signature pattens and it also reported very different clinical behaviour [11].

Basal-like subtype (BL-1 and BL-2): About 70% of the TNBC tumours are of this subtype. The genes expressed by BL1 are mainly involved in the DNA damage response, cell cycle regulation and cell proliferation. The genes expressed by BL-2 are involved in growth factor signalling, cell cycle and cell division. BL1 tumours are usually ductal of higher grade and achieves a high pathological complete response(pCR) rate when neoadjuvant chemotherapy is given, on the other hand BL-2 subtype tumours are less likely to achieve a pCR and has higher risk of recurrence in comparison to BL-1. pCR is a favourable prognostic marker for aggressive tumours such as TNBC and is benefited with long term survival [7].

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Mesenchymal(M) and Mesenchymal stem like (MSL):This subtype features higher expression of genes that are part of the epithelial-mesenchymal transition and are involved in the signalling of the growth factors. The mesenchymal type tumours demonstrate less sensitivity to chemotherapy in comparison to the BL-1, they usually metastasize the lungs. Carcinoma that are metaplastic are usually mesenchymal subtype [12].

Immunomodulatory subtype (IM):These tumours express genes that are responsible for the antigen presentation, cytokine signalling and immune processing. Among the TNBC subtypes, IM shows better prognosis. The difference between IM and Mesenchymal subtype gene over-expression in the tumour micro-environment are the infiltrating immune cells and tumour-associated mesenchymal tissues. It was also observed that these genes were not exposed in cell lines in vitro where the micro-environment was not present. Hence, micro-environments have a vital role in the growth of tumour, response to treatments and resistance to chemotherapy. In terms of immunotherapy evolution, IM subtype was actually identified as an immune modulator instead of a distinct subtype. CTL-44, PD-1 and PD-4, immune regulators are highly expressed in TNBC that are generated by the lymphocyte infiltration of the tumour, which is associated with the immune checkpoint inhibitors. There are multiple research outcomes showing Tumour infiltrating lymphocytes (TILS), that could be show better survival outcomes [13,14].

Androgen receptors (LAR): In the recent years TNBC tumours expressing androgen receptors have been the prime attraction. They are classified as luminal androgen receptors (LAR) subtype due to the apocrine histological differentiation which is similar to the luminal HR positive tumours. The LAR tumours depict a lower grade, found in post-menopausal women and lower pCR rate when treated with neoadjuvant chemotherapy. Clinically, this subtype is involved in frequent lymph node at the time of initial diagnosis, it also shows peculiar tropism with high chances of metastatic bone disease. The TNBC tumours are associated with LAR features (lobular histopathological features). LAR subtype involves in expression of genes that are related to hormone synthesis and androgen metabolism and a lower expression of genes involved in basal like proliferation [15,16].

Claudin Low: This is an intrinsic subtype that shares similar features with the basal-like subtype. The main feature of this subtype is the high expression of clusters

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that are stem cell like and mesenchymal type and lower expression of epithelial and intercellular tight-junction genes. The tumours of this claudin low type histological presentation such as metaplastic and medullary type and a high frequency of lymphocytic infiltration is observed [17].

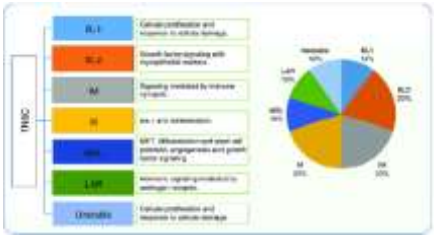

Figure 1: Subtypes of triple negative breast cancers[16].

Diagnosis of TNBC

To diagnosis TNBC, ER, PgR and HER2 testing status is required. By testing the ER, PR and HER2 cut-off status, we can determine the response of the patient to endocrine or HER2 directed therapy, so it does not directly identify the TNBC type of cancer. Over the decade the cut-offs of the ER, PR and HER2 have been varied [18].

TNBC is both of higher risk and an earlier relapse. The recurrence rate is the highest for TNBC with 2 years after diagnosis and the relapse rate after 5 years are not common. In comparison to hormone positive breast cancer (ER/PR type), TNBC has higher proportion of relapse and a very short survival rate when the disease is at a metastatic development. Survival rate is only 12-18 months for metastatic TNBC, whereas it is 5 years for patients diagnosed with ER/PR and HER2 type which is the primary reason for the need of new therapy for TNBC and identification of specified TNBC subtype targeted therapies [19].

Current management of TNBC

Systemic chemotherapy

TNBC lacks molecular targets, hence chemotherapy is the only treatment for TNBC. For TNBC patients with stage 1, TNBC-adjuvant chemotherapy is recommended. The chemotherapy that is recommended for the early TNBC is not distinct rather it is identical to other breast cancer types. Anthracycline-taxane-based therapy is recommended for stage 1-3 TNBC subtype[8].

Even though anthracycline-taxane-based chemotherapy is provided, 30-40% of the patients develops metastatic stage and die due to cancer. It was understood that a set of patients responded well to the standard chemotherapy combinations and the patients who were treated with neoadjuvant chemotherapy have a better pathological complete response (pCR)[18]. Even though we have achieved higher pCR rate, with the conventional chemotherapy, higher relapse

rates occurred in comparison to hormone receptor positive and HER-2 positive type of breast cancer, this is known as the Triple Negative paradox. This paradox states that TNBC patients with neoadjuvant chemotherapy primarily are driven by higher relapse rates. Hence, there is a need to identify subtypes in TNBC that respond well to chemotherapy by standard types and other combinations [19].

Neoadjuvant and TILS therapy

A number of analysis from clinical trials have shown that tumour infiltrating lymphocytes (TILs) can be prognostic in early stage TNBC. During anthracycline based neoadjuvant therapy, the increase and the presence of TILs suggest better response and improve the long-term survival in TNBC patients [20]. But it is very unclear if the presence of the TILs shows favourable tumour biology or if the presence of TILs is improved response to chemotherapy drugs. There TIL are not part of the clinical pathological reports, but to standardize the evaluation and reporting of TILs are part of the diagnosis is an ongoing process. At present there are no biomarkers available to check TNBC patient's response to different chemotherapies/ neoadjuvant therapy hence researchers are on the hunt for new targets for TNBC [21].

Local therapy

Surgery and radiation: there are no TNBC specific local therapies such as surgery and radiation, it is the same for all types of breast cancer, and hence no local treatment is given for TNBC specific subtypes. In the USA, the number of women undergoing mastectomy are on the rise. Women with TNBC have also opted for mastectomy [18].

Drug repurposing in drug discovery

Drug repurposing is defined as the process of identifying new indications for the already existing drugs, which is considered to be economical and an efficient approach. It has been concluded that about 75% of the already existing drugs could be used for the treatment of other diseases. In general, the time taken for the discovery of a new drug would take a very long period. The cost for the new drug development accounts to over a billion dollars and the time period for the drug to get to the market would take about 10-15 years. Even though the time taken for a drug to enter the market takes more than a decade, the success rate is only 2.01%. This causes a gap in the efficiency of the pharmaceutical research in the development of new drugs which causes a gap between the available treatments and the therapeutic needs. The recent years, the reprofiling of the already available drugs is

becoming a popular strategy for the management of various disease because of the use of de-risked compounds whole pharmokinetic, preclinical and the pharmodynamic profiles are already known, hence it can easily enter the phase III or IV clinical trial which could potentially reduce the time taken by the drug development process at a low cost and therefore is rapid [22].

Hence re-examining the efficiency of the licensed drugs and the research on drugs has been the choice of the World Health Organization (WHO) and other health related agencies are aiming to treat the health problems. There are two concepts in drug repurposing; Firstly, a single drug interacts with multiple targets, this gives researcher a new window for observing new sites of action for the new drug against the target. Secondly, targets of a particular disease those are at times involved in the biological process of pathogenesis gives way for the designation of a new interaction for the known target. In conclusion the drug that acts on common targets could be useful in the treatment of several other disorders where the target is involved.

Figure 2: Advantages of repurposing of drugs- a timeline [20]

Types of repurposing approaches

There are mainly 2 types of repurposing approaches, they are as follows:

1. Computational approach
2. Biological experimental approaches

A lot of data such as clinical trial reports, drug-target interactions, adverse drug reaction, gene expression, protein networks, electronic health records etc., are accessible for the repurposing of drugs. This storage of knowledge and omics data in the field of pharmaceutical research has led to the increase in the computational methods of repurposing of drugs that are novel in the field of drug reprofiling. The computational methods are able to integrate all the data and knowledge that is available at a very high level which helps in

identifying and studying new pathways and demonstrate the drug mechanism, understand the side effects and the drug interactions which will help in speeding up the drug discovery process[23].

1. Experimental screening approaches:

The main and the most important source of hits for the drug repurposing and drug discovery is the experimental screening approaches. The only difference being the application and the outcome of the approaches. In the drug discovery programmes that are related to the discovery of new drugs that are done by high throughput screening campaigns that demands for a very sophisticated and specialized facility for screening and creation of compound library that could contain millions of compounds. On the contrary, repurposing of the already existing drugs are targeted on known molecules that have been either approved or molecules that have failed with prior knowledge of their safety, this is repurposing is accompanied by the in-depth screening of the compounds from the already available database hence characterized by a smaller sized library of compounds. Mostly the number of compounds in a particular library will be around 500-2000 which are approved, the unapproved compounds also lie around the same number [24].

Figure3: The types of drug repurposing approaches [25]

2. In silico approach:

This type of repurposing approach is one of the sophisticated approaches to identify the existing data of associations between drug and the target. There are two main approaches:

1. Molecular approaches
2. Real world data approach

Molecular approach is an approach that mainly focuses on the drug activity and the disease pathophysiology. This type of research is mostly driven by large amount of omic data such as the genomic, proteomic and the transcriptomic data along with the data of the drug such as chemical structure and their drug targets.

Real world data (RWD): this type of approach focuses on identifying the unknown. At many times, new and

unexpected relationships between the disease and the drug is studied and based on the patients' health and their symptoms and their behaviour are all recorded and collected through different data collecting strategies [25].

Computational epurposing approaches for treating triple negative breast cancer

Based on the types of data that are collected, different types of drug repurposing approaches are identified such as target structures, drug-target interaction and transcriptomes.

There is no standardized method for the in-silico approach, hence by literature survey we have 4 types of computational approaches

Structure based reprofiling of drugs

Virtual high throughput screening: In the structure-based approach, the structure of the drug and the receptor is very important. It is mainly performed by Virtual high throughput screening of libraries of small chemical compounds from various databases such as DrugBank and ChemBank. It shows us multiple compounds that could be as the lead molecules against the potential drug target and its binding sites. VHTS method helps us mainly performed as molecular docking techniques. It predicts the affinity of the drug towards the target and binding affinity is predicted through scoring functions. There are numerous software which perform molecular docking, such as GOLD, GLIDE, UCSF DOCK, AUTODOCK VINA. VHTS can also be used to predict the target against which a set of ligands bind to [26].

Pharmacophore Mapping: This is a structure-based approaches where we search for ligands that have a particular set of molecular feature(i.e pharmacophore). The pharmacophore features include hydrogen bonds, hydrophobic groups such as chemical structures. The software that provides with the pharmacophore features include Catalyst, Unity and PharmMapper.

Ligand/receptor mapping: this is performed the guilt-by-association principle. It is performed by profiling a particular ligand and finding compounds that have similar functions and biological properties, the same applies to receptor profiling.

Transcriptional signature-based drug repurposing

> This type of reprofiling is associated with the identification of transcriptional signatures in response to a drug treatment. The transcriptomic changes are observed during the drug treatment.

ISBN: 979-85-27243-61-1

The lead molecules are identified by the negative correlation between the gene expression profile from diseases and the transcriptional signature induced by a small compound. It is done in order to find a drug that could reverse the diseased state to a normal state. A positive correlation is identidfied when the identified small molecules have a similar transcriptional signature that induces a similar gene expression. Signature based drug repurposing is also known as the connectivity mapping. The connectivity Map Database is created based on the signature principle. Other databases include Gene Expression Omnibus (GEO) and the Cancer Genome atlas (TCGA). Other tools include CMap, L1000CDS2 and KsRepo [27].

1. Network based drug repurposing: Network based drug repriposing is based on modelling biological interactions. It consists of nodes and edges, where the nodes are biological components like genes or proteins and the edges are the relations. This type od repurposing helps us in identifying new relationships between biological components, new biological networks, and new targets. The Protein-Protein interaction networks are asscociated with finding and predicting interaction of proteins with other proteins, it helps us identify the most highly connected central proteins which is known as the hubs or hub proteins. Changes in the hubs could lead to the change in the biological network that could lead to dysfunction and disease.Network based helps us in identifying new disease related targets fpr repurposing. There are a number of tools online such as PRISM (Protein Interaction by Stucture Matching), Omics Net. For drug targets interaction networks, DTI server, STITCH and Casual Biological Networks (CBN) database and KEGG database[28].
2. Data Mining based drug discovery: This type of approach is based on meta-analysis of data from the clinical trials. Researchers have used the clinical trials database along with the text mining tools such as I2E (Linguamatics) and the PolyAnalyst. Iit is performed by the retrieval of Serious adverse Events (SAE) data and the drugs that have a few SAEs, hence this can be used to identify new drugs from the testing conditions.

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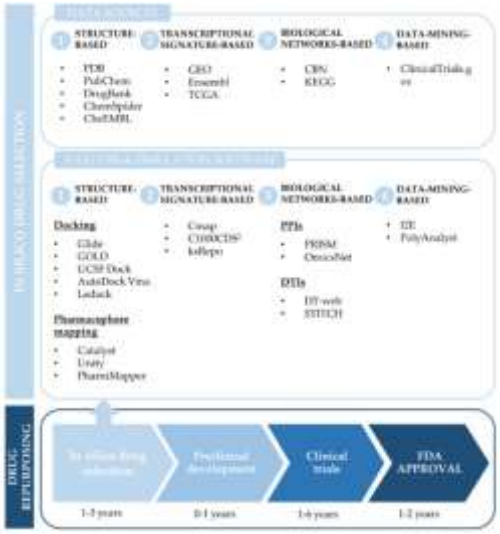

Figure 4: Computational approaches in drug repurposing [30]

2.4 Drug repurposing in Breast cancer

N-Ras inhibitor- Flunarizine drug has been approved for migraine and vertigo. But TNBC mouse models have shown to induce autophagy when treated with Flunarizine. A recent study illustrated that when metformin and hemin which are used for type 2 diabetes are combined were able to inhibit the growth of breast cancer [30].

Computational studies have shown that the expression of BACH1 has been significantly increased in TNBC, when hemin is used along with metformin mediated degradation, it sensitizes the TNBC and causes degradation of BACH1. The project on repurposing drug in oncology has given us new evidences of new drugs against the breast cancer. Originally these drugs were initially discovered for other diseases other diseases other than breast cancer. The drugs that were repurposed are as follows:

1. Mebendazole: an anti-helminthic drug
2. Nitroglycerins: Preventing heart attack
3. Cimitedine: anti-acid drug
4. Intraconazole: anti-fungal drug
5. Diclofenac: an anti-inflammatory drug
6. L-NMMA tilarginine acetate
7. Olaparib: PIM-1 inhibitor
8. L-asparginase
9. Fenofibrate

The above drugs were used for treating patients with leukaemia, helminthic infection, cardiogenic shock and viral infection. It was also used for patients with high serum cholesterol and triglycerides [31,32].

Conclusion and future perspectives

TNBC is an aggressive breast cancer subtype that lacks the hormone receptor expression such as oestrogen receptor, progesterone receptor and the human epithelial receptor which makes TNBC hard for diagnosis and prognosis. The early

relapse of the illness additionally adds to its high number of breast cancer cases among ladies. The main objectives of the health care workers is to find a solution to the increasing number of deaths due to TNBC among women, which can be accomplished by only a rapid approach of drug discovery programme, which is possible through drug repurposing. This approach saves both time and capital for the entry of a new drug molecule against TNBC into the market.

ISBN: 979-85-27243-61-1

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AMPEROMETRIC BIOSENSORS AS AN ANALYTICAL TOOL IN FERMENTATION INDUSTRIES

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ABSTRACT:

Biosensors are analytical devices that sense the presence of a biological component inside the vicinity and generate signals that may be detected by the usage of a physicochemical detector. With the advent of biosensors, traditional methodologies including the detection of contaminants in pharmaceutical industries and the diagnosis of sicknesses via biomarkers had been extended to an exceptional quantity. The review highlights the usage of Amperometric biosensors in the fermentation industry which majorly includes detection of ethanol, lactate and collective detection of various substrates produced and highlights the potential use of these biosensors in various domains of these industries. The industries can use the amperometric biosensors to control and screen the presence of various metabolites and elements that could both be of financial significance or toxic to the customers. The continuous monitoring of those metabolites is of high importance and these determine the quality of the end product. The review also offers a comprehensive description of the modifications carried out in the amperometric biosensors, using unique biomolecules and chemical compounds, along with their associated analytical capabilities.

Index Keys: Amperometric biosensors, analytical features, industrial applications, fermentation

INTRODUCTION:

Biochemical analytical techniques are extremely important in medicine, pharmaceutics, and other industries, and the development of biosensors has made continuous monitoring of processes much easier. A biosensor is an analytical instrument that uses a physicochemical detector to turn a sensitive biological reaction into an electrical signal. [1] The first biosensor as defined in 1962 by Clark and Lyons [2] [3] Based on forms of signal transduction, biosensors may be categorized as electrochemical, optical, piezoelectric, and thermal sensors. Based on the type of electrical property measured, electrochemical biosensors are categorized as potentiometric, amperometric, and conductometric sensors. The utility of biosensor areas are clinical, quality control, diagnostics, medical applications, process control, bioreactors, agriculture and veterinary medicine, bacterial and viral diagnostics, drug production, wastewater treatment from industries, mining, military defence industry etc. Amperometric biosensors are integrated devices that are based on estimating the current created by the oxidation or reduction of an electroactive organic component, resulting in a discrete quantitative analytical data set [4] When a redox response occurs, amperometric biosensors monitor the current flow between the anode and cathode, and the resulting current is found to be proportional to the electroactive element in the solution. [5] Glucose biosensors are one of the most actively used and researched amperometric biosensors [6].

Tracking lactate, ethanol, glucose, fructose, lactic acid, and glycerol is critical in the fermentation business for product quality and health concerns. The analysis is currently being carried out using spectrophotometric methods. Several immobilized oxidases on the electrodes are oxidized to produce hydrogen peroxide. The current produced by oxidation is proportional to the concentration of the analyte. The amperometric biosensor can therefore detect the analyte concentration.

This review aims to provide information on recent developments in amperometric biosensors for industrial applications. The biosensors mentioned have been put through their trials in the lab and have been tuned to work in mass production and processing. Amperometric detection of analytes in different industries is a vast opportunity for economic benefits, and improving food safety, and scientists across the world have been trying new modifications to improve its analytical parameters and increase its efficiency. Many new and modified additional components, such as nanomaterials, nanotubes, platinum, hexadecane, gold electrodes, matrices such as sodium alginate, resydrol polymer for bioreceptor immobilization, and others, have been utilized to improve biosensor qualities such as conductivity and sensitivity. The use of various features put into amperometric biosensors for possible applications in the fermentation industry is highlighted in the review.

The proposed IUPAC definition states that a biosensor is an analytical device that is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element that is in direct spatial contact with a transducer element. [7] . The sugars in the raw materials are transformed into ethanol and carbon dioxide. This process is termed fermentation. Fermentation is monitored to determine the output quality [8]. Fermentation is a large portion of the food processing business, however, there is a lot of need for biosensors in this particular sub-sector. Parameters such as starch, glucose, lactate, and ethanol need constant monitoring in this industry for obtaining exceptional quality end products and for other economic reasons. [9] **Figure 1** illustrates a glucose biosensor, which monitors the fermentation process of hardwood hydrolysate by glucose monitoring using glucose oxidase as the recognition element. The microbial load present in the primal matter and the range of raw products also decide the grade of the final product. [10] Certain substrates that are specific to the analytes like, oxidases are immobilized on the electrode surface, which binds and reacts with the analytes and produces H_2O_2, whose oxidation

produces current, measured by the amperometric transducer. The reaction is as follows:

Substrate + O_2 → Product + H_2O_2

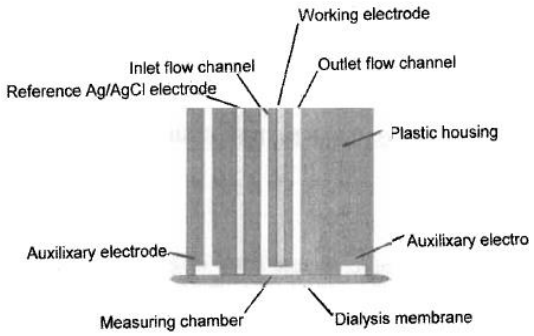

Figure 1: Diagrammatic representation of a glucose biosensor, which monitors the fermentation process of hardwood hydrolysate. [11]

(i) ***Detecting the presence of ethanol in alcoholic beverages:***

Ethanol concentration is important since it determines its economic value as well as the character of the end product. For ethanol analysis, many amperometric biosensors have recently been developed and employed. Sensors have recently been modified to boost selectivity and other properties such as sensitivity and measurement, range, accuracy, reproducibility, and response time. [12]

Table 1: Chemical reaction occurring in substrate containing ethanol to which biosensor is in contact [13]

Enzyme	Analyte	Enzymatic Reaction
AOX	Ethanol	Ethanol+O_2→Ethanal +H_2O_2

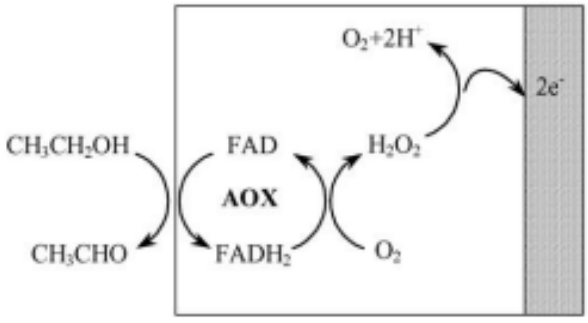

Figure 2: Reaction scheme of direct H_2O_2 detection from catalytic oxidation of ethanol by AOX [14]

(a) Ethanol detection using an ADH-based biosensor:

M. Boujtita et al. reported an ethanol biosensor based on ADH that focuses on the oxidation of ethanol to acetaldehyde. This process is catalyzed by NAD+. The biosensors amperometrically monitor the conversion of NAD+ to NADH and the oxidation of NADH, which releases electrons. Alcohol dehydrogenase (ADH) and NAD+ modified carbon paste electrodes are used in the biosensor. The research also shows the response of the sensor studied with different types of pasting liquid, which

includes analyzing the concentration of ethanol in wine, beer, and it was compared favourably with spectrophotometry. The hexadecane electrode is used because it showed more sensitivity and linearity. The matrix has minimal effect on the amperometric current measured and does not interfere with the result of the ethanol analysis given by the carbon paste electrode modified with ADH/NAD+. The sample must be prepared, i.e. dilution, because the enzyme electrode is dependent on the dilution factor. The biosensors based on ADH have limited operational strength and are reliant on the coenzyme NAD+, which requires constant recovery in the analysis, raising the overall cost of biosensor manufacture. [12]

(b) ***Development of Amperometric biosensor with ethanol oxidase (AOX) based Polyvinyl chloride(PVC) cell***:

Vinita Hooda. et.al. have developed an amperometric bio-enzymatic biosensor. Alcohol oxidase is incorporated into the PVC reaction cell in these biosensors. Nanomaterials modified working electrodes are employed for the fast quantification of alcohol. Alcohol oxidase-based alcohol biosensors work by utilizing just molecular oxygen to oxidize ethanol into the corresponding aldehyde with the arrival of H_2O_2, without the addition of any other cofactor. The use of multi-walled carbon nanotubes (MWCNTs) and silver nanoparticles (AgNPs) facilitates the direct transfer of electrons between the Horseradish Peroxidase (HRP) enzyme and the electrode. The immobilized AOX onto the outside of the PVC container holds great electrocatalytic movement at physiological pH, and detection takes place amperometrically. Electrochemical cells are constructed using PVC because of their properties like reusability, improved analytical performance, and stability. The currently used alcohol biosensor was compared with this biosensor by conducting experiments with wine samples, and the analytical parameters were improved. Response time is 8 seconds at pH 7.5 at 35℃ incubation temperature. The result was compared with that of standard analytical methods. [15]

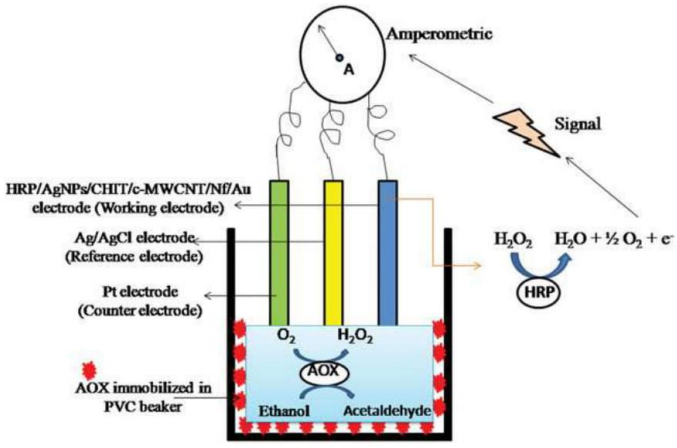

Figure 3: Amperometry in this biosensor was conducted in PVC cells because of its reusability [15]

(c) Microbial amperometric biosensor:

Many microbial amperometric biosensors based on T. brassicas, S. cerevisiae, Acetobacter acetic, or Acetobacter xylinum have been reported for ethanol detection. [16] [17]L. Rotariu et al. described a microbial amperometric biosensor using yeast cells (Saccharomyces ellipsoids) mounted on Clark-based oxygen electrodes. The products generated by the microorganism's respiratory activities are used to determine the amount of ethanol by the biosensor. A two-minute reaction time is required. This biosensor has a second Teflon membrane that protects the biocatalytic yeast layer, allowing for selective ethanol detection in the presence of glucose. It has been found that incubating the biosensor with ethanol improves its selectivity. The selective biosensor was used to determine ethanol concentration in alcoholic beverages. The results were compared with the spectrometric method with alcohol dehydrogenase and a good correlation was observed.

$R\text{-}CH_2OH+O_2 \rightarrow R\text{-}CHO+H_2O_2$ [18]

(ii) *Detection of lactate concentration in wine:*

LAB (lactic acid bacteria) performs malolactic fermentation, which is a type of secondary fermentation. When LAB undertakes a series of metabolic actions, lactate is produced. It is vital to maintain track of the levels of this process because it can impact the beverage taste and other features and attributes. The development of lactate biosensors has been reported from all over the world. The current measured by the amperometric biosensor is proportional to the concentration of lactic acid. Amperometric biosensors are divided into two types: lactase oxidase-based biosensor (LOx) and lactate dehydrogenase (LDH)-based biosensor. [19]

Table 2: Chemical reaction occurring in substrate containing Lactate to which biosensor is in contact [20]

Enzyme	Analyte	Enzymatic Reaction
LOX	Lactate	$Lactate+O_2 \rightarrow Pyruvate+H_2O_2$
LDH	Lactate	$Lactate + O_2 \rightarrow Pyruvate + NADH$ $NADH \rightleftharpoons NAD^+ + H^+ + 2e^-$

(a) Amperometric biosensor based on lactate oxidase with platinum electrode:

This amperometric biosensor is used to determine lactate levels. The study investigates two approaches for immobilizing lactate

oxidase(LOX) on the surface of SensLab platinum printing anodes using different types of absorption. Lactate hydrolysis is accompanied by the formation of hydrogen peroxide, an electrically active molecule whose breakdown results in the creation of electrons detected by the amperometric transducer. The enzyme was immobilised onto the transducer surface in the study using two methods: physical adsorption in Resydrol and electrochemical deposition in PEDT(poly-3,4-ethylenedioxythiophene). The sensor with lactate oxidase immobilized by physical adsorption in Resydrol polymer appears to have both a smaller dynamic range (0.004–0.5 mM lactate) and more sensibility (320 nA/mM). The immobilization strategy has no impact on results, and this report was obtained when these biosensors were tested. The results of employing an amperometric lactate biosensor and standard chromatography techniques to analyze the lactate concentration in wine and must during fermentation have a satisfactory correlation. According to the report by L.V. Shkotova et al., the biosensor developed can be used in the food business to improve quality and can also be used to monitor the ageing process of wine. [21]

(b) Bienzymatic biosensor for determining l-lactate concentration:

Sandra Perez et al. described an a mperometric enzymatic biosensor that uses the phase inversion appr oach to fuse Horseradish Peroxidas e (HRP) and Lactate Oxidase (LOx) enzymes into a carbon nanotube/p olysulfone film on screen-printed electrodes (SPEs). Horserad ish peroxidase is a biochemical that was utilised as an enzyme in this in vestigation [22]. The layer was coated with Ferrocene, which is said to improve sensitivity, working potential, and allow for a reduction

in H_2O_2 at 100mV. The estimations were done in phosphate buffer solution at pH 7.5 under batch conditions, according to the report. The reaction time of the biosensor was 20 seconds. Detection was carried out on a vari ety of wine and beer samples. They found functional correlations by c omparing the results of the biosens or with the results of the spectrop hotometric measurements. This de vice was discovered to have high a ccuracy, sensitivity, a low limit of detection, and fast throughput. However, it is also reported that the shelf-life stability isn't very high and needs improvement in future trials. [23]

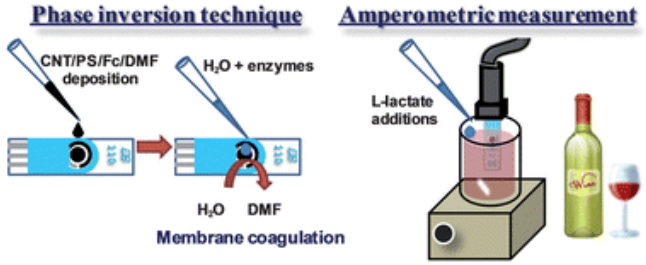

Figure 4: Phase inversion technique of fusion of Horseradish Peroxidase (HRP) and Lactate Oxidase (L Ox) enzymes into a carbon nanotube/polysulfone fil m on screen-printed electrodes (SPEs) and the amperometric measurement of L-lactate. [23]

(c) Lactate detection using a nanoparticle-enabled biosensor:

Nanoparticles are atom clusters with sizes ranging from 1 to 100 nanometers. H. Kang et al. have successfully demonstrated how nanoparticles may be employed to improve the efficiency of biosensors. [24] .L. Shkotova et al. modified and evaluated amperometric biosensors with palladium and platinum nanoparticles for lactate concentration analysis in the final product during fermentation. They observed an increase in bio selectivity upon the incorporation of Pd and Pt NPs. This modified biosensor finds an effective application for maintaining the excellent quality of wine and for selective detection of lactate in raw material during fermentation. Research has shown that the lactate biosensor function of amperometric transducers has improved sensitivity and selectivity with the incorporation of Pt And Pd nanoparticles along with the Nafion protective membrane. [25]

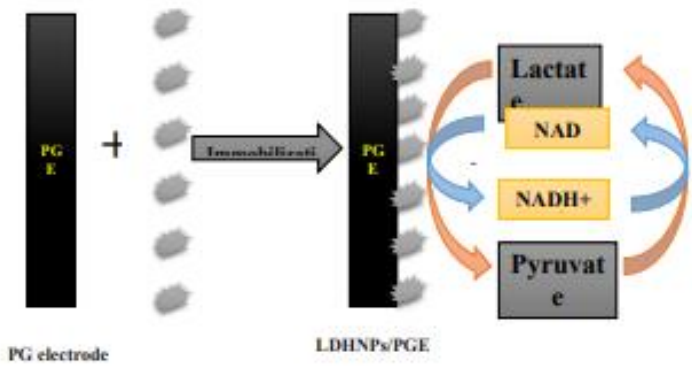

Figure 5:The chemical reaction that takes place during the creation of the LDHNPs/PG electrode is depicted in this diagram. [26]

(iii) **Detection of multiple enzymes:**

Multiple analytes are to be maintained in the industrial fermentation process, so multiple oxidase-based biosensors are developed to serve this purpose. Analysis of various enzymes is important in the industrial fermentation process and they are real-time tracking is also vital. Multiple oxidases are implemented on respective electrodes to obtain biosensors. These biosensors serve the purpose of maintaining multiple analytes concentrations in fermentation.

Table 3: Chemical reaction occurring in substrate containing Ethanol, Lactate, glucose to which biosensor is in contact

Enzyme	Analyte	Enzymatic Reaction
AOX	Ethanol	Ethanol+O_2→Ethanal +H_2O_2
LOX	Lactate	Lactate+O_2→Pyruvate+H_2O_2
GOX	Glucose	Glucose+O_2→Gluconolactone +H_2O_2

(a) Amperometric biosensors using platinum (printed) electrodes and immobilized enzymes:

Goriushkina T. B et al. reported a biosensor for application in wine analysis that concentrates on the determination of the concentration of ethanol, glucose, and lactate by immobilization of respective oxidases on printed platinum electrodes. The immobilized oxidases react with the analyte and produce H_2O_2 upon oxidation. The current produced is measured by the amperometric transducer. The data of linear response to ethanol, glucose, and lactate within the concentration range is mentioned in **Table 4**. Even after two months of manufacture, the biosensor activity for ethanol and glucose did not change. The operational stability of the lactate biosensor activity, on the other hand, lasted four days. During the fermentation of wine, must, and for the examination of ethanol, glucose, and lactate in wine and must during fermentation, the research also revealed great selectivity to the substrate and commercial applicability. The results of several varieties of wine, such as red, white, and rose wine, as well as dry, strong, and sweet forms of must, have been correlated with other amperometric biosensors and regular methods. [27]

***(b) Real-time monitoring of alcoholic fermentation using screen-printed biosensors*:**

Screen-printed biosensors for the determination of glucose, ethanol, and fructose are blended with handy instrument ation for real-time monitoring. S. Piermarini et.al reported the application of these biosensors to screen micro alcoholic fermentation (micro ALFs) processes in red wine. The ethanol and glucose biosensors were created using grap hite screen-printed sensors that were then modified using Prus sian Blue and an oxidase enzyme. On the applied potential, the electrochemical mediator permits for delicate and s pecific placement of H_2O_2 (50 mV). A standard screen-

printed sensor was coated with fructose hydrogenase for fructose and an electrochemical mediator phenazine methosulphate was used for acting as an enzymatic co-substrate. They have stated the working range, reproducibility of probe fabrication, and its stability. The research indicates that they have collected samples of fortified must-wine, which were tested to monitor the micro-ALFs in the samples induced by two strains of yeast. The data was also compared to spectrophotometry, which discovered a strong correlation. The data was used to determine the normal fermentation levels by analyzing the fermentation profiles at various periods. They also mentioned the discrepancies in the strains' metabolic activity. [28]

(c) ***Detection of enzymes using carbon film resistors***:

Carbon film resistors are used as transducers within the advent of several oxidase-based enzyme electrode biosensors. Prussian Blue (PB) was used to exchange the resistor electrodes, and eventually, a layer of enzymes was covalently immobilized. The enzyme substrates are diagnosed through a hydrogen peroxide reduction response towards Ag/AgCl with a reaction time of 1-2 mins. Ultimately, there maybe electrochemical biosensors were used to analyze complicated food matrices—yoghurt, wine, and must. The outcomes of the preceding test have been compared to those of a spectrophotometric reference approach, which showed a great correlation with the outcomes and indicated that it can be used to analyze wine and meals. The particular functions of those biosensors cause them to an excellent material for disposable or effortlessly renewable sensors. Voltammetric techniques and EIS have been used to test its stability closer to carbon resistor substrates. because the electrodes are tested and assessed in wine samples, this studies primarily specializes in wine process control. In terms of sensitivity, those biosensors' detection limits are corresponding to the ones of screen-printed biosensors and platinum-based biosensors. They are cheaper and are being evaluated for a variety of analyses. As a result, they have been decided to be suitable for food screening. The various designs allow organic substrates and chemical compounds to be immobilized quickly. The enzyme biosensors with carbon film resistors had been also found to be appropriate for complex matrices in the study. [29] [30]

The various important analytical characteristics of the biosensors used in the fermentation industry are mentioned in **Table 4**.

The electrode used in the Biosensor	Analyte	Limit of detection	Linear detection range	Potential	Sensitivity(A/mM)
Alcohol dehydrogenase (ADH) on NAD+ modified carbon paste electrode. [12]	Ethanol	n.r.	0 M to 11 mM	0.7V	n.r.
Alcohol oxidase coupled PVC reaction cell on nanomaterial modified electrodes. [15]	Ethanol	0.0001µM	0.01mM to 50mM	-0.75V to 1.2V	155µ Am/Mcm²
Yeast cell immobilized on Clark type oxygen electrode. [17]	Ethanol	1.5mM	3mM to 50mM	-650mV	n.r.
LOx in resydrol polymer on the platinum printed electrode. [21]	Lactate	n.r.	0.004mM to 0.5mM	0V to 3000mV	320nA/mM
LOx and HRP in Carbon nanotube on SRP. [23]	L-lactate	5.6 X 10^{-7}M	1.1x10^{-6} M to 5.6 X10^{-4}M	-100mV	1168.8µA/mM
Platinum and palladium nanoparticles. [26]	Lactate	0.1µM	0.05mM to 0.8mM	0V to 1.0V	3.03 nAm/Mcm²
Oxidases immobilized on electrodes. [27]	• Ethanol • Glucose • Lactate	• 0.03mM • 0.04mM • 0.008mM	• 0.3mM to 40 mM • 0.04mM to 2.5mM • 0.008mM to 1mM	0 to 600mV	n.r.
Graphite screen-printed sensors modified with Prussian Blue coupled with oxidase enzyme. [29]	• Glucose • L-Glutamate • L-Lactate • Ethanol	1µM	• 10µM –800 µM • 10µM–700µM • 10µM–500µM • 10µM–700µM	n.r.	• 8µA/mM • 7µA/mM • 10.4µA/mM • 5.5µA/mM
Screen-printed sensors modified with Prussian Blue coupled with oxidase enzyme with carbon film resistors as transducers. [28]	• Glucose • Fructose • Ethanol	n.r.	• 0.02mM–0.7 mM • 0.05mM–0.5 mM • 0.05mM–0.5 mM	70mV	n.r.

ISBN: 979-85-27243-61-1

All the above biosensors mentioned in **Table 4** have amperometric transducers.
n.r. : not reported

Industrial Application:

Even though biosensors offer very high sensitivity and reduce cost, there are still factors that have to be considered for industrial application like a limited lifetime of the biological component, Handling and manufacture. There is ongoing research towards the mass application of biosensors in the industry, but only a few have succeeded, which is mentioned in **Table 5.** Autoanalyzer, manual laboratory devices, and portable systems are among the commercial biosensors available. **Table 5** also mentions the commercial application of other electrochemical biosensors.

Table 5: Industrial applications of electrochemical biosensors [31]

Biosensor	Analyte(s)	Electrode Biocomponent(s)	Company
AM2 & AM3	Ethanol	AOX	Analox instruments
AM5	Methanol	AOX	(UK and USA)
GL6	Glucose, ethanol, lactate, methanol and glycerol	GOX, AOX, LOX, GK GPOX	www.analox.com
LM5	Lactate	LOX	
Answer 8000	Glucose	GOX+HRP	Gwent sensors (UK) www.g-s-l.co.uk
Microzyme	Lactate	n.r.	Biosentec (France) www.biosentec.fr
Per Bacco 2000 Per Bacco 2002	Glucose Lactate Malate	GOX LDH MDH	BioFutura s.r.l. (Italy) www.biofutura.com

Conclusion:

Because of the inefficiency of traditional techniques of analysis of resultant products, amperometric biosensors are seen as having promising industrial applications. To improve biosensor properties, increase operating efficiency, and prepare for mass use, several types of electrodes, matrix, and immobilization procedures have been employed and explored with samples. The demand for biosensors in the fermentation industries is immense. The use of biosensors in the field of medicine has made the diagnosis of diseases very rapid yet reliable and biosensors employed in the pharmaceutical industries have made a quality analysis of the components very simple and rapid. Biosensors have

successfully replaced these industries' existing time-consuming procedures, and they have shown significant potential in all other industrial areas as well. Because of their low cost, low (Limit of detection) LOD, high sensitivity, and other improved analytical features over traditional methods, biosensors are the future of these sectors. Many MNCs have invested in biosensors for their application in the industries. The industrial application of these biosensors brings economic and public health welfare, hence enhancing the quality of life. Even though a prototype of these biosensors has been developed, tested, and adapted for mass use, industrial use of these biosensors has yet to befall. Continuous research is, however, required to overcome the limitations of these biosensors to generate high-quality fermented products that are safe for society.

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A REVIEW ON THE NATURAL SUPER FOODS AND THEIR PROCESSING

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Abstract:

The term 'superfood ' refers to foods that offer maximum nutritional benefits for minimal calories and they are packed with vitamins, minerals and antioxidants. Sweet potato are good source of fiber, potassium and other essential nutrients. Spinach is a nutrient dense and very low in calorie and rich in iron. Soya bean is used as an alternative to meat in vegetarian diet. It has the perfect blend of protein and fiber. Pomegranate which is called the divinity fruit has antioxidants, antiviral and anti tumor properties. Carrots high in vitamin A which boosts immunity and benefits eye health. Strawberries and blueberries are diabetic superfoods because they are packed with antioxidants and fiber. Eating apple on regular basis may help prevent type 2 diabetes as well as keep blood sugar level stable. Guava has high content of dietary fiber but storage, handling, processing and transporting becomes difficult due to high moisture content so different types of drying process is important for the colour quantity and nutritional value. Guava is rich in dietary fiber that helps in ease constipation. Avacado incredibly nutritious with high amounts of potassium. Dehydration of fruit and vegetable are forms of food preservation techniques to increase it's shelf life. Pre drying treatments prepare the raw product for drying. Raw product preparation includes selection and sorting , washing, peeling cutting and blanching for some fruits and most of the vegetables including leafy vegetables such as spinach. Blanching effects on nutritional quality. Drying and dehydration removes majority of the water contained in fruit and vegetable. Sun drying is limited to climate with hot sun and dry atmosphere. Hot air drying is cheap and commonly used. Drying process of sweet potato generally reduces the physio chemical properties, color and nutritional quantities of it's flour. Blanching, steam blasting and vaccum freeze drying steps involved in it's processing affects the flour nutrient composition. Following the drying/ dehydration, the superfoods are blended into powder acts as a complete nutritional supplement which may be consumed as replacement for daily food and can be formulated according to the taste that fits best to the consumer. This paper is mainly based on processing of various superfoods to increase it's shelf life and retain the nutritional value.

Keywords:

Super foods with nutritional contents,
Dehydration and pretreatment method,
Pre drying treatment (selection and sorting, washing, peeling , cutting and blanching),
Drying Process (common hot air and sun drying methods).

MICROORGANISM USE IN PLASTIC DEGRADATION: A REVIEW

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Abstract- **Plastic pollution is one of the major problems faced all over the world. More than hundred millions of tonnes of plastic are manufactured each year. The majority of plastic ends up on land or in the water, causing several problems. Every year, this percentage rises. Natural decomposition of plastic takes hundreds of years. As a result, various types of bacteria have been identified from soil and other sources that are capable of decomposing plastic more quickly. These bacteria are then inoculated in plastic-containing medium to determine polymer breakdown properties, time, and enzyme. This sort of study aids in the solution of the plastic problem by boosting the rate of breakdown of plastic.**

Keywords**– Plastic degradation, biodegradation, microorganisms isolation, plastic pollution**

I. INTRODUCTION

Plastics are man-made long chain polymeric units. Plastics have become an indispensable part of our life. A key breakthrough came in 1907, when Belgian-American chemist Leo Baekeland created Bakelite, the first real synthetic, mass-produced plastic [1]. In a wide range of items, plastics are utilised and other formerly used materials for applications dominated by plastic, such as wood, metals and glass have been removed. In 2019, the global production of plastics reached 368 million metric tons [2]. Europe was responsible for production of 57.9 million tons of plastic in 2019 China is one of the world's major plastics manufacturers, with more than a quarter of worldwide output[2]. Plastic imports are continuously growing in the United States and China is the leading provider. In the previous ten years, Chinese plastic exports have expanded significantly; in2009, the value of exports came to USD 14.4 billion, and increased by 2019 to USD 48.3 billion [2]. Since the early 1950s, researchers estimate that over 8.3 billion tons of plastic have been manufactured [3]. About 60% of this plastic ended up at a deposit or in the natural environment [3]. Fig: -1 displays the annual plastic output between 1950 – 2020. Production is expected to decline by around 0.3 percent in 2020 owing to the impact of COVID-19 on the sector [2].

The most popular types of plastic include PET, LDPE, HDPE, PP, PVC, PS and others. These seven are plastics that cannot be degraded. Fig 2 shows the product of these seven plastics and their identification. Excluding these, the most popular biodegradable plastics are PLA, PVA, PBS and others.

ISBN: 979-85-27243-61-1

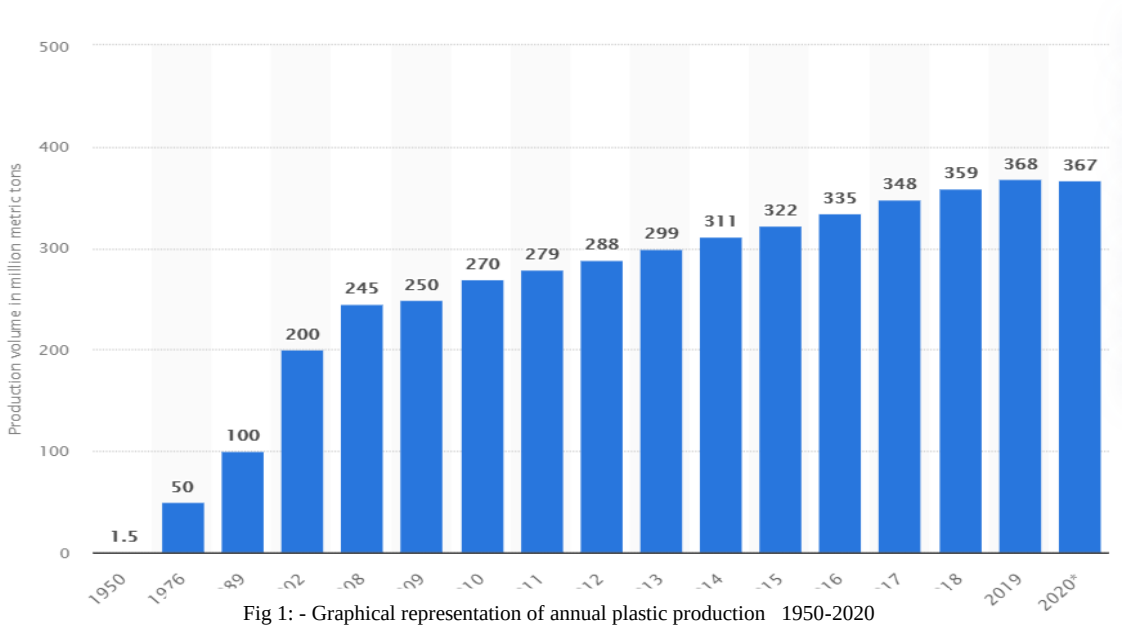

Fig 1: - Graphical representation of annual plastic production 1950-2020

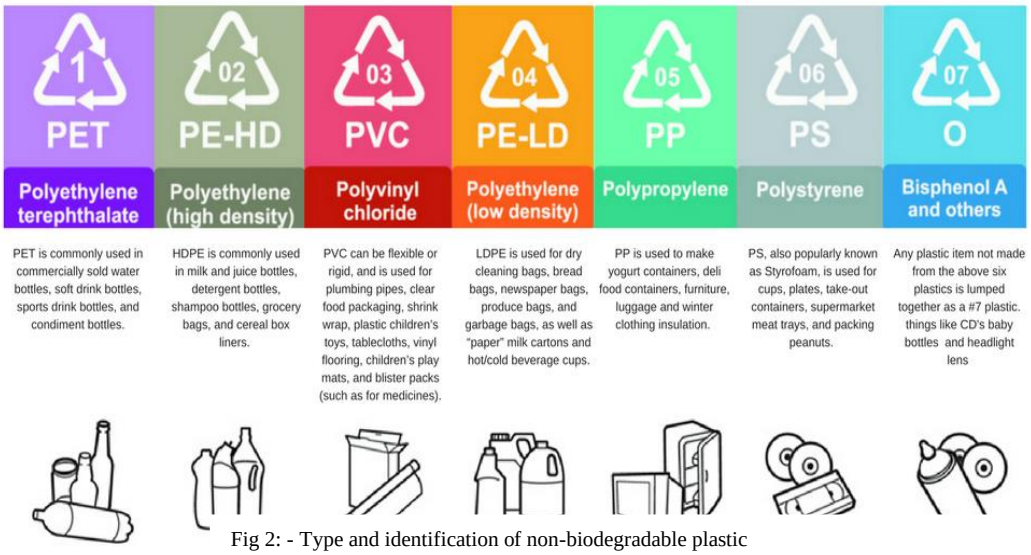

Fig 2: - Type and identification of non-biodegradable plastic

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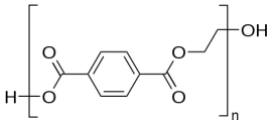
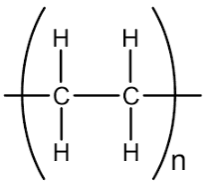
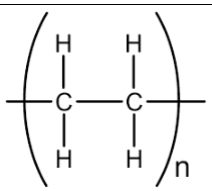
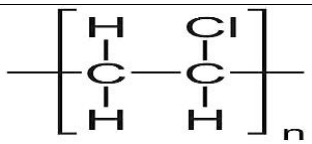
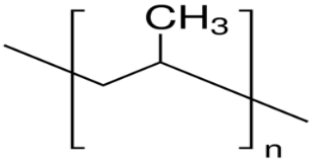
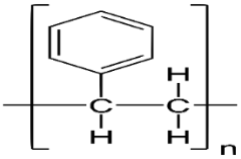
Name	Description	Structural Formula
PET (Polyethylene Terephthalate)	This is one of the plastics used most frequently. It is light, durable, often transparent and commonly used in food packaging and manufacturing (polyester).	
HDPE (High-Density Polyethylene)	High-density petroleum-based polyethylene is humidity and chemical resistant, which makes it excellent for cartridges, containers, tube and other construction components.	
LDPE (Low-Density Polyethylene)	Polyethylene with lower petroleum density is a version of HDPE's which is more soft, clearer and more flexible. It is generally utilized as a liner in cards and in corrosion-resistant surfaces and other items.	
PVC (Polyvinyl Chloride)	PVC is strong, stiff and chemicals-resistant and weathering resistance, making it desirable for construction and building applications.	
PP (Polypropylene)	PP is heat-resistant to other types, so that it is suitable for food and food storage products that are intended to store hot product or heat themselves.	
PS (Polystyrene)	PS is cheap and is extremely effectively isolated, making it an essential element in the food, packaging and construction sectors.	
Others	This category is a comprises of additional plastic kinds, which do not fall into any of the other six classes or are numerous type combinations. For this plastic kind, the code for recycling is 7. The most significant thing about this is that the plastics generally cannot be recycled.	

TABLE 1: Non- biodegradable plastics and their structure

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II. PLASTIC DEGRADATION

Any modification of the polymer or chemistry owing to a variety of environmental factors, including light, temperature, humidity, chemical conditions or organic activity. Those processes that, due to physical, chemical or biological interactions, alter the polymer properties are known as polymer breakdowns. The microorganism produces an extracellular enzyme in microbial degradation that may break bonds between carbon polymers and utilize it as a source of carbon. These enzymes breakdown plastic, or return the polymers to their monomer state before they are produced.

III. FACTORS AFFECTING PLASTIC DEGRADATION

There are several various factors that affects the plastic degradation rate.

Moisture content : Water is very crucial for existence of micro organisms.The microbial activity is said to be increased when there is a proper amount of water content is present .

pH: The variation of pH level effects the growth of micro organism and thereby hinders the rate of plastic degradation also.

Temperature: Whenever there is a increase in temperature , this leads to destruction of micro organisms .Therefore the rate of plastic degradation gets affected.

Molecular weight: As the weight of plastic sample increases, it also takes more time for the micro organisms to degrade the plastics. We can say that molecular weight is inversely proportional to the rate of plastic degradation.

Surface area: As the surface area is increased, this facilitates the micro organisms to degrade plastic easily and smoothly.

IV. MATERIALS AND METHODS

A. Isolation of plastic degrading microorganism:

The 10 g soil samples in 100 ml synthetic medium have been injected. Furthermore, 300 mg of partially degraded polyethylene material was added as substrate collected from same place to the specific media. The mixture of bacteria was purified by a spread plate method after 12 weeks of incubation, and subsequent clean cultures were kept on slant nutrients agar. Furthermore, plastic bacteria have been tested for degradation [5].

B. Screening of Plastic degrading microorganism

Plastic degradation was evaluated in isolated individual bacterial colonies. Polyethylene plastic samples were taken and sterilized by distilled water and 70% ethanol. Plastic samples were inserted into conical flask containing synthetic media, bacterial culture of specified quantity is inoculated into the media. Bacteria was allowed to degrade plastic in incubator shaker at room temperature with agitation speed of 120-150 rpm for a period of 1 month. After the completion of specified time plastic samples were taken out sterilized by distilled water and ethanol. Samples were air dried to take accurate weight. [6]

C. Assessment of plastic degradation by microorganisms

As the process of plastic degradation experiment starts with incubation for several days, the sample is analyzed after specific time (week) duration. There are a number of methods to determine the rate, potential and degree of biodegradation of polymeric materials.

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- Microscopic examination (SEM analysis) of the plastic sample that gives the structural information on the plastic like dimension, pore nature, etc.[7]
- Typically, aerobic biodegradation of plastics is characterized by the utilization of oxygen and results it the formation of constituents such as CO_2, H_2O, SO_2 etc. Therefore, the Biological Oxygen Demand (BOD) of the incubation period of the plastic sample serves as a parameter to measure the degree of biodegradation.[10]
- Gas Evolution Tests (CO2or CH_4) are used to determine direct measurement of mineralization which is a step in Biodegradation. Also, as the final outcome of bacterial respiration is CO_2, its detection using gas chromatography is being consistently used.
- The disintegration of plastic products during composting process is determined by passing the obtained compost through series of sieves that usually have average mesh size of about 2mm. The sample if undergone degradation, its particles effortlessly go through the sieves with compost. This is the ISO200 method of testing. [11]
- Also, other physical parameters analysis such as tensile strength, weight percentage, molecular weight distribution, gravimetric weight loss, Sturm test are done to determine the biodegradation. [8]
- The rate of biodegradation is expresses as the weight loss percentage of the plastic sample during the course of experiment.

$$\% \text{ Weight loss} = \frac{(\text{Initial weight} - \text{Final Weight})}{\text{Initial Weight}} \quad [7]$$

- Fourier Transform Infrared Spectroscopy uses Beer's law and is a preferred infrared spectroscopy method to detect the degradation of plastic. It does so by comparative spectrum analysis of neat plastic and its degraded form.[12]

V. RESULT

- Required microorganisms were isolated from soil and the biodegradation experiment of plastic under laboratory scale was conducted. It was observed that the process started with the interaction of bacteria with plastic by forming a biofilm on the surface of the plastic.
- After weeks of incubation, degraded plastic sample was analysed to check the weight loss in percentage by using the above mentioned formula.
- Also CO_2 monitoring of the process is done and if present in high amount, it indicates biodegradation of plastic.
- The visualisation of the plastic sample by SEM analysis shows small holes on the plastic surface.
- The rate of biodegradation is found to be dependent on ecological conditions such as temperature, pH, oxygen content, the type of plastic, etc.

VI. CONCLUSION

Plastic is obviously one of the main components of our lives. In our everyday existence plastic is most often used. The manufacture of more than 100 million tons of plastic annually and the fact that it cannot be reused and recycled leads to plastic being deposited in land or on water, which

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increases pollution and affects the habitat around it. The greatest creation of man was once now his worst enemy. The future solution to the whole problem is the microorganism used in plastic breakdown. Enzyme generated by microorganism helps to break the polymerase chain. Identifying and modifying such an enzyme may improve the degradation process.

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ISBN: 979-85-27243-61-1

SARS-CoV-2 main protease inhibition by compounds isolated from Sarsaparilla using molecular docking

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ABSTRACT- In this project the study is carried out HPLC analysis for the sarsaparilla sample and extracted the two pharmaceutical compound that is steroid saponin. It will help to inhibit the SARS COV2 proteins, these two saponins have many advantages like it will act as anticancer, ant leprosy, will help to reduce infection also helped in post menstrual problem as well as act as antibacterial.

The sample which used for this project that is Sarsaparilla is a plant sample contain many plant metabolites such as flavonoid, carotenoid saponins plant hormones etc. It helps to reduce infection to treat cough fever and act as an Anti-rheumatoid arthritis. This sample is homeopathic sample, in this project I used one tincture and one dilution 200 ml.

Tools which used in this project are PYRX, OPENBABEL, PYMOL Docking Studies which helped to complete third objective of this project.

Application of this project is it will also help to inhibit the SARS COV proteins which is responsible for replication in human body. Because this sample which is used by this project contain steroid saponins which will help in instant immunity booster for corona patient.

Keyword: Protein database, corona virus, docking, affinity, binding

INTRODUCTION

Sarsaparilla is a tropical plant from the sort *Smilax*. The climbing, woody plant fills somewhere down in the overhang of the rainforest. It's local to South America, Jamaica, the Caribbean, Mexico, Honduras, and the West Indies. Numerous types of *Smilax* will come under the classification of sarsaparilla:

1. *S. officinalis*
2. *S. japicanga*
3. *S. febrifuga*
4. *S. regelii*
5. *S. aristolochiaefolia*

THE ADVANTAGE

Sarsaparilla contains more number of plant synthetic compounds thought to beneficially affect the human body. Synthetic substances known as saponins would help lessen joint torment and skin tingling, and furthermore eliminate microorganisms. Different synthetic substances might be useful in diminishing irritation and shielding the liver from harm. Note that human investigations for those cases are either exceptionally old. The examinations referred to beneath utilized the individual dynamic segments in this plant, singular cell studies, or mice considers.

HPLC it is another type of column chromatography which work on high performance in other word high pressure. It is used for separation of unknown component from the liquid mixture.It used in protein extraction and many other manufacturing, purpose.

APPLICATION

1. It is used in drug discovery
2. It used in clinical application
3. It has high sensitivity
4. It has good repeatability
5. It used in proteomics
6. It is also used in pharmaceutical applications
7. Measurement of quality of drinks and other beverages
8. Detection of liquid mixture
9. It used in biotechnology and food analysis

SARSCOV-2

Towards the end of December 2019, a novel coronavirus (2019-nCoV/SARS-CoV-2) with human to human transmission, originated in Wuhan, China, and caused several human infections and disorders in the respiratory system.10,11 This viral disease is a pandemic that has become a global challenge and the number of newly infected patients has been increasing day by day.

Since the SARS-CoV-2 outbreak, different traditional herbs with promising results have been used alone or in combination with conventional drugs for the treatment of infected patients. There exist numerous uncertainties surrounding the novel coronavirus behavior; thus, it is too early to conclude whether medicinal plants, spices, or isolated compounds and molecules could be used as preventive drugs or as appropriate therapeutic compounds against COVID19

REAGENT USED

- Reagents Used In This Project Are HPLC WATER, METHANOL, DILUTIONSAMPLE, ACETONITILE and TINCTURE
- Acetonitrile and HPLC water as solvent, for reverse column cleaning purpose after 5 runs we need to clean column by HCL and NAOH that is called acid base regeneration.

- METHANOL for syringe cleaning. Used for sample injection in HPLC

TOOLS AND TECHNIQUES

PyRx

Fig 1 PyRx window

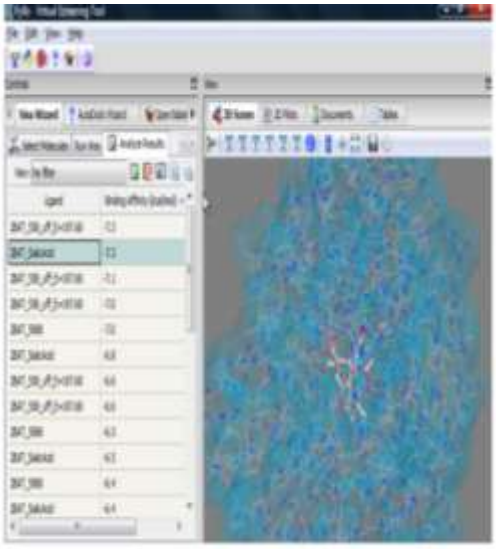


Fig 2 PyRx visualization of docking

- It is a virtual screening tool which used in Bioinformatics for docking of ligand and protein.
- It does not required any commands it is auto-dock just you have to add some parameters and you can dock the proteins and ligand .
- You can dock more than one protein and ligand together.
- It is free tool does not required any sign up and login.
- It is not paid, it is totally free to use

PYMOL

- PYMOL is a visualization tool, which is totally free to use no need to pay mostly used in bioinformatics to visualize the docked protein and ligand.

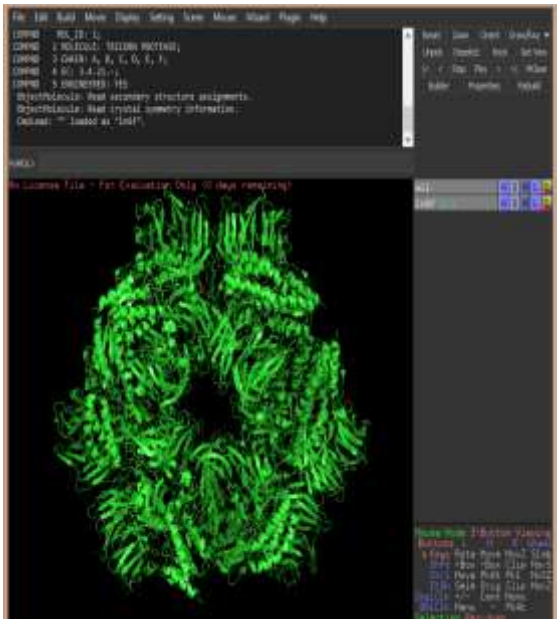


Fig 3 PYMOL window.

- You can download it on any platform like Linux, windows etc.
- It has many parameters like you can change color of protein and ligand according to your wish.
- It is 3D visualization tool.
- Maintained by Schrodinger
- Official site is pymol.org

OPEN BABEL

Open Babel it is a chemical toolbox discover to speak too many languages of chemical data. It's an open, synergetic project which can allow anyone to convert, search, store, or analyze data from molecular modeling.

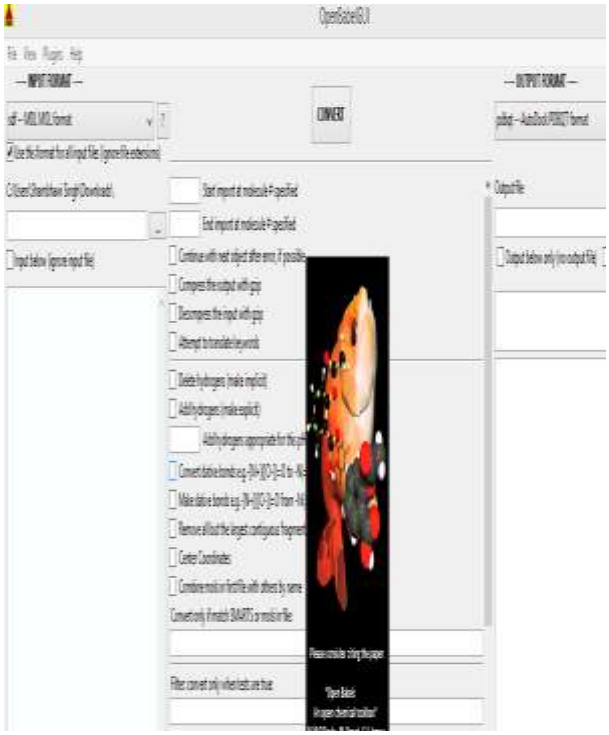


Fig 4 open babel logo

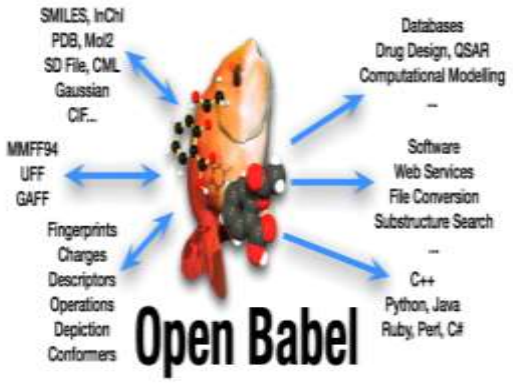


Fig 5 application of open babel

THEORY OF PROJECT

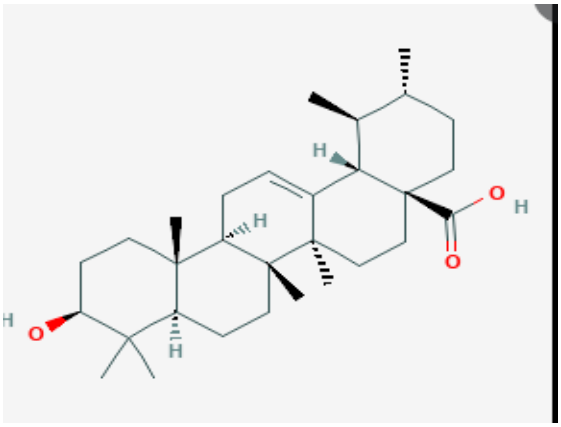


Fig 6 structure of ursolic acid

- Ursolic acid is a steroid saponin obtained from plants like sarsaparilla , it obtained from trees ,part of plants like root, bark and trunk .
- Molecular formula C30H48O3
- It is also called pentacyclic triterpenoid.
- It will act as an anti-fungal, anti-tumor, anti-bacterial as well as anti- rheumatoid, it will also help in pain relief.
- Other names are – PRUNOL MALOL URSON
- Molecular weight 456.7 g/mol

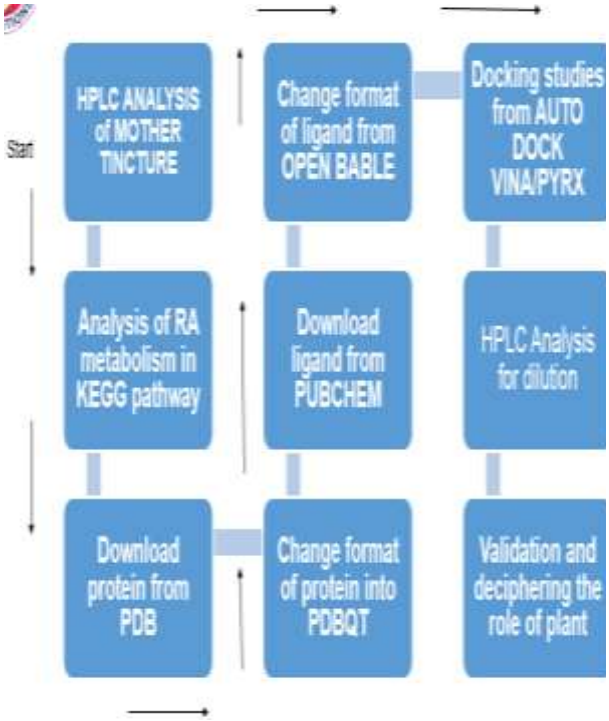
DOCKING STUDIES

- Docking studies also called molecular docking this tools and study basically used in bioinformatics tools.

- It will give energy binding and affinity between ligand and protein in all rotations.
- Many tools are available VINA GOLD PYRX AUTODOCK etc.

- It is open you can install in windows as well as Linux and it required no programing language but if you have skills on PYTHON and R then it will help to create command and troubleshooting.

METHODOLOGY



RESULT AND ANALYSIS

Saponins which are extracted from sarsaparilla sample

- First we need to download ursolic acid 3D structure from PUBCHEM this should be in SDF format, then we have to convert it to pdbqt this is the format which we will use for docking in PyRx tool which is a Docking tool, for converting SDF to pddbqt I used OpenBabel tool

- Above saponins extracted from sarsaparilla sample by HPLC, This is used as a ligand for docking

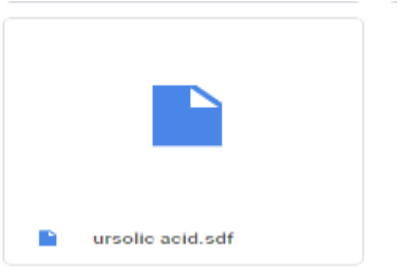


Fig 7 SDF format of ursolic acid

Proteins Which Is Responsible For SARS COV2

- Above pdbqt formats are for proteins which is downloaded from PDB ,protein data base , we need to download it in PDB format then we need to convert it in pdbqt format by OpenBabel. These ID's are taken from PDB proteins ID 2HSX
- above information I have refer from reference no 5 [Thao Quyen Cao *et al* ,2021]

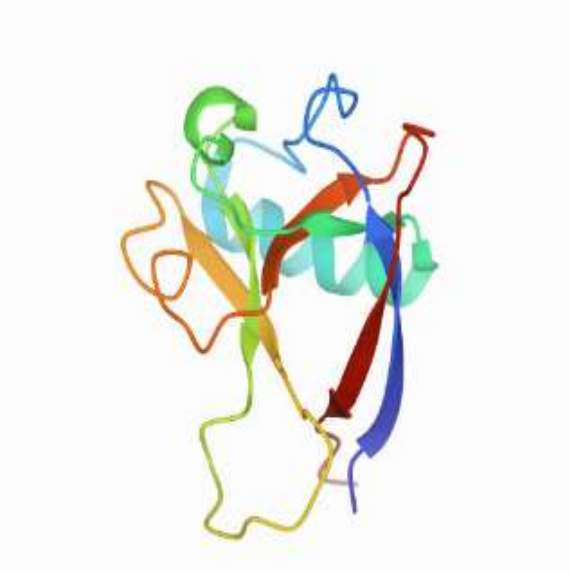
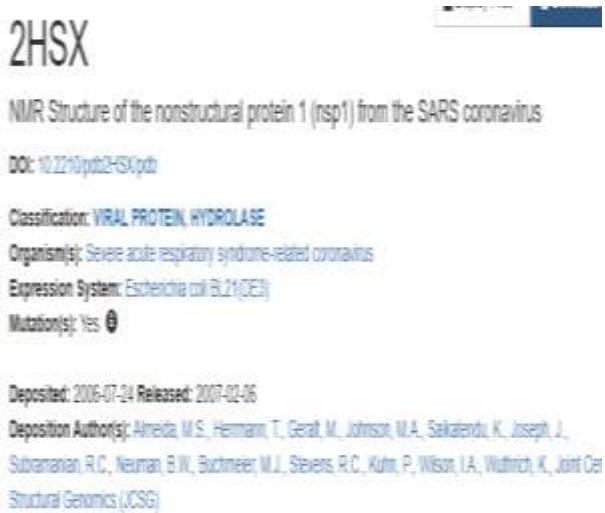


Fig 8 structure of SARS COV proteins



HPLC REPORT FOR SAPONINS

Table 1 retention time of chromatogram

	RT	Area	% Area
1	14.545	266453	3.58
2	14.996	5739877	77.20
3	17.607	1428410	19.21

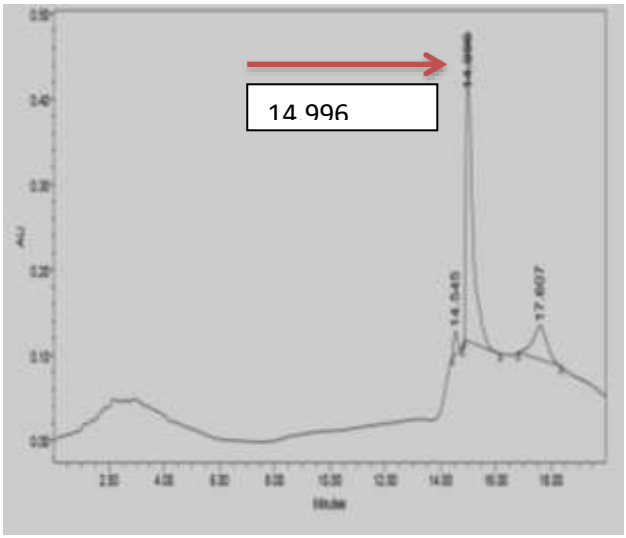


Fig 9 chromatogram obtained by HPLC

1. Above report is for URSOLIC ACID which identified by HPLC analysis RT is **14.996** matched with reference paper reference no is 21 (Tamanna Talreja *et al* 2017; 6(1): 89-92

- standard of URSOLIC ACID is 15. Exact 15 we have not got because this project has done in lab in which temperature humidity is not maintained so little variation is there that is acceptable .

- Area % indicate purity of compound.

RESULT OF DOCKING STUDIES

Fig 10 docking of protein and ligand

Table 2 result of docking

2hsx_ursolic_acid_1	-7.6
2hsx_ursolic_acid_1	-6.2
2hsx_ursolic_acid_1	-6.1
2hsx_ursolic_acid_1	-6.1
2hsx_ursolic_acid_1	-6.1
2hsx_ursolic_acid_1	-6
2hsx_ursolic_acid_1	-5.9
2hsx_ursolic_acid_1	-5.8
2hsx_ursolic_acid_1	-5.8

- Above result is of docking studies of ligand URSOLIC ACID and protein SARS COV 2 that is 2HSX as you can see its perfectly docked sky blue color represent protein while green color represent ligand as URSOLIC ACID
- Binding affinity is very less as you can see in table **-7.6 ,-6.2,-6.2,-5.9** and -6 respectively
- Lower the value of docking that means more the negative score is better binding
- Above information I have refer from paper reference no 23 [Simone C.B. *et al* ,2005]

CONCLUSION

This article state that the saponins find from sample sarsaparilla is binding with protein which is responsible for SARS COV 2 protein that is 2HSX it will inhibit this protein from further replication in human body. However other proteins are also there for covid in future can be check binding affinity for other proteins. Hence we can not claim say that this saponin are going to treat completely from corona virus but steroid saponin that is URSOLIC acid is helping as immunity booster for corona patient .

FUTURE SCOPE

Future scope of this project this will treat coronavirus SARS COV 2 protein which is responsible for inhibition of protein which is responsible for corona virus replication inside Human body. So saponins extracted from the sample Sarsaparilla. it contain steroids and steroid is using to enhance the immunity system for the Corona patients so it might inhibit the protein and it will also increase the immune system in the coronavirus patient.

It will also treat other infection like antibacterial and antimicrobial according to literature survey further investigation will focus on it. It will also help to cure Rheumatoid arthritis as well.

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A BOON FOR EARLY DISEASE DIAGNOSTICS- THREAD-BASED BIOSENSOR

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ABSTRACT

A device or substance which can detect disease or medical condition is known as diagnostics and the use of threads for this purpose helps in the fabrication of thread-based diagnostic devices. Threads, with their excellent flexibility and wicking property, are widely used for the fabrication of biosensors. These low-cost microfluidic devices are cost-effective, long-lasting, with early and simple diagnostics. The threads employed should be hydrophilic, allowing them to transfer both aqueous and non-aqueous fluids by capillary action. Threads transfer liquids via capillary action and thus reduces the need for an external pumping system or any other resources. Threads also showcase various advantages over paper and because of which they are becoming powerful tools. Thread-based analytical devices have various applications in medical diagnostics, environmental testing, food quality analysis, and also in various industrial applications. In this review of ours, we have tried to give an overview of the application of thread-based biosensors in medical diagnostics. These low-cost micro Thread based analytical devices (μTADs) have huge potential in developing countries because of their cost, easy fabrication, and huge point of care (POC) diagnostics.

Keywords: Biosensor, threads, Thread based analytical devices (μTADs), Point Of Care (POC), Diagnostics

INTRODUCTION

Biosensors are emerging as effective (sensitive and selective) and affordable analytical diagnostic tools for early-stage disease detection, which is required for personalized health wellness management. Low-level detection of a targeted disease biomarker (picomolar level) has proven to be extremely useful in assessing disease progression while on therapy. Efforts are ongoing to promote cutting-edge biosensing technology as a next-generation non-invasive disease diagnostics methodology.[1] New concepts and applications of low-cost, portable, and field-based diagnostic technologies have piqued the interest of researchers. The recent rise of paper-based microfluidic devices demonstrates the allure of low-cost and field-based technologies.[2]

otton thread, an ancient material used in civilization, is also an appealing material for the fabrication of low-cost and low-volume microfluidic diagnostic devices for healthcare and environmental assays. This is due to the fact that the gaps between the fibers provide capillary channels for liquids to wick along the thread. Natural cotton thread is not wettable by aqueous liquids; this is due to the presence of wax on the surface and in the fiber wall of natural cotton fibers. As a result, the natural cotton thread must be dewaxed or surface treated to allow for aqueous liquid wicking. Cotton and polyester threads, for example, can be used for diagnostic purposes. Natural cotton has a wax layer on its surface, which prevents it from being wet by aqueous liquids. This means that natural cotton thread is hydrophobic and must be treated with plasma to become hydrophilic. By increasing the concentration of carbon and carbon-oxygen on the thread surface, plasma treatment prepares the thread for wicking.[2]

Microfluidics is a powerful tool for downscaling devices, particularly flow-based ones because it provides an integrated platform for sample preparation, delivery, and measurement with a high degree of automation, small amounts of reagents, and faster assay time. As a result, researchers have focused their efforts on developing point-of-care (POC) assays for a wide range of applications using microfluidic-based devices. Microfluidic devices are typically low in cost and have a simple fabrication process.[3]Thread-based devices were successfully developed for the development of a biosensor capable of detecting targeted biomarkers at a very low level (picomolar and femtomolar)[4], [5]. A biosensor with such a low detection limit is critical for detecting a disease-specific biomarker at an early stage of disease onset and monitoring disease progression while on prescribed therapy. The virus replicates at low levels that are undetectable using polymerase chain reaction (PCR) and enzyme-linked immunosorbent assays (ELISA).[4] This approach is also highly recommended for managing a variety of other diseases (including cancer and stress), as well as environmental, agricultural, and food security issues. This electronics-based approach is useful for designing and developing portable biosensors with reduced form factors, which are the foundation of diagnostics at the point of care (POC) application to perform personalized diagnostics. However, all of these techniques necessitate highly skilled labor, as do all of the time-consuming procedures and storage processes.[1]

ISBN: 979-85-27243-61-1

A Brief History

Point of care (POC) diagnostics has always attracted scientists because of the fast results that they give with the limited use of resources precisely and rapidly. These quick detection techniques will help to fasten the treatment process because of the detection in an early stage and thus reduces the risk of death. More importantly this diagnostic technique help in the reduction of time required for the collection of the sample, to do the test, and to obtain the result thus reducing the need for a highly qualified professional to do the test. POC method of diagnostics can help a developing country as well as a highly developed nation. In 1962 blood glucose analysis was developed which was a POC device. After 1977 self pregnancy testing kits were successful because of the POC detection technique. All of these are termed POC's as the testing is done on-site without the need for an expert. On-site diagnostics is a trend in the current era and used in telemedicine where the diagnostics are performed at home and shared with medical practitioners for advice on treatment and prevention of diseases.[5] Hence emphasis should be laid to develop more advanced POC's.

Around 1700 litmus paper was being used as POC devices to test for acidic and basic characteristics which was a sensational invention at that time. In the 1930s and 1940s, West produced metal spot studies based on this principle, replacing pH-sensitive chromophores with ligands that changed color in the presence of particular metals. This lacked sensitivity along with selectivity which led to the discovery of paper-based sensors such as LAF(lateral flow assay) in 1970. This was able to detect biomolecules from labeled antibodies by using a calorimetric and or a fluorometric device, also these were efficient in environmental monitoring. Paper-based sensors are very much effective, efficient, and they work due to capillary action on the paper strip which is preloaded with reagents. The quantitative and qualitative results can be obtained at the end of the reaction based on the intensity of color obtained by the addition of reagent on paper.[8]

The development of glucose-based biosensors was so much in trend from 1946 to 2012. Scientists developed various glucose monitoring biosensors to quantify the blood glucose level of diabetic patients.[6] . The glucose detection paper biosensor will be coated with enzymes(glucose oxidase or dehydrogenase) whereupon the addition of a drop of blood the resulting enzymatic reaction helps in the analysis of glucose levels in patients. The stability of these strips was notably good for many years at a temperature of about 45℃. This was one of the best paper-based biosensors and it was fabricated in the 1970s. The use of paper-based analytical devices has been useful in numerous disease diagnostics. Another material that has similar or better properties than paper would be thread and hence we are providing an overview of thread-based diagnostics in various fields.

Recent researches imply that thread-based sensors can be well substituted for paper-based point-of-care devices. One of the key benefits of using thread instead of paper to build microfluidic sensors is that thread comes in a wider range of materials. While paper can be made from fibers other than cellulose, using current papermaking technology will be more costly and difficult. Thread is therefore a valuable substitute for paper in the production of low-cost

ISBN: 978-93-5724-8-6

microfluidic sensors. Currently, various types of threads are being used and some of which include cotton, polyester, and much more. The capillary action of the threads along with a better wicking property helps in the proper usage of threads in point-of-care diagnostics. The usage of natural cotton threads is restricted if they are untreated with plasma treatment because as to remove the cellulose coating in order to get its wicking property. Various types of diseases including cancer, heart diseases, pancreatic disorders, and including COVID -19 can be detectable at an early stage by the use of thread-based point of care devices.[9]

APPLICATIONS

MEDICAL DIAGNOSTICS

Micro Paper-based analytical devices (PADs) were introduced around 2007 which has much more potential as POC tools for the diagnostics of various diseases and pathogens, including Hepatitis C, understanding the genetic material of various organisms, glucose, and BUN (Blood Urea Nitrogen) concentrations, and NO2 in saliva. Thread-based diagnostic processes can be used in the detection of glucose and urea nitrogen in the blood. Contemplation of the blood glucose level and Blood Urea Nitrogen (BUN) is very much required for diabetic patients and also for patients suffering from Renal diseases. For the detection of BUN and blood glucose levels, thread-based diagnostic systems have been devised. The threads are coated with Polyvinylchloride (PVC). This helps in the on-site detection of glucose and urea nitrogen electrochemically in whole blood. Paper-based micro-fluidic detection and cotton thread-based detection systems are currently in trend because of their speed of detection, easy fabrication, low cost in terms of production and they also possess high compatibility. But they are conjugated with practical problems which include breakage and the amount of the wetting capability of cotton threads. The thread which is being used for the BUN and glucose detection is coated with a number of enzymes such as glucose oxidase, urease, and catechol which is used as a moderator prior to coating with PVC in order to prevent the vaporization of these enzymes and this also helps to bring down the Joule's heating effect. The sample should be applied to the thread at -0.28 V. The principle of this system is the sample digestion by the immobilized enzymes on the thread and then the product obtained by this process can be detected by various downstream processes. Before adding the PVC to the thread, the thread is dopped by various enzymes and is put into a buffer solution. This is done to halt the penetration of PVC into the fibers of the thread. Notably, this water-based buffer solution has an immiscible nature with PVC and thus it helps in the accomplishment of a thin layer on the thread surface. Once this is done an electric field can be applied to the thread dropped with the sample for capillary electrophoresis separation. Coating the enzyme soaked thread with PVC has helped in the better propagation of the sample and progressing results were obtained. In comparison to the normal glass microfluidic channel, the threads coated with PVC layer displayed good EOF mobility, as per the results. The designed thread-based microfluidic device also has a wide linear range for detecting GLU and BUN, according to the results. The blood cells were concurrently lysed and filtered using the thread to detect GLU and BUN in whole blood. The mode of detection is represented in [7]**Fig**. 1.

ISBN: 979-85-27248-61-

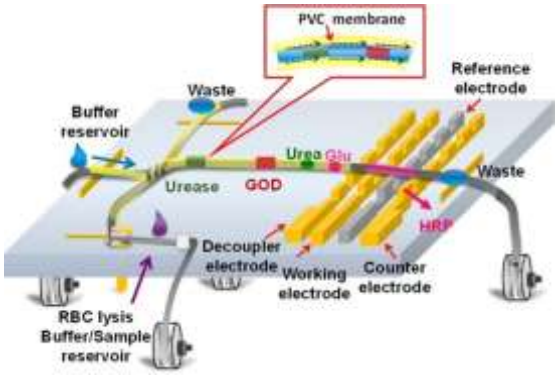
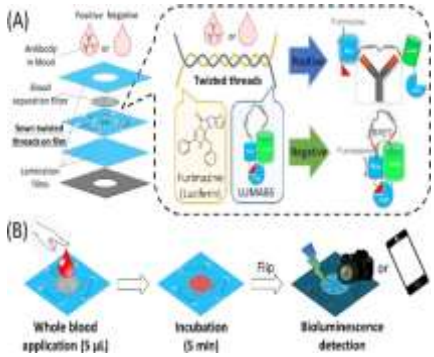


Fig.1: Schematic of the enzyme-doped thread coated with PVC membrane for high-performance CE-EC detection. [7]

Antibodies are special proteins that help our body to fight against the foreign invaders that are the antigens and enhances the working ability of our immune system to fight the approaching harmful microbes in the near future. Determination of various



antibodies is very much important for various therapeutic applications, diagnosis of autoimmune diseases, and contagious diseases. But the detection of antibodies in the laboratory requires all the sophisticated technology along with a highly skilled workforce making it cumbersome. This scenario can be eliminated by switching to thread-based detection techniques which can be used as a Point-of-Care system in the lab. Microfluidic thread-based analytical devices (µTADs) use bioluminescence resonance energy transfer (BRET) switching detector proteins to detect antibodies. The devices are made up of vertically stacked layers that include a blood separation membrane and a plastic sheet with a woven cotton thread on which BRET sensor proteins and the substrate furimazine have already been pre-deposited as shown in **Fig** 2. This is based on intensity-dependent analysis and results can be obtained on smartphones. These µTADs in amalgamation with BRET helps in the quantification of antibodies present in a single prick of blood (5 µl). [8]

A thread-based diagnostic approach has also been used for blood grouping. Using 2 µl of blood from a single prick the ABO and Rh blood grouping can be achieved with the use of µTADs. The fibers of the threads help in the separation of agglutinated red blood cells from plasma. Also in this system with the use of chromatographic techniques has improved the quality of detection. The further development of this system can be helpful for diagnostics even in remote areas of the world obliging better and faster results. For this process, polyester threads were used due to the outstanding color projection properties so that the propagation and reaction which occurred can be seen early in the capillary channel of the thread.[9]

Fig 2. (A) Schematic illustration of the mechanism of bioluminescence reaction on µTADs where LUMABS and its bioluminescent substrate (furimazine) are pre-deposited separately in dry form on two intertwisted cotton threads; the emitted bioluminescence color shifts from green to blue in response to a specific antibody. (B) Schematic illustration of the analytical procedure for antibody detection with proposed µTADs: applying a single drop of a whole blood sample to the device (5C), followed by capturing the bioluminescence signal by a digital camera or a mobile phone camera 5 minutes after sample application [8]

ISBN: 978-93-5427-243-1

The thread-based diagnostic approach has also paved the way for the estimation of lactate present in saliva. It is a discomfort for patients to retrieve blood for various tests to determine any particular disease which they suffer from. The collection of the saliva samples is very effortless and can be cumulated by even an inexperienced worker. Test sample assemblage in the form of saliva is found to be useful for people with hemophilia, neonates, patients undergoing chemotherapy, specially-abled people, and also in the case of certain elderly patients. Knowing the amount of lactate present in the body is very much required for patients in the medical crisis

units, patients having lactic acidosis which is a condition that can weaken the muscles which in turn can cause a heart attack.[10] Lactate concentration in the saliva can be determined by using a cotton fabric biosensor. Primarily the cotton fabric used for this experiment was treated with Na2Co3 to make it more hydrophilic in nature. Using the template patterning process all necessary electrodes for a three-electrode configuration system were incorporated on the treated cotton fabric. Using AutoCAD 2010 software, a template for patterning electrodes was created, with the counter(CE) having a significantly higher surface area than the working electrode(WE) and Reference electrode(RE). To enable optimal charge transmission within the device, the three electrodes were built with a minimum spacing between them. A digital craft cutter was used to print the template on self-adhesive vinyl sheets. The printed template being bonded to the cotton fabric surface, and the template apertures for the WE and CE were filled with Carbon graphite paste modified with Prussian blue (C-PB), while the RE was filled with Ag/AgCl paste. This was

followed by placing the cotton fabric in the oven at 60°C. Using the included connector clips,

the three electrodes on the device were connected to the mSTAT400 portable potentiostat and the electrochemical impulses subsequently analyzed and exhibited via DropView software. The template method, regardless of the uneven and non-planar surface of the substrates, produces a rapid and high-quality transfer of the electrode patterns, making it suitable for extending the molding process to a variety of materials that are inconsistent with basic screen-printing protocols.[4]. The process of electrode fabrication and detection is enumerated in **Fig**. 3 and **Fig**. 4

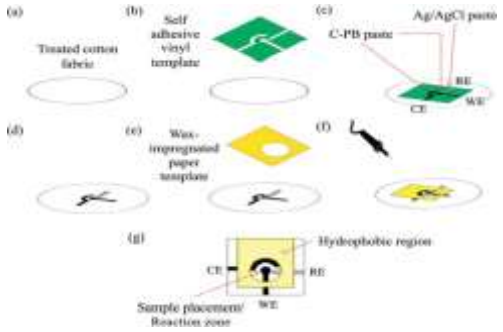


Fig 3: Schematic illustration of the fabrication process of the FED. (a) The platform for FED is treated cotton fabric.

(b) For patterning the electrodes, the self-adhesive vinyl template was used. (c) C-PB paste was applied for both the

WE and CE, while Ag/AgCl paste was applied for the RE. (d) After the template was removed, the substrate was cured at 60 C for 30 min in the oven. (e) The template for patterning the sample placement/reaction zone was printed on wax-impregnated paper. (f) The wax-impregnated paper

ISBN: 978-93-2723-07-1

template was placed accordingly and heat treatment was used to transfer the wax onto the substrate at 150 C using a soldering iron. (g) The ready-to-use device. RE, reference electrode; WE, working electrode; CE, counter electrode[4]

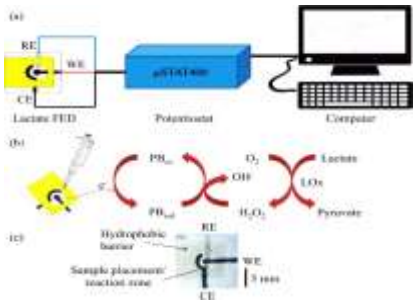


Fig. 4. Overview of FED technology. (a) The instrumental setup for lactate determination. (b) The reaction that occurs at the C-PB/LOx electrodes of the FED. (c) Picture of the fabricated FED (15 15 mm). RE, reference electrode; WE, working electrode; CE, counter electrode. [4]

CONCLUSION

Threads can be used as a very promising tool for the making of POC due to their flexibility and easy portability. Based on various material comparisons threads have proved to be more cost-effective and also can be tuned out easily. The propagation of sample solution on the thread by capillary action eliminates the need for external devices to help with fluid proliferation. Various varieties of threads are available who thus possess a choice of material based on the need. A large number of samples can be analyzed simultaneously with the usage of threads as biosensors. Mass production is possible which again makes it more affordable and making it available at a cheaper rate for the public.

ISBN: 979-85-27243-61-1

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Microbes as Biofertilizers

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Abstract

The ever-increasing demand for food, feed and fiber from the growing population has created undue pressure on the land that leading to degradation of soil quality. Soil is the basic unit for the practice of agriculture, without which farming cannot be accomplished. Human greed has led to the exploitation of the soil to a great extent in recent times due to the usage of chemicals to increase the supply to growing demands. Chemicals in the form of fertilizers, pesticides, fungicides etc. are used in modern agriculture. Consumption of food polluted with chemical fertilizer leads to hemoglobin related disorders in humans. Biofertilizers were made to change the fortunes of agriculture. The use of microorganisms to provide the plants with the necessary nutrients such as nitrogen, phosphate, etc. is the concept of the biofertilizer**.** In order to supply nutrient-wealthy foods, the structural, biological, and mineral fitness of the soil (N, P, K) should be taken into account**.** Plant Growth Promoting Rhizobacteria (PGPR) colonize the root of the plant and influence the growth of the plant directly or indirectly. It decreases the global dependence on hazardous agricultural chemicals. Apart from providing nutrient supply and protecting against pathogens, PGPR plays a crucial role in increasing soil fertility, helping plants in stress management and also in bioremediation for eco-friendly sustainable agriculture.

Keywords – Food, Agriculture, Organic fertilizer, PGPR

1. Introduction

Soil is the habitat of numerous dwelling organisms that have interaction with different organisms. Living soil organisms manipulate water infiltration, mineral density, and the nutrient cycle. Microorganisms integrate with natural count within the soil, so as to interrupt it down into even smaller molecules, earthworms digest natural count, recycle nutrients, and as a result enhance the soil surface. In addition to the residing component, the soil incorporates minerals and nutrients. In order to supply nutrient-wealthy foods, the structural, biological, and mineral fitness of the soil (N, P, K) should be taken into account [1]. Plants need various nutrients from the soil such as nitrogen, phosphorus, and sulphur to grow. However, the nutrient content material of the soil can lower over the years while plants are harvested due to the fact the nutrients do now no longer go back to the soil. Therefore, those critical nutrients want to be balanced via the herbal decomposition manner as vegetation die and decompose and the nutrients removed from the soil go back to the soil, or truly via means of including fertilizers. Intensive agriculture, the improved.extraction of minerals, has brought about an impoverishment of soil fertility and a loss of subordinate and micronutrients, which lowers the water table [2].

India is the fourth biggest consumer of chemical fertilizers, and its soil quality gradually decreasing due to extensive gap among additions and utilization [3]. Biofertilizers can responsible for improvement of soil atmosphere and crop yield in sustainable agriculture [4].

2. Role of Chemical Fertilizer

Fertilizers are compounds which are used to improve the nutrients in soil so that it will encourage soil fertility and resound plant growth. Today fertilizers have come to be imperative for current agriculture so that it will feed the developing population. The use of fertilizers, particularly chemical fertilizers, has added advantages to mankind which have helped reduce starvation and demise in unique elements of the world. Chemical fertilizers are made up of various synthetic compounds specifically designed to increase crop yields. Although chemical fertilizers boost crop production, it is hackneyed, has accustomed the soil, reduced fertility, elevated pesticides, contaminated the air and water, and left out greenhouse gases that present threats to human fitness and the surroundings. As a result, scientists and researchers are perceiving debating and taking into consideration natural fertilizer to be the fine approach to keep away from soil contamination and lots of different threats to the surroundings and existence from the hackneyed of chemical fertilizers. Continuous use of these chemical fertilizers depletes vital soil nutrients and minerals that are glaringly positioned in fertile soil. When we use chemical fertilizers; they no longer assist top off soil nutrients and its fertility. And we recognize phosphorus no longer breaks down in water and it is overplayed, leading to hardening of soil. Likewise, alkaline fertilizers, sodium-nitrate increase alkalinity in soil, decreasing its fertility and making it unproductive. Hence, soil fertility and plant life rely on a good deal at the balanced delivery of critical nutrients and minerals. Although chemical fertilizers assist vegetation to develop quickly; vegetation will now no longer be healthful and sturdy as vegetation grown in that way no longer have sufficient time to evolve to expand an excellent root growth, sturdy stems, or nutritious end result and vegetables. Even they will be much less likely to continue to exist due to the fact they'll be greater liable to pests and illnesses as they need appropriate immune devices and sufficient resistance in opposition to those forces. Besides this, chemical fertilizers can source root burn or fertilizer burn, as chemical fertilizers do

ISBN: 979-85-27243-61-1

now no longer permit sufficient water consumption for the vegetation. Chemical fertilizers are soaked up in nitrogen salts, and whilst the nitrogen is absorbed with the aid of using soil too rapidly; it is going to desiccate and dry the plant.

3. Soil Degradation

Soil degradation is described as "the pace of negative changes in soil attributes resulting in a decrease in land productive capacity owing to a process mostly produced by human intervention." The value of soil to humanity cannot be overstated. It supplies food, fodder, and job opportunities. Soil is home to a diverse range of ecosystems, biodiversity, and groundwater. As a result, soil deterioration can have a variety of negative repercussions. It is critical to guarantee that developments in agriculture and industry do not damage the soil on which they rely in order to ensure growth and economic progress. Soil degradation is exacerbated by agricultural and other

human activities such as land clearing, deforestation, industrial development, incorrect waste management, mining, and overgrazing. Land use has changed as a result of rising population and needs for housing, food, and abuse of natural resources, resulting in deterioration of soil quality.

Agriculture is India's economic backbone, and it makes a significant contribution to the GDP. In the last 50 years, India's agricultural output has expanded from 50 metric tonnes to more than 250 metric tonnes. Agricultural practises that do not include any conservation strategies contribute to soil degradation by diminishing soil fertility, resulting in low yields. So that the environment is not negatively harmed, sustainable farming methods must be followed. Soil is a valuable resource that must be protected at all costs. The contamination of the soil has been caused by intensive agriculture combined with the use of inorganic fertilisers and pesticides, particularly during the Green Revolution. India's crop production has climbed to 193.million tonnes in 40 years. Fertilisers, high producing varieties of seeds, insecticides, and irrigation infrastructure have all contributed to this. According to a study, only 23% of fertiliser is used by crops, while the remaining 77% is leached away, resulting in nitrate pollution of ground water. Soil degradation is exacerbated by fertiliser subsidies [5].

4. Biofertilizer

Biofertilizers, also known as bioinoculants, are compositions that hold in living or dormant cells of microorganisms that aid crop plant nutrient uptake through interlinkages within the rhizosphere after being applied via seed or soil. It speeds up bound microorganism processes in the soil, increasing the amount of nutrients that can be easily incorporated [5]. Microbes are very small, but they are extremely potent and useful. In reality, microorganisms can give all of the components used in chemical fertilisers, particularly nitrogen (N), phosphorus (P), and potassium (K) [6]. Biofertilizers provide a number of other advantages, including being a cost-effective, environmentally friendly, and renewable supply of plant nutrients, making them an important component of integrated nutrient management. The efficacy of the microorganism isolates, and the right application technology are the two most important factors in inoculation success.

Biofertilizers will refine soil fertility and crop productivity. Though the advantages of legumes in rising soil fertility was notable and their role in biological N-fixation was uncovered over one hundred years ago. Advantages of using biofertilizers are that they raise crop yield by 20-30%, return chemical N and

ISBN: 978-81-7048-61-1

phosphorus by 25%, revive plant growth, trigger the soil biologically, reinstate natural soil fertility, offer defence against drought and a few soil borne diseases.

5. Population and Biofertilizer

Rigorous agriculture and high amounts of nutrient extraction have resulted in a loss of soil fertility, secondary and micronutrient deficiencies, and a drop in water table level. All of these factors have contributed to soil depletion and mortification to the point that the correct approaches, machineries, and education can restore this asset to the underlying soil. Land degradation has become a major issue, and it is necessary to strike a balance between the numerous goals of penury reduction, food supply, and long-term agronomics.

6. Role of microbes in Biofertilizer

The bio-remediation method, which incorporates plant-microbial interaction, is gaining popularity around the world as a way to improve the productivity of salt-affected soils. Microorganisms have the potential to adapt quickly to changes in the environment and deterioration and can thus play a critical part in the preservation and competitiveness of any environment. Synthesis of suitable solutes, bio-control potential, salt strain forbearance, genetic diversity, fabrication of plant growth enhancing hormones, and interactions with agricultural plants are just a few of the characteristics that microorganisms have. They play a substantial role in mitigating salt ramifications on crop plants if their properties are properly harnessed [7]. The widespread utilization of artificial nitrogenous fertilizers in farming is a major cause of concern around the world. Alternatives to nitrogen fertilizers must be developed as soon as possible due to environmental concerns. Biological Nitrogen Fertilizer (BNF), a microbiological procedure that transforms atmospheric nitrogen into a plant-functional form. These systems are a cost-effective and environmentally friendly way to reduce external inputs while enhancing internal resources. Inorganic nitrogen fertilisers have a very high production cost. Biofertilizer is used to address nitrogen deficiency in crops in a sustainable manner. Biofertilizers like cyanobacteria can fix less than 10 kg of nitrogen per hectare [8].

6.1 PGPR

PGPR are microorganisms that colonise the plant root and aid plant improvement either directly or indirectly. These soil bacteria could colonise roots and promote plant improvement. These soil bacteria can colonize roots and promote plant growth. *Azospirillum, Rhizobium, Azotobacter, Bacillus, Enterobacter, Gluconoacetobacter, Arthrobacter, Pseudomonas, Clostridium, Serratia* and *Azoarcus* are among the PGPR species. PGPR may have a straightforward or indirect influence on plant growth. Furthermore, PGPR are recognized as potential microorganisms that can protect plants from a variety of environmental challenges in both normal and stressed conditions. PGPRs, notably N_2 fixers, phosphate and potassium solubilizers, are recommended as a long-term option for increasing plant nutrient uptake and crop yield.

1. Phosphorus solubilizing bacteria

Phosphorus-solubilizing bacteria that fix atmospheric nitrogen, such as *Azospirillum*, and *Azotobacter* can increase the solubility and accessibility of phosphorus to plants, and hence crop output is increased. *Azospirillum* also has

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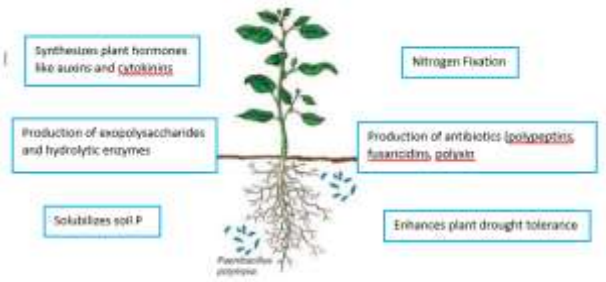
other advantages, such as the ability to produce growth- promoting chemicals, disease resistance, and drought tolerance [9].

The main mechanism for mineral phosphate (P) and other mineral solubilization useful for plant growth is the production of organic acids. Under in vitro conditions, most strains of A. brasilense and A. lipoferum use organic acids, such as malic, succinic, a-ketoglutaric, gluconic, or lactic [10,11,12] as their preferred carbon source, rather than producing them.

2. Potassium solubilizing bacteria

Paenibacillus polymyxa (formerly lus polymyxa), a potassium-solubilizing bacteria that is an agriculturally essential microorganism that has been extensively explored for its plant developing qualities. *P. polymyxa* is an endospore-forming microbe that has the potential to colonise a variety of ecological environments. The capacity of *P. polymyxa* to operate as a biological control agent against a wide range of plant diseases is well known. In both lab and field circumstances, it may create antifungal chemicals like fusaricidin and antibiotics like polymyxin, which could inhibit pathogen multiplication. *P. polymyxa* strains are too familiar for their ability to solubilize phosphate, fix atmospheric nitrogen, and produce phytohormones, and hence can be used as an effective biofertilizer in commercialized agriculture. *P. polymyxa* aids in biological N fixation, producing phytohormones and influencing nutrient uptake from the soil, and thereby rising length of the plant and biomass, as well as all-inclusive yield of the crop (Figure)



Paenibacillus polymyxa strains - Primary plant growth-promoting (PGP) features [11]

Diazotrophic (N-fixing) strains have huge promise as biofertilizers because nitrogen is regarded to be the most critical mineral nutrient necessary for plant growth and maintenance. *P.polymyxa* can now release phytohormones, fix N and generate antifungal and antibiotic chemicals, among other things [13].

3. Silicon solubilizing bacteria

Bacteria that can dissolve silicon. Silicon (Si), the second most abundant element (27 %), is usually found in the unavailable forms of aluminium, magnesium, calcium, sodium, potassium, or iron silicates (SiO3). Chemical and biological interactions in the soil control its accessibility to the plant roots. Plants absorb Si as soluble monosilicic acid, which strengthens cell walls through a variety of methods. Si fertilization in plants, which has previously been shown to activate defence responses, improves their resilience to biotic stresses [14].

Induced systemic defence responses in plants have been reported as one of the mechanisms by which organisms reduce the diseases in plants in conjunction with other mechanisms including direct antagonism, antibiosis and siderophore production. Induction of defence responses by *Bacillus* spp. and Trichoderma spp. is largely associated with production of pathogenesis related proteins like b-1,3-glucanase and the defence enzyme phenylalanine ammonia-lyase and oxidative enzymes like peroxidase, polyphenol oxidase and superoxide

ISBN: 979-85-27243-61-1

dismutase [15-19]. Apart from controlling diseases, these biocontrol organisms also promote plant growth by production of plant growth hormones like IAA and GA3 coupled with increased availability of nutrients [20-22].

7. Conclusion

The green revolution resulted in incredible increases in food grain output, but with little regard for agriculture's long-term viability. Biofertilizers are becoming increasingly important in agriculture's long-term viability. For agriculture's long-term viability, many complementary combinations of microbial inoculants for key nutrient control are required. This study's combination focuses on N_2 fixation, potassium solubilization, silicon solubilization, and phosphate solubilization. These critical nutrient supplies allow for soil fertility enrichment, which increases output. The proper formulation of the biofertilizer results in efficient usage of the biofertilizer as a chemical fertilizer substitute.

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Curcumin promotes mitochondria-mediated apoptosis in human laryngeal cancer cell line (HEP-2) by miRNA-203 mediated down regulation of survivin

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Abstract:

Curcumin (CUR) is a phenolic compound, and its pharmacological profiling confirmed its potential anti-cancer property as it exhibit pleiotropic action on cancer. It selectively induces apoptosis in tumor cells through mitochondria-related intrinsic and death receptor-associated extrinsic pathways. Recent studies found that CUR plays a vital role in cancer progression by changing specific miRNA expressions in a variety of cancers. CUR inhibits cell proliferation and promotes apoptosis in laryngeal cancer cells through Bcl-2 and phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt), and by up-regulating microRNA-15a (miR-15a). MiR-203 is an antitumor miRNA that is downregulated in pancreatic cancer cells and laryngeal carcinoma cells, specifically targeting survivin expression. Its expression is inversely related to the expression of the survivin gene by inhibiting the caspase-3 and caspase-7activity results in inhibition of cell apoptosis. The present study aimed to investigate whether curcumin inhibits cell proliferation and promotes apoptosis in laryngeal cancer cells through miRNA-203 mediated down-regulation of survivin.

We studied CUR induced apoptosis by evaluating the differential expression of apoptosis related genes Bax and Caspase-3 levels were significant increased along with increased miRNA-203 ($p<0.01$) expression. Mitochondrial membrane potential was measured as direct indicator of induction of apoptosis. CUR treatment showed concentration dependent cell viability and is positively correlated with mitochondrial potential. Apoptosis in CUR treated cells were also showed by concentration dependent increase in nuclear fragmentation, chromatin condensation and blebbing of the membrane.

In conclusion; the present study demonstrates that CUR promotes mitochondria-mediated apoptosis in a human laryngeal cancer cell line (HEP 2) by upregulation of miR-203 and targeting survivin. We suggest that curcumin may offer an important therapeutic advantage in the clinical management of refractory laryngeal cancer over other standard treatments.

1. Introduction

Laryngeal cancer is one of a group of head and neck squamous cell carcinoma (HNSCC) that affects the larynx. Globally, it accounts for about one-fourth of HNSCC cases and 2% of the total world cancer cases [1]. In India, approximately 25,000 new cases have been diagnosed, and more than 17,000 die every year due to this cancer. The incidence of laryngeal cancer in Indian men was about 3-6% comprised of all cancers. The 5-year survival rate is approximately 28% which is much lower when analogized with the other Asian nations [2]. Indian studies have indicated that incidence of laryngeal cancer have risen over the years, primarily due to tobacco smoking, smokeless tobacco, alcohol intake habits, and human papillomavirus (HPV) [3].

Survivin is one of the smallest members of the inhibitor of apoptosis protein (IAP) family. It inhibits apoptosis by deactivation of both extrinsic (caspase-8) and intrinsic (caspase-9) pathways respectively.. Several studies have confirmed that survivin expressed remarkably high in many human cancers such as pancreatic, melanoma, breast, lung,

colon, brain, and neuroblastoma [5]. A meta-analysis research has shown that survivin plays a key role in the occurrence and development of laryngeal cell carcinoma and its increased expression results in the poor prognosis of laryngeal cancer patients [6]. A Genome-wide searches has shown that selective expression of survivin in the tumor rather than the healthy tissue encourages further research into the development of survivin-mediated therapy [8].

MicroRNAs (miRNAs) are small non-coding, single-stranded RNA molecules having 18-25 nucleotides in length. MicroRNAs regulate the expression of genes at the post-transcriptional level by base-pairing with the 3′ untranslated region of their target mRNAs [9]. The miRNAs have been reported to control the cell proliferation, apoptosis, migration, invasion and cell cycle progression in different cancer cell lines. MiR-203 is an antitumor miRNA which is downregulated in both pancreatic and laryngeal carcinoma cells and explicitly targets survivin expression [10, 11]. The increased in expression of miR-203 is decreased the expression of the survivin gene by inhibiting the caspase-3 and caspase-7activity results in inhibition of cell apoptosis [12]. *In-vitro* and *in-vivo* studies confirmed that miR-203 regulates cell proliferation, apoptosis, and cell cycle progression in pancreatic and laryngeal carcinoma cells by directly targeting survivin [10, 11].

Curcumin (CUR) is a phenolic compound, and its pharmacological profiling confirmed that potential anti-cancer property as they exhibit pleiotropic action on cancer [14]. It selectively causes tumor cells to undergo apoptosis through mitochondria-related intrinsic and death receptor-associated extrinsic pathways mediated by phosphatidylinositol 3-kinase-Akt (PI3K/Akt) signaling pathway [15]. Recent studies found that CUR plays a vital role in cancer progression by changing expressions of miR-21, miR-215, miR-192-5p, miR-31, and miR-26a in a variety of cancers such as colorectal, prostate, lung, oral and pancreas [16]. Xiaofeng Zhu et al. revealed that CUR inhibits the growth of laryngeal squamous cell carcinoma by up-regulation of mir-145 and suppression of the PI3K/Akt/mTOR pathway [17]. Zewu Li et al. showed that zinc finger protein 217 (ZNF217) is essential for cell proliferation and has been implicated in colorectal cancer which is suppressed by the miR-203 expression [19]. Nan Wang et al. study showed that miR-203 suppresses the proliferation, migration, and promotes the apoptosis of lung cancer cells by targeting SRC [20]. Saini S et al. study confirmed that miR-203 expression was down-regulated in human bladder cell lines by hypomethylation of the miR-203 promoter [21].

However, the present study aimed at whether CUR promotes mitochondria-mediated apoptosis in a human laryngeal cancer cell line (HEP 2) by upregulation of miR-203 and targeting survivin.

Materials and Methods:

2.1. Cell Culture

The HEP 2 cells were cultured in MEM medium containing fetal calf serum (FBS) (10%) and Antibiotic-Antimycotic solution (1x). It was maintained in a CO2 incubator (Galaxy 170R, Eppendorf, New Brunswick, Germany) at 37 °C, 5% CO2 with optimum humidity.

2.2. Cytotoxicity

We performed cytotoxicity by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. In each well, approximately 5×103cells were seeded in a 96-well flat-bottom polystyrene microtiter plate (NEST-Biotechnology) and maintained at 37ºC in saturating humidity and 5% CO2 overnight. The cells were treated with different concentrations (100, 50, 25, 12.5, 6.25, 3.125 1.560 µg/mL) of CUR and incubated for 24, 48, 72 hrs. The wells were washed twice with PBS. The 20 µL of the MTT reagent solution (5mg/ml in PBS) was added to each well and the plate was incubated for 4h in CO2 incubator. After incubation, 100 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals. The absorbance was recorded at 570 nm using a microplate reader (Bio-Rad, California, USA) [22].

Formula: Surviving cells (%) = Mean OD of test compound /Mean OD of Negative control ×100

2.3. Detection of mitochondrial transmembrane potential

The cells were seeded at a density of approximately 1×105 cells/well in a 12-well flat-bottom microplate containing coverslips (Cat no-TCP017) and incubated in a CO2 incubator at 37°C for overnight. Hep2 cells were treated with CUR 30, 20, 10µg/mL for 12 hrs. After the incubation, wells were washed with DPBS, and a 4% paraformaldehyde solution was used for fixation. After cell fixation, JC-1(5µg/ml) was incubated for 20 min and observed under the fluorescence microscope (Olympus BX41, New York, USA) at 10x magnification [23].

2.5. DAPI staining

The cells were seeded at a density of approximately 5×104 cells/well in a 24-well flat-bottom microplate containing coverslips and maintained in the CO2 incubator overnight. Cells were treated with CUR 15µg/mL for 24, 48 and72 hrs. After the incubation time, cells were washed with DPBS and a 4% paraformaldehyde solution was used for fixation. After fixation, cells were treated with DAPI and observed under the fluorescence

microscope (Olympus BX41) at 40× magnification [24].

2.6. Apoptosis by Flow cytometer

The cells were seeded in a 24-well flat-bottom microplate and maintained at 37ºC in the CO2 incubator overnight. The IC50 value of each compound was treated at 12 hrs. After the incubation, cells were washed with PBS. Centrifuge for 5 minutes at 500 x g at 4°C. Discard supernatant, and resuspend the cell pellets in ice-cold 1X binding buffer at 1 x 105 cells per mL. Keep tubes on ice. Then add 1 µL of Annexin V-FITC solution and 5 µL PI and Mix gently. Keep tubes on ice and incubate for 15 minutes in the dark. Add 400 µL of ice-cold 1X binding buffer and mix gently. Analyze cell preparations within 30 minutes by flow cytometry [24].

2.7. Quantitative expression of apoptosis-modulating genes and miRNA-203

The effects of CUR on the expression of miRNA-203 and apoptosis-modulating genes were listed in Table No 1[23]. ß -actin served as a housekeeping gene. The treated and untreated cells were lysed, and the mRNA was isolated and purified using RNeasy mini kits (Qiagen, Hilden, Germany) and was stored in RNA Save at –80°C. High-quality RNA samples with 260/280 ratios (1.8–2.0) were used in further steps. The mRNA was reverse-transcribed into cDNA, and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analyses were performed to quantify mRNA expression of apoptosis-modulating genes. Quantitative RT-PCR was performed in 20 µl reaction containing cDNA, nuclease-free water, primers and SYBR® Green Real-time PCR Master Mix (Thermo Scientific, Massachusetts, United States) according to the manufacturer's instructions [22].

For miRNA-203 expression, isolation of miRNA was performed using the mirVana™ miRNA isolation kit. cDNA was synthesized using the iScript kit (BioRad, Copenhagen, Denmark) following the manufacturer's protocol. Quantitative PCR (qPCR) was performed using the KAPA SYBR® Fast Universal Master Mix (Kapa Biosystems Inc., Massachusetts, USA) on the ABI StepOne Plus system (Applied Biosystems, Foster City, CA, USA). Oligonucleotide sequences and qPCR conditions were used as previously described. All samples were run in duplicates, and U6 was used for normalization. Relative expression was calculated using the formula $2^{-\Delta\Delta Ct}$ [25]

2.8. Statistical Analysis

Statistical analysis was performed using GraphPad PRISM (version5.0). Experiments on cell lines were conducted in triplicates and repeated three

times. Results are expressed as mean and SEM and one way ANOVA followed by Dunnet multiple comparison tests to evaluate treated groups and control. It was used to assess differences between means. The difference was considered significant when $p < 0.05$. Using graph Pad Prism Version5.1, we calculated the IC50 for each compound.

Results

3.1 Cytotoxicity

The cytotoxicity of CUR concentrate on Hep2 cell line was evaluated at different periods using MTT assay. The IC_{50} value for CUR at 24, 48, and 72 hr was 31.43, 22.62, and 14.89µg/mL respectively. Only at a lower concentration, i.e. 1.56 µg/ml concentrations at 24, and 48 hr of treatment was not shown the significant decrease in cell viability 97.6% and 96.13% respectively compared to the control. Otherwise, CUR was showing a highly significant ($P<0.01$) decrease in cell viability compared to control for the remaining concentrations of different time intervals as shown Fig.1

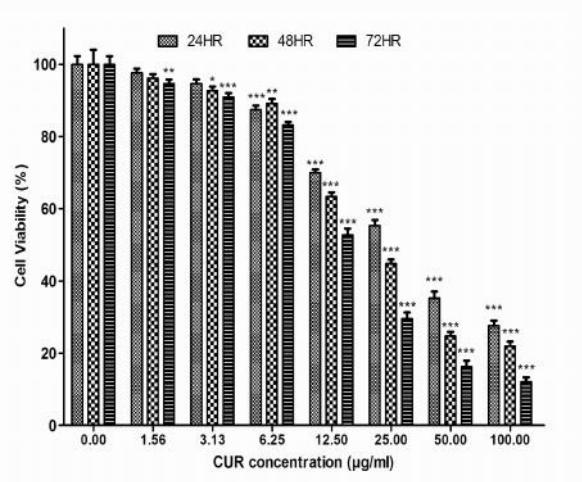

Fig. 1 Cytotoxicity effect of CUR on Hep2 cell line.

3.2. Effects of curcumin on the loss of mitochondrial membrane potential (ΔΨ) of Hep 2 cells

An impact of CUR on the loss of mitochondrial membrane potential m (Δ Ψ) of Hep 2 cells signifies the early stage of apoptosis. After overnight incubation, CUR-treated cells showed a significant decrease in red to green fluorescence intensity ratio in comparison with the untreated cells indicating collapse mitochondria membrane potential. CUR showed a concentration-dependent collapse in mitochondrial membrane potential as shown in Fig 2.

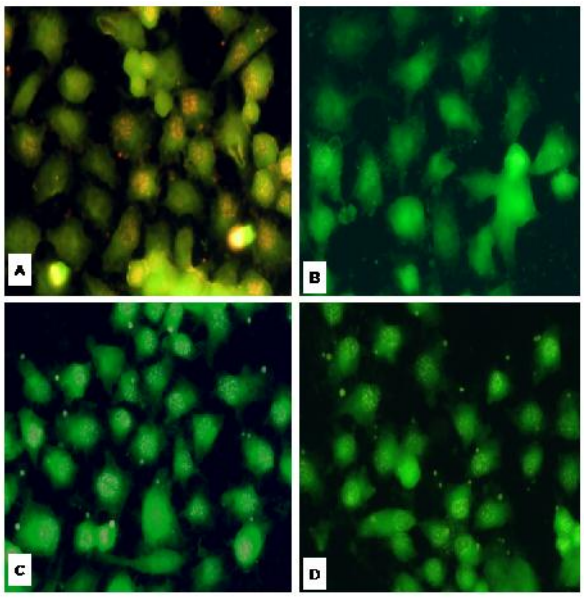

Fig. 2 Fluorescence microscopic images showing the mitochondrial transmembrane potential of curcumin in a Hep 2 cells A. Untreated B. Curcimun treatment (IC_{50}-at 24 hr), C. Curcimun treatment (IC_{50}-at 48 hr) D. Curcimun treatment (IC_{50}-at 72 hr)

3.4. DAPI

The apoptosis-inducing potential of the CUR was determined by DAPI staining. Untreated cells were showing without any cellular morphological changes. Whereas, 24 hrs treated CUR causes chromatin condensation in a few cells as seen with 35 % ($P<0.01$) cells undergoing apoptosis. Similarly, for CUR 48 and 72 hrs treated cells show nuclear fragmentation, chromatin condensation and blebbing of the nucleus were seen with apoptosis 60% and 80% ($P<0.01$) respectively which is significantly higher when compared to control cells as shown in Fig.3

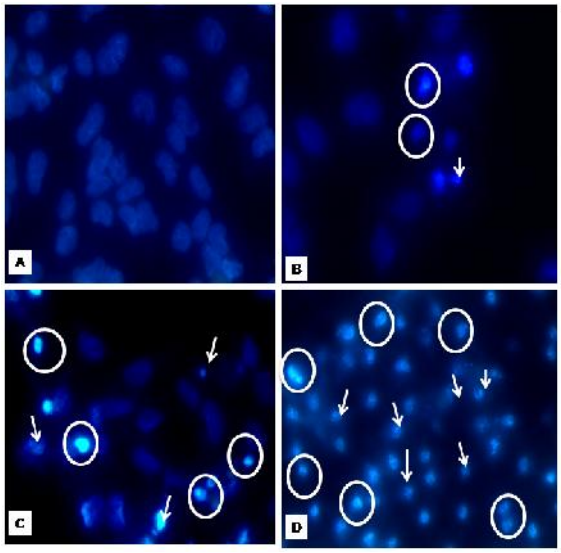

Fig. 3 Fluorescence microscopic images showing the Apoptosisof curcumin in a Hep 2 cells A. Untreated B. Curcimun treatment (IC_{50}-at 24 hr), C. Curcimun treatment (IC_{50}-at 48 hr) D. Curcimun treatment (IC_{50}-at 72 hr)

3.5. Apoptosis by Flow cytometer

We investigated the apoptosis-inducing potential of CUR in HEP2 cells using the Annexin V-FITC/PI staining technique. After predetermined incubation of CUR, The uncontrolled cells with 91.09 % live cells with 7.92% apoptotic and 0.6 % dead cells. Whereas in treated, live-cell percentage decreased from 91.09 % to 35.17% with early apoptotic cells and late apoptotic cells were 2.44% and 56.17% respectively. Also, dead cell percentage was increased from 0.63 % to 6.22 % as shown in Fig 4

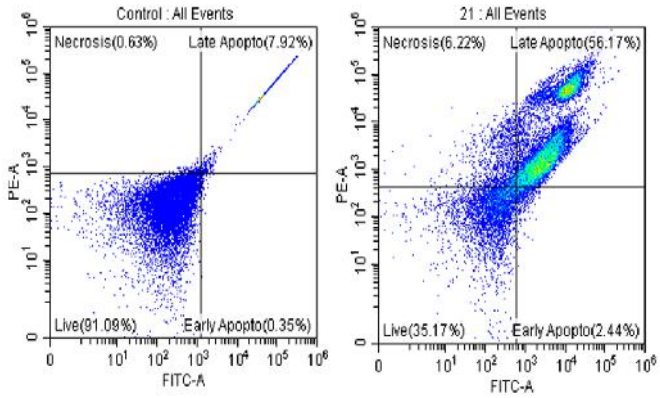

Fig. 4 Histograms of flow cytometric analysis displaying the effect of CUR in a Hep 2 cells.

3.6. Effect of curcumin on the mRNA expressions of apoptosis-modulating genes (Bax, Bcl-2, Bcl-xL, caspase-3, & survivin) and miRNA-203

We investigated the effect of CUR on the expression of apoptosis-modulating genes (Bax, Bcl-2, Bcl-XL, caspase-3, and survivin), to the relative expression of the housekeeping gene ß-actin. As shown in Figs.5 the anti-apoptotic genes (Bcl-2, Bcl-xL, and survivin) were down-regulated at the transcriptional level in Hep-2 cells after treatment with CUR for 12 h.

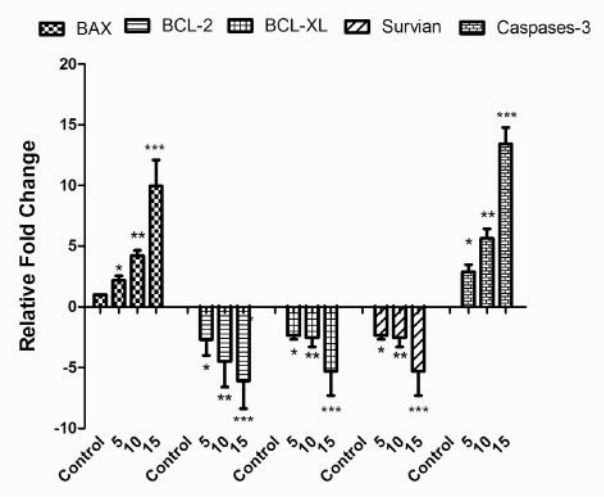

Fig. 5 The mRNA expression levels of apoptosis-modulating genes (Bax, Bcl-2, Bcl-XL, caspase-3, and survivin)

CUR decreased Bcl-2, Bcl-xL and survivin genes expression by 3.08, 2.8 and 2.7 fold change at 5µg/mL concentration respectively. But it was significant compare to control. Similarly, it significantly decreased the expression of Bcl-2, Bcl-xL and survivin genes by (6.78, 4.46, 6.66, $P<0.01$) and (12.73, 7.66, 14. 66 $P<0.001$) fold at 10 µg/ mL and 15 µg/ mL concentration, respectively when compared to untreated. CUR significantly increased caspase-3 gene expression by 4.2 fold at 5µg/ mL concentration whereas Bax gene expression significantly increased by 2.7 fold compared to untreated cells. Similarly, it significantly increased Bax and caspase-3 genes expression by 8.5 and 6.7 fold compared to untreated at 10µg/mL concentration. At 15 µg/mL concentration, it's a significant increase in Bax and caspase-3 expression by 13.83 and 13.1 fold compared to untreated.

The effect of CUR on the miR-203 expression of human laryngeal cancer cells was determined by RT qPCR. As shown in Fig.6 CUR treatment significantly increased the expression of miR-203 by 4.5, 8.7 and 19.2 fold at 5, 10 and 15 µg/ mL concentration compared with the untreated control cells ($P<0.05$).

ISBN: 979-85-27243-61-1

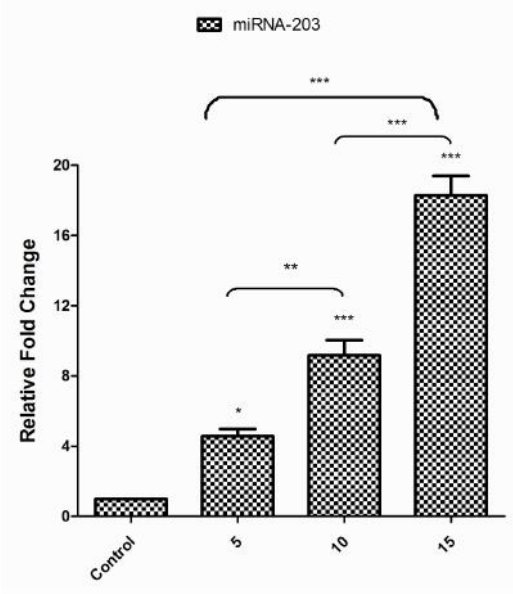

Fig. 6 The miR-203 expression of expression in CUR treated Hep 2 cells.

4. Discussion

The poor survival statistic of HNSCC cancer patients due to the emergence of resistance to chemotherapy treatment, their off-target toxicity has forced researchers to turn to naturally occurring herbal products[26]. Herbal products can alter the activity of multiple targets of carcinogenesis; polyphenols can inhibit the growth of cancer cells[27]. Over 6000 years CUR has been used in flavoring, coloring, preservative agent and traditional Ayurvedic & Chinese medicine[27]. In the present study, the anti-proliferative activity of CUR against the Laryngeal cancer cell line was experimentally proved. Curcumin showed good cancer cell inhibition, as indicated by the IC50 value, which ranged from 15 to 32 µg/ml. Maria et al. reported the maximal death at the maximum concentrations of 150 µmol/L for a slow-growing laryngeal cancer cell line (CCL23) and 300µmol/L for more aggressive oropharyngeal cancer cell line (CAL27) [28]. CUR decreased cell viability by time and concentration-dependent manner. Mitochondrial mediated pathway is one of the major apoptosis signaling pathways induced by the CUR in many cancer cells. Mitochondrial membrane potential collapse is an early step in the apoptotic cascade confirmed by numerous studies [23, 29]. The collapse in the mitochondrial membrane potential was observed after the increase in CUR concentration treatment in the current research. Similar results were shown in Jin-bo Wang et al. who showed the CUR induces the mitochondria-mediated apoptosis in human colon cancer cell line HT-29[23]. Apoptosis (programmed cell death) is a dynamic physiological process of cellular self-destruction, which is caused by specific morphological and biochemical changes in the nucleus and cytoplasm [32]. In the present study, we proved that the treatment of curcumin induces nuclear fragmentation, chromatin condensation and blebbing of the nucleus results into apoptosis. CUR induces apoptosis time-dependent manner. Similar results have been reported in M.R param et al. where 12µg/ml of free curcumin causes significant apoptosis in the human melanoma cell lines

(A375)[24] and Jin-bo Wang et al. who showed the CUR at 20, 40, and 60 µM concentration was capable to significantly induces apoptosis of cells with increased in a dose-dependent manner[23]. Similarly, we confirmed the apoptosis-inducing potential of CUR in HEP2 cells using a flow cytometer. The previous study showed that 50 µmol/L of curcumin treatment in a slow-growing laryngeal cancer cell line (CCL23) causes 27% of late apoptosis and 6% early apoptosis. Similarly, 50 µmol/L of curcumin treatment caused increased apoptosis in the oropharyngeal cancer cell line showed 33% of late apoptosis and 17 % early apoptosis{LoTempio, 2005 #58}.

Cancer cells evade apoptosis by overexpressing anti-apoptotic genes, particularly of BCL2, Bcl-xL, and survivin [35]. Bcl-2 is dominant regulator apoptosis via caspase activation and maintaining the integrity of the mitochondrial and endoplasmic reticulum (ER) membranes [36]. Survivin has a dual function, displays cell death repressor activity and mitotic progression[37].CUR caused a decrease in the expression of BCL2, Bcl-xL and survivin genes inducing apoptosis. When treated with CUR, the ratio of Bax and caspase-3 gene expression was significantly increased suggests induced apoptosis. Previous studies have shown that BCL2 and survivin genes were significantly decreased its expression at 40, 60, 80 µmol concentration of CUR whereas the Bcl-xL gene significantly decreased its expression at 60, 80 µmol concentration of CUR. Bax gene expression was significantly increased its expression at 40, 60, 80 µmol concentration of CUR. However, the caspase-3 expression doesn't significantly increase its expression at all concentrations of CUR treatment [23]. Shi-Geng Pei et al. study revealed that survivin plays a dual role by inhibiting apoptosis and promoting cell proliferation thus helps in tumor progression [7]. This dysregulation of survivin expression in cancer due to increased promoter activity, demethylation of survivin exons, amplification of the survivin locus, increased upstream signaling in the phosphatidylinositol 3-kinase or mitogen-activated protein kinase pathways [8].

Understanding of miRNA deregulation in diverse cancers is a potential therapeutic approach in the field of cancer. Many studies confirmed that miR-203 controls cell proliferation, apoptosis and cell cycle progression, migration, invasion in various cancers [10]. MiR-203 play role as an oncomir by directly target oncogenes such as Bcl –w, DNp63 in esophageal, E2F transcription factor 3(E2F3) in melanoma and nasopharyngeal carcinoma, Runx2 in breast cancer and metastatic bone eukaryotic

initiation factor 5A2 (EIF5A2) in colorectal cancer and ABL1 in hematopoietic malignancies[11]. In-vitro and in-vivo studies confirmed that miR-203 regulates cell proliferation, apoptosis and cell cycle progression of pancreatic cancer cells and laryngeal carcinoma cells by directly targeting surviving respectively[10, 11].

CUR increases miR-203 expression doses dependently at 5, 10, 15 µg/ml concentration in human laryngeal cancer. Shaofeng Mou et. al study proved that significant up-regulating miR-15a after treatment 20 µmol and 40 µmol CUR concentration in human laryngeal cancer [38]. Similarly, Sharanjot Saini et. al showed that curcumin(10 µM)concentration signifying increase microRNA-203 expression in bladder cancer by targeting the Src and Akt2 results into inhibited the proliferation and increased apoptosis of bladder cancer cells[21]. Using real-time PCR and in situ hybridization techniques confirmed that both prostate carcinoma cell lines and clinical biopsy showed down-regulation of miR-203 expression. Further, in-vitro and in-vivo functional assays conclude that miR-203 is an "antimetastatic" miRNA and potential molecular target for advanced prostate cancer [13].Zewu Li et al. showed that zinc finger protein 217 (ZNF217) is essential for cell proliferation and has been implicated in colorectal cancer which is suppressed by the miR-203 expression [19]. Nan Wang et al. study showed that miR-203 suppresses the proliferation, migration, and promotes the apoptosis of lung cancer cells by targeting SRC [20]. Saini S et al. study confirmed that miR-203 expression was down-regulated in human bladder cell lines. Further, microarray and miR qRT-PCR expression confirmed the up-regulation of miR-203 in CUR treated bladder cancer cell lines by hypomethylation of the miR-203 promoter [21]. However, the present study aimed at whether CUR promotes mitochondria-mediated apoptosis in a human laryngeal cancer cell line (HEP 2) by upregulation of miR-203 and targeting survivin.

The schematic diagram elucidating the possible mechanism of action of CUR on survivin. CUR increases expression of antitumor miR-203 which in turn inhibits survivin expression. Survivin is one of the smallest members of the inhibitor of apoptosis (IAP) family. Survivin is one of the

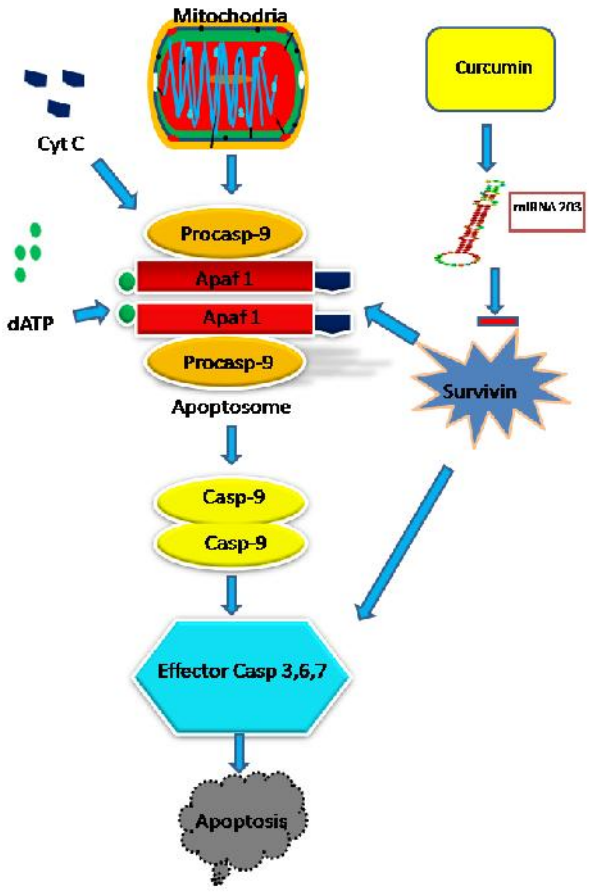

Fig. 7 The schematic diagram elucidating the possible mechanism of action of CUR on Hep 2 cells.

smallest members of the inhibitor of apoptosis (IAP) family of proteins. It inhibits apoptosis by deactivation of caspases [4]. It inhibits apoptosis by deactivation of the caspase-8 in the extrinsic pathway. This is the initiator in the extrinsic pathway through the binding of ligand such as FasL, TNF to death receptor of the cell surface. Similarly, the intrinsic pathway survivin inhibits apoptosis by deactivation caspase-9 (initiator caspase) which part of apoptosome complex results into inactivation of caspase-3 (effectors caspase). Further, prevent activation of Caspase-activated DNase (CAD) and inhibitor of CAD (ICAD) which are requiring for the cleavage and fragmentation of DNA [32].

5. Conclusion

In conclusion, the present study demonstrates that CUR promotes mitochondria-mediated apoptosis in a human laryngeal cancer cell line (HEP 2) by up-regulation of miR-203 and targeting survivin. Because of our present results and the relative non-toxicity associated with the use of curcumin, we suggest that a combination of curcumin may offer an important therapeutic advantage in the clinical management of refractory laryngeal cancer over other standard treatments alone.

In conclusion, our study results recommend that curcumin can significantly inhibit cell proliferation in Hep-2 cells. It induces apoptosis through mitochondrial-mediated as evidenced by the collapse of oxidative DNA damage, and nuclear fragmentation. Further curcumin- modulates the apoptotic genes which include decreases in Bcl-2/Bax and Bcl-xL/Bad ratios followed by the activation of caspase-3. These results indicated that curcumin could be used as a novel and cost-effective therapeutic agent for the treatment of laryngeal cancer.

ISBN: 979-85-27243-61-1

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Synthesis of nano sized TiO_2 particles for resisting the growth of microbial consortium in various applications.

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Abstract:
In this experimental study, the impact of antimicrobial activity of nano sized Titanium oxide ($nsTiO_2$) particles on microbial consortium has been analysed under batch conditions. The antimicrobial activity studies were carried out by ($nsTiO_2$) which has been prepared by varying pH conditions. In addition to that, the structural and thermal property of the nanoparticles were tested by using UV spectrophotometer, FTIR, XRD and Thermogravimetric analysis. From the obtained result, the characterization studies confirmed the size of synthesized nanoparticles, band gap, and degradation temperature of around 40 nm, 3.74 eV and above 1000℃ respectively. Moreover, the efficacy of antimicrobial activity of ($nsTiO_2$) particles were depicted by suppression of growth rate of *Pseudomonas aeruginosa* with respect to the function of time.

Keywords: Titanium oxide, Anti-microbial activity, Nanoparticles, *Pseudomonas aeruginosa*, XRD.

1. Introduction

Due to toxic effect and the increasing of resistance towards certain antibiotics, the scientific community move towards developing or synthesizing of in-organic antimicrobial substances such as pure metal salts and metal oxide nano-particles. The metal oxide has a significant property to resist or inactivate the activity of microbial DNA, restricting bacterial replication, hindering the activity of metabolic enzymes in electron transport chain. There are several metal oxide nanoparticles namely Zinc oxide (ZnO), Manganese oxide (MgO), Titanium oxide (TiO_2) and Iron oxide (Fe_2O_3) which are extensively used as an antimicrobial agent due to its physiochemical properties in the application of biological domain. But TiO_2 has an excellent semi conducting, higher resistance to chemical corrosion and higher antimicrobial and antifungal property among the other nanoparticles. In addition to that, TiO_2 nanoparticles has higher surface area to volume ratio and non-toxic in nature [1]. Generally, the antimicrobial activity of nanoparticles is influenced by several inherent properties such as morphology, size, chemistry and nanostructure. Moreover, the antimicrobial activity of nanoparticle also depends upon morphological, structural and textural properties. According to the structural property, the titanium oxide nanoparticles exhibit various crystalline polymorphous phases such as anatase, rutile and brookite [2]. Several literatures stated that, the anatase phase has more antimicrobial activity than the other phase due to the formation of hydroxyl free radicals during photochemical process. In this research work, we mainly concentrated on study of the

antimicrobial effect by using various crystalline phases of TiO_2.

2.Materials and methods:

Chemicals, reagents, and solvents used during this work include distilled water, Titanium Iso-prop oxide (Sigma Aldrich), propanol (Merck), sodium hydroxide (Merck), nitric acid (SRL, India) and ethanol (SRL, India). All these chemicals and reagents are of analytical grade and were used in this work without any further purification.

3.Test procedure for the synthesis of Titanium oxide nanoparticles with respect to pH

65ml of 1.0 N NH_4OH solution was added to titanium isopropoxide in an ice bath at 10°C to form titanic acid [Ti $(OH)_4$]. It is then dissolved with 33 ml of 1.0 N HNO_3 to form titanyl nitrate [TiO $(NO_3)_2$]. Deionized water containing citric acid and 1.0 N NH_4OH were added to adjust the pH value of the solutions ranging from 2 to 6 for different samples. The white precipitated sol was obtained after adjusting the final range of pH of the solution. This was then washed and dried in an oven at 80°C for 24 h. The white gel was calcined in the muffle furnace from 400 to 800 °C for 2 hours. Five samples were prepared at final pH of 2, 3, 4, 5, and 6 [3].

3.1 Test procedure to observe the impact of antimicrobial effect by using $nsTiO_2$

In this study, the antimicrobial activity of $nsTiO_2$ was delineated by varying the mass of the TiO_2 in the range of (1 to 5 gm). Then the powdered form of nanoparticles were dispersed in 1 ml of distilled water and then the samples were sonicated at 20°C for 1hr to maintain the homogeneity of nanoparticles in the solution. After that, a piece of Carboxy methyl cellulose membrane (CMC) was immersed for 30 min in the samples for the adsorption of nanoparticles on the surface of selected membrane. Eventually, the membrane was placed in the well grown bacterial medium in petri dish to enumerate the antimicrobial activity with respect to time**.**

3.2 Characterisation studies of the $nsTio_2$:

Functional groups present in the synthesized powders were identified by FTIR conducted on a 630 1B diamond ATR module in the range of wavenumbers 4000-400cm $^{-1}$(2000 scans). The test was conducted on five samples. In addition to that, the absorption spectra of $nsTiO_2$ were recorded by using UV spectrophotometer (Thermo scientific, UK) between the wavelength of 200 to 800 nm [4]. Thermal gravimetric analysis (TGA) of the biosynthesized NPs was carried out using a simultaneous differential thermal analysis DTA-TGA (DTG-60H, Shimadzu Co., Japan) and was used to determine the calcination temperature. The crystalline structure and the average crystalline size of the titanium oxide NPs were investigated using an X-ray diffractometer (XRD-7000, Shimadzu Co., Japan) and were recorded with 2θ from 10 to 80 using CuKα (λ = 1.5

A^{O}) radiation operated at 40 kV and 30 mA [5].

4.Results and discussion

4.1 Ultraviolet-Visible (UV-Vis) Analysis

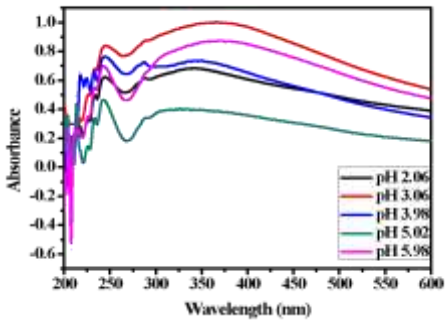

Fig. 1a Absorption spectra of $nsTiO_2$ by using UV Spectrophotometer.

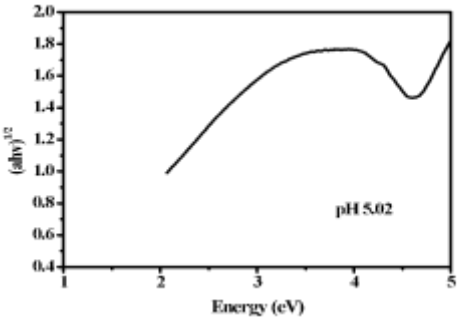

Fig. 1b Tauc Plot of $nsTiO_2$

Fig 1(a-b) shows the absorption spectra and Tauc plot of chemically synthesised $nsTi0_2$ particles at different pH (2-6) conditions. The optical property of the nanoparticle was analysed between the wavelength of 200 to 600nm. From the obtained result, Fig 1a shows that, the absorption spectral peak of $nsTiO_2$ was observed between 225 nm to 275nm. In addition to that, the maximum absorbance achieved by the synthesised nanoparticles was around 0.8. Likewise, the Fig 1b Tauc plot delineated that, the band gap energy of $nsTiO_2$ is around 3.14 eV of at pH 5 [6].

4.2 Fourier Transform Infrared (FTIR) Spectroscopy

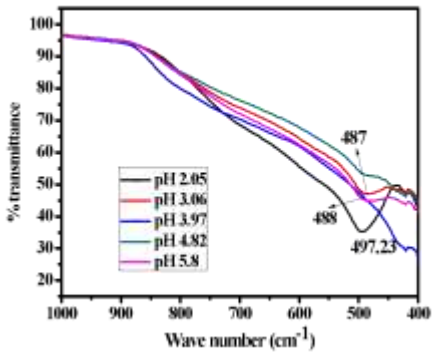

Fig 2. FTIR spectrogram of $nsTiO_2$

Fig 2 shows the FTIR spectrogram of chemically synthesised TiO_2 nanoparticles at different pH conditions. The obtained result depicted that, the absorption peaks of the $nsTiO_2$ existed between 480 cm^{-1} to 500 cm^{-1}. In addition to that, the intensity of absorption peak of TiO_2 increases at lower pH conditions due to the agglomeration of nanoparticles [7].

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4.3 X-Ray diffraction analysis of nsTiO2

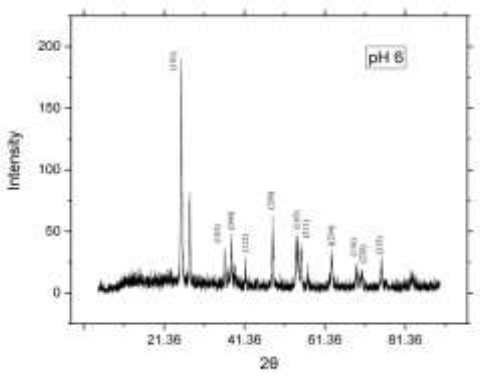

Fig. 3 XRD analysis of nsTiO2

Fig 3 enumerates the XRD pattern of synthesised TiO2 nanoparticles. From the analysis, the diffraction spectral speaks were observed at the 2θ values of 25.3, 26.6, 37.3,48.35, 41.36, 56.3,61.36 and 72.35 along with the miller indices planes (1 0 1), (1 0 3), (0 0 4), (1 1 2), (2 0 0), (1 0 5), (2 1 1), (2 0 4), (1 1 6), (2 2 0) and (2 1 5). The analysis confirms that the synthesized titanium oxide nanoparticles are in a tetragonal crystal structure without any impurities and the scanned region within the scanned region done from 10° to 81.36° [8]. In addition to that, the angle 2θ at 25.3 and 48.35 confirms the presence of anatase phase. Likewise, the XRD was also analysed for TiO_2 nanoparticles synthesised at pH conditions.

Note: The antimicrobial activity results are not included in this paper due to some ethical issues.

5. Conclusion:

In this experimental study, the characterization studies confirmed that the synthesised nanoparticle belongs to anatase phase and size of nanoparticles are around 34 nm to 61 nm. Moreover, the UV analysis and Tauc plot delineated that, the absorption spectra achieved between 225 to 275 nm and the energy band gap is around 3.14 eV. Finally, the FTIR revealed that the absorption peaks were observed at 480 cm^{-1} to 500 cm^{-1}.

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Applications of Calcium Phosphate nanoparticles in medicine and therapeutics

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Abstract: Doped Calcium Phosphate Nanoparticles are promising materials for treatment in the field of Orthopedics and Dentistry. Bio ceramic Calcium Phosphate samples composed of hydroxyapatite and tricalcium phosphate are used in dentistry for filling up bony defects and used as root repair materials, apical fill materials, aids in regeneration, etc. It is also used in new born formation in regions like alveolar bone, sinus lifts and defects caused by tumor. Its properties can be studied through X-ray diffraction, dynamic light scattering, electron depressive the grounds of unique osteo conductive and bio-active properties, it is widely preferred as the biomaterial of choice in both dentistry and orthopedics. As it is similar to human heart tissue like bone and teeth, special focus is dedicated to the loading capacity, controllable release of drugs under internal biological stimuli wherein it can be used to transfer nucleic acids or drugs.

Keywords: Calcium Phosphate Nanoparticles, Hydroxyapatite, Dentistry, Orthopedics, Drug Carrier.

I.INTRODUCTION

Nanoparticles have gained the spotlight in technological advancements due to their tunable physicochemical characteristics such as Surface area, wettability, Melting point, catalytic activity, electrical and thermal conductivity, light absorption and scattering properties. [1] Calcium phosphate nanoparticles having average particle size of 15-40nm and surface area of 30-60m^2/g is mainly used for biomedical applications but also used in other sectors such as cosmetics, catalysis, water remediation, and agriculture. [2]

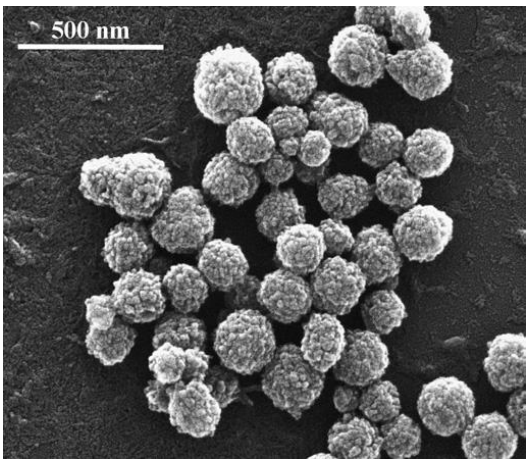

Fig 1. SEM Picture of functionalized Calcium Phosphate nanoparticles [3]

Calcium phosphate materials are quite similar to bone in composition and in having bioactive and osteoconductive properties. Calcium phosphate materials in different forms such as cements, composites, and coatings are used in many medical and dental applications. [4] Calcium phosphates belong to the group of bioactive synthetic materials and its commonly used forms are the hydroxyapatite and the tricalcium phosphate because of their properties like chemical composition similar to the skeletal tissue, osteo conductivity, crystallographic structures. [4] In case of dentistry, Calcium phosphate is used in the repair of periodontal defects, tooth replacement, used as scaffolds in tissue engineering for bone or dentin regeneration etc. [4] In the field of orthopedics, Bone defects or loss of bone, congenital disorders or diseases like osteoporosis can affect the self-healing potential of bones. Therapies that involve the administration of therapeutic factors to promote bone tissue regeneration requires administration of larger doses and has side effects. Therefore, the

ISBN: 979-85-27243-61-1

development of biomaterials that can help in the targeted delivery of drugs, therapeutic factors in the form of carriers to treat diseases, promote bone healing and can overcome some of the limitations in case of systemic delivery. [5]

II. TYPES

The two types of calcium phosphate biomaterials focused here are hydroxyapatite and tricalcium phosphate.

Hydroxyapatite is a bio-ceramic which is porous, granulated in nature, biocompatible, non-toxic, non-inflammatory and even non-immunogenic agent. [6]

Tricalcium phosphate show resorbable characters during bone regeneration and can be completely substituted for bone tissue after stimulation of bone formation. [58]

III. SYNTHESIS AND CHARACTERIZATION

Numerous methods of Calcium nanoparticle synthesis aimed towards obtaining nanoparticles with diverse size usually within nanoscale dimension, composition, morphology and less agglomerated forms. Calcium nanoparticles are majorly synthesized by co-precipitation method or hydrothermal treatment where by varying reaction parameters size and morphology can be controlled corresponding to biological applications [26]

In general, calcium phosphate is synthesized by various methods like wet-chemical precipitation, sol-gel chemistry, flame spray pyrolysis, and solid-state reactions. The precipitation from water is easy, cost-efficient, and environmentally friendly, as no organic solvent is required. It has some advantages like the possibility to control particle crystallinity and size by varying pH, concentration, temperature, and precipitation time [25]. The sol–gel synthesis is based on the reaction of a calcium source and a phosphate source, usually in an organic solvent. It offers different possibilities to fabricate a wide range of structured nanomaterials, including coatings on metallic implants. The sol–gel synthesis is advantageous due to its simplicity, high versatility, comparatively homogeneous product composition (e.g., Ca:P ratio), and low synthesis temperature. [41] Flame-spray pyrolysis is a versatile method for the large-scale synthesis of calcium phosphate nanoparticles. A solution or a dispersion of the precursors is injected into a flame where the particle formation occurs at high temperature. The possibilities for precursor selection and reactor system engineering make this method suitable to produce particles with variable properties, also for biomedical applications. The method is adjustable with respect to particle morphology, crystallinity, and size [27]. Pulsed laser ablation has also been applied to prepare calcium phosphate nanoparticles from synthetic and biological calcium phosphate substrates [29]. This method is based on the ablation of nanoparticles from a solid substrate and has turned out to be very versatile to prepare metallic and ceramic nanoparticles. However, its applicability to prepare calcium phosphate nanoparticles is probably limited in comparison to other methods where particle size and composition can be controlled more easily. When comparing all methods for synthesis of calcium phosphate nanoparticles for biological application, a precipitation from aqueous solutions has distinct advantages compared to the other methods. It allows to load biomolecules into the particles or/and to functionalize them on the surface, leading to reproducible and uniform nanoparticles in stable colloidal dispersions. It also avoids organic solvents [36].

Amorphous CP-NPs have been prepared through a modified co-precipitation method

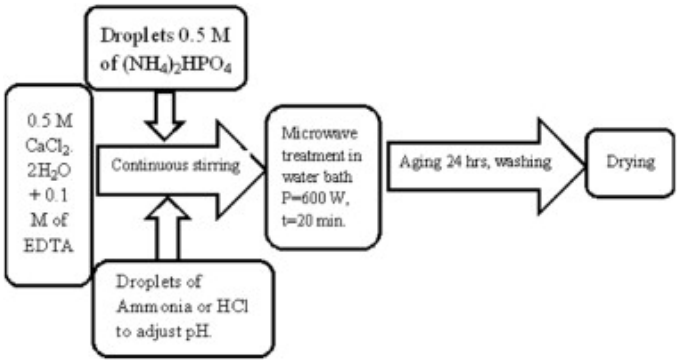

Figure 2. Synthesis by co-precipitation method [56]

By hydrothermal method: Following Other methods, Homogeneous hydroxy Apatite (HAp) nanoparticles were obtained by a hydrothermal treatment Of CaCl2-Na2HPO4- NaOH aqueous solution

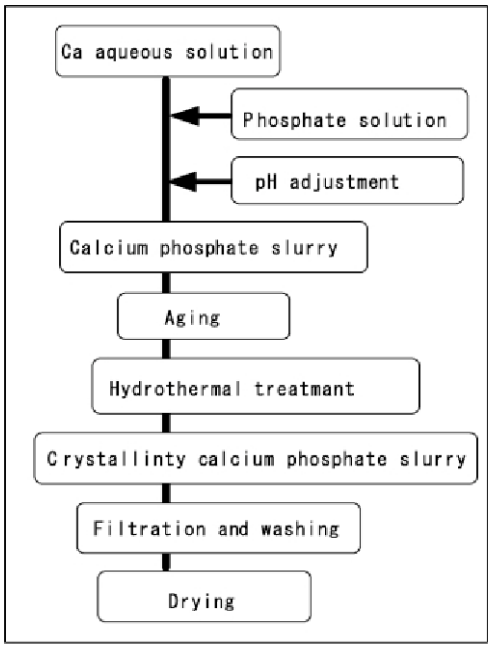

Figure 3. Synthesis of CaP-NP by Hydrothermal method [57]

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Method	Advantages	Limitations
Precipitation from aqueous solutions	Bulk synthesis possible; low cost; incorporation of organic or biological compounds possible; only water as solvent	Upscaling can be difficult and requires a continuous process
Sol-gel method	Low cost; control over chemical composition	Upscaling can be difficult; organic solvents required
Flame-spray pyrolysis	Good crystallinity; possibility for scale-up	Particle agglomeration; no incorporation of organic molecules possible; special equipment necessary
Pulsed laser ablation	Control over product properties possible by adjustable laser parameters	Tendency for particle agglomeration; high-end laser equipment needed; difficult scale-up
Solid-state synthesis (high-temperature methods)	Easy and low cost; well-crystallized particles; high yield	Agglomerated particles; poor redispersability; application of organic compounds possible only after the synthesis

Table 1. Summarises the various methods for synthesis along with their advantages and limitations

In general, different methods are usually applied for the characterization of calcium phosphate nanoparticles, as it is generally recommended for nanoparticles. Besides electron microscopy, dynamic light scattering (DLS) is probably the most prominent method to analyze the size and the surface charge (zeta potential) of dispersed calcium phosphate nanoparticles.[51] DLS is a fast and appropriate method if the particle size distribution is monomodal and narrow. This has to be confirmed by other techniques like electron microscopy or disc centrifugation [48]. In polydisperse systems, DLS tends to produce artifacts due to the fact that large particles scatter the light much more intensely than smaller particles.

If prepared for biomedical use, the calcium phosphate nanoparticles must be thoroughly purified to remove excess reagents from the synthesis and unwanted synthesis by-products like the inorganic counter ions of calcium phosphate. Otherwise, these will render the interpretation of biological results impossible. Commonly applied purification techniques are centrifugation, nanofiltration, and dialysis. Centrifugation is the best option for calcium phosphate nanoparticles due to their density and comparatively large diameter (typically 50 to 100 nm). After purification and size characterization, the amount of cargo must be quantitatively determined [55]. This is more difficult than generally assumed and not always done in the literature. Auto fluorescent or fluorescently labelled drug molecules can be easily detected by UV-spectroscopy or (less accurately) by fluorescence spectroscopy [54].

The number concentration of calcium phosphate nanoparticles in a dispersion can be determined by elemental analysis. Typically, AAS and ICP-MS are the methods of choice. From the calcium and/or the phosphate concentration, the concentration of calcium phosphate in the dispersion can be derived if its stoichiometry is known. Usually, the stoichiometry of the most common calcium phosphate phase hydroxyapatite, $(Ca_5(PO_4)_3OH)$, is tentatively applied [49]. Furthermore, the particle density of the calcium phosphate (hydroxyapatite: 3,140 kg m^{-3}), the particle shape (usually spherical), and a monodisperse particle size distribution must be assumed. As not all parameters are exactly known, the particle concentration in a dispersion is accessible only with limited accuracy [50]. Even Scanning and transmission electron microscopy is performed for characterization.

Method	Possibilities	Limitations
SEM and TEM	Size and shape of nanoparticles (only calcium phosphate core); phase identification if combined with electron diffraction (ED) and/or energy-dispersive X-ray spectroscopy (EDX) (important for calcium phosphates due to different possible phases)	Imaging in the dry state; only a small number of particles can be analysed; possible contamination by other compounds (e.g., salts or biomolecules from the dispersion medium); beam damage may cause artifacts, especially with hydrated or amorphous calcium phosphate phases
DLS	Easy and fast determination of particle size and surface charge (zeta potential)	Works well for monodisperse systems and poorly for polydisperse systems
DCS	Easy determination of particle size	Works well for calcium phosphate nanoparticles due to their density

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		(about 3,000 kg *m*-^{3})
AUC	Discrimination between particles of different size and density	Works well for calcium phosphate nanoparticles due to their density
NTA	Direct determination of particle size (hydrodynamic radius); direct visualization of dispersed particles	Analysis of only a small number of particles; large particles may sediment and remain undetected
UV-VIS	Determination of the loading with drugs and biomolecules	Fluorescent labelling of analytes is usually necessary
XRD	Identification of crystalline calcium phosphate phases; determination of nanoparticle crystallinity and domain size; detection of crystalline impurities	Only for crystalline particles; broad diffraction peaks; amorphous phases may go undetected besides crystalline phases
SAXS	Determination of hydrodynamic radii and agglomeration state; calcium phosphate is well suited due to its density	Complex data analysis
ICP-MS	Determination of overall particle composition with high precision	Analysis of single particles possible if not too big; calcium phosphate nanoparticles are usually too large (40 nm or more)
AAS	Determination of metal content, for example, calcium or substituting ions	Lower sensitivity than ICP-MS; not possible for phosphate

Table 2. Summarises the different characterization techniques with the possibilities and limitations of each

IV.APPLICATIONS:

a) Dentistry

ACP-NPs are prepared via co-precipitated technique from the solution of Ammonium hydrogen phosphate and calcium chloride as well as NaOH for the adjustment of pH 8, which constitutes the chitosan solution [7]. ACP-NPs in chitosan solution is used to treat the existing enamel decalcification or as a preventive measure in the form of a mouthwash during orthodontic treatment. Chitosan acts as an antibacterial, anti-inflammatory agent, prevent plaque, bad breath and decreases gingivitis. This is preferred over synthetic polymers as they are biocompatible, biodegradable, easily available and functions as a stabilizing material [7].

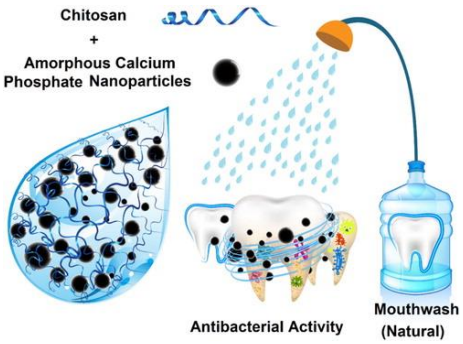

Figure 4. The scheme of antibacterial effect of synthesized ACP-NPs.[7]

b) Orthopedics

Bone regeneration involves various complex biological processes. Many experiments have been performed using biomaterials in vivo and in vitro to promote and understand bone regeneration. Among the many biomaterials, calcium phosphates which exist in the natural bone have been conducted a number of studies because of its bone regenerative property. It can be directly contributed to bone regeneration process or assist in the use of other biomaterials [8]. Since the 1900s, synthetic calcium phosphates have been actively studied for clinical use [9]. Thereafter, bone regenerative applications such as bone cements, scaffolds, implants, and coating techniques using calcium phosphates have emerged, and some have been commercialized [10].

Although calcium phosphate has been widely used for bone treatment as a raw material itself, many studies have been made using processed calcium phosphate applications for better utilization. It is used as coating materials for improving bioactivity of bone implants. And also, it is used as composites with biomaterials to alter mechanical properties, control biodegradability, and encapsulate drugs [8]. CaP-NPs mixed with other materials can be utilized in various applications because of difference in ion release, solubility, stability and mechanical strength. The release of Ca and P ions regulates the activation of osteoblast to facilitates bone regeneration. CaP-NPs as lipid carrier offer an attractive method of treatment against osteosarcoma. Nano structured implants mimic the environment of native bone, stimulate implant Osseo integration and surrounding osteogenesis to a greater degree than conventional implants [11].

c) Gene Silencing

Genetic disorders can also be treated by gene silencing techniques, that is, RNA interference (RNAi), which is based on the specific inhibition of the protein synthesis

ISBN: 979-85-27243-61-1

in a cell [14]. Calcium phosphate (CaP) nanoparticles are promising gene delivery carriers due to their bio-[1]resorbability, ease of preparation, high gene loading efficacy, and endosomal escape properties [12]. Calcium phosphate nanoparticles carries siRNA but has certain drawbacks like physical instability and low transfection inefficiency which can be overcome when combined with materials like PEG and liposome. PEG coated CaP has hydrophilic and neutral features, enhances stability and immunogenicity. Liposome coated CaP shows effective protection of siRNA against serum nucleases, excellent stability against electrolyte induced flocculation, and effective target gene silencing [12].

d) Miscellaneous

Drug delivery- Calcium phosphate nanoparticles are well suited to transport organic or biological molecules into cells and tissue. They are well tolerated by cells and organisms and have a clearly defined degradation pathway, also due to their almost ubiquitous presence in the body. Promising applications for them are seen in drug and gene delivery and also in immunization, for example, for vaccination [13]. As an account of properties like target ability, stability under physiological condition, variable solubility in cells, non-cytotoxicity, etc. Calcium phosphate nanoparticles are used for drug delivery. Example: CaP-NPs bind with ceramide, a waxy lipid which when targeted to tumor cells, induces apoptosis [15].

Transfection- The introduction of foreign plasmid DNA into eukaryotic cells is called transfection. As DNA alone is not able to cross the cell membrane and also prone to enzymatic degradation, a suitable delivery system is required. Different kinds of nanoparticles, dendrimers, and liposomes have been developed for transfection, including calcium phosphate nanoparticles [16]. DNA-loaded calcium phosphate nanoparticles are usually prepared by a precipitation of calcium phosphate from calcium- and phosphate-containing solutions, followed by a rapid colloidal stabilization with polyelectrolytes, including nucleic acids [17].

Tissue engineering- Tissue engineering is a promising tool for regenerative medicine, but the search for suitable scaffolds composed of a bioactive degradable substrate is still a challenge [18]. In general, calcium phosphate nanoparticles are well suited in tissue engineering for hard tissue regeneration (mainly bone) due to their presence in bone, causing their biocompatibility, biodegradability, bioactivity, osteo conductivity, and osteo inductivity. Scaffolds for hard tissue regeneration can be supplemented by bioactive calcium phosphate nanoparticles that are loaded with stimulating biomolecules. For instance, it is possible to stimulate tissue growth by releasing proteins or DNA for transfection, loaded to calcium phosphate nanoparticles [18]. It is also possible to enhance the mechanical properties of organic scaffolds by the mineral calcium phosphate [19]. Accordingly, calcium phosphate has been incorporated into different natural and synthetic polymers to produce nanocomposites with specific mechanical and biomedical properties [20]. Chitosan, collagen, gelatin, polycaprolactone, and poly (lactic acid) are examples of polymers which are widely used as biodegradable matrix for such nanocomposites [21].

V. SCOPE

Nanoparticles have a high surface-to-volume ratio which causes their specific physicochemical, biological, optical, electrical, and catalytic properties [22]. In general, he application of nanoparticles in biology and medicine is a rapidly growing field, for example, the efficient targeted delivery of drugs and biomolecules in vitro and in vivo in cancer therapy and immunology. Besides polymeric nanoparticles and biological nanoparticles, inorganic nanoparticles like iron oxides silica, gold and calcium phosphate have gained high attention due to their mechanical stability and integrity, ease of preparation, tunable size, and versatile surface chemistry [24]. Among inorganic nanoparticles, calcium phosphate nanoparticles have distinct advantages, mainly their high biocompatibility and biodegradability. In bulk form or as coating, calcium phosphate is a well-known biomaterial which in nanoparticulate form has found many applications in vitro and in vivo. Within the organism, calcium phosphate is present in biomineralized hard tissue, usually as nanoplatelets embedded in a softer protein matrix (collagen) [27]. There, it is the mineral component of bones and teeth, usually as calcium-deficient hydroxyapatite with different ionic substitutions. Unlike many artificial nanoparticle materials like iron oxide, polymers, silica or nano diamonds, calcium phosphate is almost ubiquitous in the body due to its presence in bone, teeth, saliva, and blood, leading to a high biocompatibility and an intrinsic non-toxicity [25].

VI. CHALLENGES

Conclusion/ Discussion: Among inorganic materials, calcium phosphate nanoparticles are well suited to transport organic or biological molecules into cells and tissue. They are well tolerated by cells and organisms and have a clearly defined degradation pathway, also due to their almost ubiquitous presence in the body. If they are used as carriers, they are advantageous to encapsulate sensitive cargo like proteins or nucleic acids inside to protect them from enzymatic degradation, something that is impossible with solid nanoparticles. A surface functionalization further enhances their potential for a targeted delivery, for example, to tumors or cells of the immune system. Polymeric or liposomal particles are competing strategies, but it is advantageous that calcium phosphate nanoparticles contain the same components as biomineralized mammalian tissue (unlike synthetic polymers) and that they have a solid core with high mechanical stability (unlike liposomes). Therefore, we expect more widespread applications in different areas of cell biology and medicine in the future as the basic

ISBN: 979-85-27243-61-1

synthetic steps have been explored and several in vivo studies have demonstrated their potential. Promising applications are seen in drug and gene delivery and also in immunization, for example, for vaccination [22].

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ISBN: 979-85-27243-61-1

Bioplastics and Its Properties- A Review

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Abstract: - Plastics are essential for modern life in various ways, but these create environmental problems such as pollution and degradation. To overcome this issue the conventional plastic has been replaced with bioplastic. Starch based organic sources like potato peel, banana peel and other sources of starch have been studied for their potential use in preparation of bioplastic. Starch is a biopolymer consisting of two polymer types of glucose namely amylose and amylopectin. The purpose of starch based bioplastic is to achieve cost effectiveness, biodegradation and holds other advantages like good oxygen barrier in dry state, abundance, etc [1].

The bioplastics have mechanical properties like tensile strength, transparency, heat resistance and elongation at break. Hence, it can be used in the industry for various applications such as molding and packaging. Therefore, bioplastics can be best suited as an alternative to improve healthy life and sustain a pollution-free planet [1].

This review focuses on the effect of plasticizers such as glycerol and sorbitol on the tensile strength of bioplastic. The chemical and morphological characteristics are analyzed using FTIR and SEM and the biodegradation, water absorption capacity of the bioplastic has been studied.

***Keywords*- Starch, Bioplastic, Biodegradability, Tensile strength, Elongation, plasticizers, FTIR, SEM, water absorption capacity.**

I. INTRODUCTION

Plastics are an essential part of modern life. Plastic is a broad name given to different polymers with high molecular weight, the term plastic is commonly used to refer to synthetically created materials that we constantly use in our daily lives [1]. Plastics have been incredibly useful to us in various ways. However, its overuse has been causing our planet many environmental problems such as pollution, degradation, and cancer [2]. Conventional plastics are not just polymers which can be molded or extruded into desired shapes but often contain additives that improve their performance. Some additives include the following:-Antioxidants, Stabilizers, Plasticizers, Blowing agents, Flame retardant, Pigments. Disadvantage of conventional plastics is their non biodegradability [2]. Bioplastics are produced from renewable natural materials and have been observed to have the potential of being an alternative to plastic packaging due to their environmental friendliness and easy degradation [3]. The development of most bioplastics is assumed to reduce fossil fuel usage, and plastic waste, as well as carbon dioxide emissions. The biodegradability characteristics of these plastics create a positive impact in society, and awareness of biodegradable packaging also attracts researchers and industries [4].

Currently, thermoplastics such as polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), and polystyrene (PS) make up a total of 60% of the overall plastic demand in Europe. While these plastics are traditionally petrochemical derived, there is a growing demand for the production of plastics using renewable resources (so called

"bioplastics") as alternatives to their petrochemical derived counterparts). All bioplastics are produced starting from natural resources. However, not all are biodegradable. Bioplastics are a family of plastics that can be divided into two categories, biodegradable and non-biodegradable [5]. Bioplastics are easily degraded by microbes, and the degradation process does not take a long time. Starch is one of the most common raw materials used for bioplastic fabrication in replacing plastic polymers. The bioplastic produced from starch has a high biodegradability (quickly decomposes) in the soil. Further, in agricultural countries such as Indonesia, starch is largely available and relatively [6].

Starch is a biodegradable, bio-based polysaccharide consisting of amylose and amylopectin and is synthesized by most plants via photosynthesis. Starch-based polymers represented 21.3% of global production capacities of bioplastic in 2019 [5]. Approximately 50% of the bioplastics used commercially are prepared from starch. The production of starch-based bioplastics is simple, and they are widely used for packaging applications. The tensile properties of starch are suitable for the production of packing materials, and glycerol is added into the starch as a plasticizer. The required characteristics of the bioplastics are achieved by fine-tuning the quantities of the additives. For trade applications, the starch-based plastics are regularly mixed with eco-friendly polyesters [4].

Effect of plasticizer on properties of bioplastic

Bio plastic from starch based samples reinforced with natural fibers like wool, cotton, hair & jute, were prepared and tested for their mechanical strength [7].

Normally, starch based bioplastics are slightly hard & brittle in nature, they break easily when folded. The brittleness is removed by decreasing the tensile strength of bioplastics [7].

The tensile strength of bioplastics depends on the amount of fiber reinforced in it and the strength increases on increasing the amount of fibers up to a certain limit. Strength of fiber reinforced bio plastics also depends on the distribution of fibers throughout the sample. The tensile strength of bio plastics reinforced with wool fiber showed the maximum average tensile strength of 25.7592 Kg/cm^2 as compared to bioplastics reinforced with cotton, hair and jute fibers. This may be due to proper mixing and uniform distribution of natural fibers within the bioplastic samples. Bio-composites on the base of thermoplastic starch reinforced with natural fibers, both of which are renewable materials, can be a potential alternative for conventional plastics [7].

Bioplastics with other additives in plasticizers and fillers affect the bioplastics final quality on the tensile strength parameter. Glycerol was the most widely used plasticizer because Glycerol has the best interaction ability compared to other plasticizers. However sorbitol in bioplastic production is highly dependent on the characteristics of the starch used. Sorbitol needs to be supported with certain fillers that can increase the tensile strength of the bioplastic produced according to the characteristics of the starch used. Fillers that were commonly used are chitosan, clay and ZnO [8].

The use of chitosan in making bioplastics has concentrations ranging from 2 - 41.7%. This shows that chitosan could fill bioplastics with various types of plasticizers, starch, and different concentration variations. Bioplastics of multiple kinds of starch and plasticizers using chitosan produce tensile strength values that meet bioplastic standards. The chitosan added by ZnO had a tensile strength value of 3.29 MPa. This value was not more significant than the chitosan filler bioplastic without ZnO or vice versa. The result was that the increase in ZnO and clay concentrations causes the stiffness of the plastic produced [8].

A corn starch based film with 25% fructose recording the highest tensile stress (6.8 MPa) which is higher than that recorded with 25% sorbitol (4.52 MPa) and 25% urea (0.62 MPa) counterparts. The expected interpretation of the high tensile stress at low plasticizer content is related to hydrogen bonds, which formed between starch and plasticizer molecules, these bonds in many studies have detected the reduction in tensile strength of starch-based films due to increased concentration of plasticizers [9].

The tensile strength from fructose films was higher than those fabricated plasticized films by mixing cassava starch with 30% fructose and achieved 4.7 MPa. Similarly, cassava starch with 30% urea and obtained 0.68 MPa. In general, the tensile strength values for CS-fructose-plasticized films were higher than the cornstarch films plasticized by glycerol, cornstarch with stearic acid and glycerol, and cornstarch with xylitol and glycerol. The F-plasticized films particularly, 25% fructose film offered the best combination to be used for the application and development of biopolymer films [9].

Characteristics of the bioplastic

SEM examination demonstrated the microbial activity of degradation on the bioplastic samples. The surface structure of the material had lost its evenness, and flaws were evident. The sample exhibited a substantial variation in the structure and

got broken when touched. Therefore, from the weight loss method and the SEM analysis, it could be concluded that the bioplastics prepared from starch are biodegradable [4].

Jackfruit seed starch plasticized with glycerol were developed and characterized. Morphology and structure were analyzed only for bioplastics made with 2 and 3% starch with 30 and 60% glycerol. SEM images were produced with magnification of 8000 times.SEM images shows that the film surfaces exposed to air are rough with some grooves. The micrographs show some intact starch granules, which means that the starch was not fully gelatinized during the film forming process [13].

The chemical characterization of the bioplastic produced can be analyzed by using Fourier Transform Infrared Spectroscopy (FTIR). According to Fatimah et al., (2017), C-O, O-H, C-H, OH, C=O, C=C and =C-H will be found by using spectrum 100 Perkin Elmer FTIR with the spectra were taken in 256 scans between 4000 and 400 cm^{-1}. The C=C and =C-H were only found in the sample of bioplastic with 4 % of corn starch. There is no presence of OH (deflection of water) in any other sample of bioplastic [15].

Biodegradable plastic from the rice waste and chitosan with glycerol as plasticizer, and acetic acid as a catalyst, has a functional cluster that is a combination of specific functional groups that have constituent components such as CH, OH, NH, C≡C, C = O, and C=C, and also have amide and ester functional groups in biodegradable plastic film samples, so that plastic from the rice waste can be degraded and can be said to be green-plastic [16].

Bioplastics containing glycerol absorb moisture over time which is likely due to the hydrophilic nature of glycerol. The bioplastic sample containing 2mL of glycerol shows maximum gain in the absorption content of 63.15% in the same interval of time. This could render this plastic unsuitable for most applications as the resulting water absorption would alter the properties of the plastic, reducing its tensile strength. However, the trend line indicates that with addition of glycerol, moisture absorption capacity of bioplastic decreases. It demonstrated the maximum moisture absorption capacity among all the samples which makes it unsuitable for many applications. As for the aspect of moisture content, it would be beneficial to examine the possible uses of other materials that would limit the amount of moisture content change that would occur with starch based plastics as it has been demonstrated that this area has a significant effect on the overall properties of the plastic.[14]

When comparing the amounts of plasticizer used in fabricating bioplastics from starch, it was found that the sample containing 2mL glycerol provided the best mechanical properties like low density and high tensile strength. However, it demonstrated the maximum moisture absorption capacity among all the samples which makes it unsuitable for many applications. One area of interest that requires further research is the continued modification of the starch based bioplastics by altering the percentages of each component used as well as using different materials to make bioplastics in order to reach the optimum properties for other applications.

Analysis shows that the maximum equilibrium moisture content for Chitosan materials at low relative humidity (43 %) was 7.64 % at 10 ^{0}C while it was a low of 3.47 % at 50 ^{0}C. As relative humidity rises, the EMC reached a high of 19.44 % at 10 ^{0}C and a low of 13.19 % at 50^{0}C. It is also noticed that changing the relative humidity from 43 to 95% leads to an increase of 11.80 % in the moisture content of the material at 10 C temperatures. On the other hand, increasing the temperature from 10-50 ^{0}C caused a decrease of 4.17 % in the equilibrium moisture content of Chitosan material, while at the higher temperatures and relative humidity (50 ^{0}C and 95 %), increasing the relative humidity from 43 to 95% at 50 ^{0}C caused an increase of 9.72 %, whereas it was 6.25 % when the temperature increased from 10-50^{0}C at 95 % relative humidity.

The results revealed that the equilibrium moisture content of all materials under study increased with increasing the relative humidity but it decreased with increasing the temperature. The temperature and relative humidity play an important role in the microorganism activity which can attach and degrade the bio materials.

Biodegradation of bioplastics

Bioplastics are biodegradable as they are made from renewable sources. Renewable sources are mainly made up of starch components such as potato starch, corn starch, cassava, banana stems, jute, jackfruit seeds etc [17]. Employments of new techniques for manufacturing of bioplastics that promote sustainable solutions and reduce the plastic waste have been greatly encouraged in recent years [18]. Production of bioplastics involves the use of starch as an organic source. All green plants produce starch which is white in color, granular, organic chemical, soft, tasteless powder, and insoluble in cold water and other solvents. It is polysaccharide consisting of amylose and amylopectin. The biodegradation tendency is increased in the bioplastics because the amylose molecules

lose water and the plasticizer property increases due to the presence of the amylopectin molecules. Bioplastics produced from different components have shown a change in biodegradability property. A plastic can be considered biodegradable if a significant change in the chemical structure i.e., degradation, occurs in the exposed material resulting in carbon dioxide, water, inorganic compounds and biomass [19]. The biodegradability seen in bioplastics made from different components like polysaccharides, lipids, or products of microorganisms through aerobic and anaerobic digestion processes. Poly(hydroxyalkanoate)s (PHA)s which are class of polyesters are involved in anaerobic degradation of bioplastics and are able to degrade to carbon dioxide and water in 5-6 weeks and show 85% of degradation within 20 days. These can be used directly as bioplastic without any modification. Another component used for making bioplastic is starch blends which have starch content varying from 5-90wt%. These show 90% of degradability within 30 days. Bioplastics are also produced using pectin-cellulose biofilms which are produced from orange waste through a solution casting method [20]. Due to the presence of pectin, these biofilms show 90% of degradability within 15 days and with an average production of methane over 300NmL/g COD [18].

Another abundant natural polymer called chitin prepared by deacetylation process in presence of alkali is used for making biofilms. Chitin/Chitosan along with glycerol and plasticizer show 82.38% biodegradability for 28 days [10]. This acts as a transparent film which improves the quality as well as storage life of products. The most attractive material from a packaging point of view is Polylactic acid [PLA] which has excellent biodegradability and biocompatibility [11]. The degradation of PLA in the environment is not easy because, under ambient conditions, PLA in soil or in sewage is resistant to microbial attacks. Hence PLA is first hydrolyzed at a elevated temperature [approx of 58˚C] to reduce molecular weight before biodegradation process. The degradation process is considered as a 100 day long process where the small pieces of PLA have faster degrading rate when compared to large pieces because the total surface area of the plastic has less contact with the soil. The smaller pieces have a degradability rate of 60% over 40 days, the rate of degradation varies depending on the source used for carrying out biodegradability [12].

Thus biodegradability is an important property required for production of a bioplastic which provides the biodegradation of bioplastics in various environments, environmental conditions and degree of biodegradation.[10]

II. CONCLUSION

From this study, it can be concluded that the synthesis of bio-plastic by using starch instead of conventional plastic often degrades more quickly and it does not leach out toxic chemicals. The chemical, mechanical, and thermal properties of the products were analyzed were it explains the variations in the concentration of sorbitol and glycerol plasticizers which affect the mechanical properties of bioplastics namely tensile strength and percent elongation[22]. The equilibrium moisture content of all materials under study increased with increasing the relative humidity but it decreased with increasing the temperature. The temperature and relative humidity play an important role in the microorganism activity which can attach and degrade the biomaterials [21]. The tensile strength of the bioplastic decreases with the increase in percentage of elongation. Study also showed that different types of starch and ratio of bioplastic will affect the water absorption and tensile strength properties [9]. The FTIR and SEM analysis conclude that the bioplastics from starch are biodegradable [4]. Thus bio-based plastics have exhibited good thermal and mechanical properties with high biodegradability that makes alternative ways to reduce synthetic plastic and create an eco-friendly environment.

Hence, the research and development in the field of bioplastics is much needed and should be encouraged [11].

ISBN: 979-85-27243-61-1

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Folk Knowledge on Medicinal Plants of Kodagu and Dhakshina Kannada districts of Karnataka

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Abstract: India is known for its rich flora and fauna, Western Ghats being one of the eight major hot spots for biodiversity by UNESCO for its densely covered forest areas, consists of large variety of plants especially ethnobotanical importance plants with high medicinal value. The factors are adequate rainfall, soil type and temperature make rich diversified plant species which have paved high importance in local traditional medicine and Ayurveda. Throughout ages traditional healers have practised medicine from one generation to another without being documented. These ethnic medicines are prepared from all parts of the plant such as the roots, bark, flower, leaf, stem, seeds etc. These plant parts are converted into tradition medicines such as Kashaya, lehya or powder form. Basic plant information was collected from traditional medicine practitioners through questionnaire for local plant names and their medicinal uses. This paper summarizes the scientific names and their applications of these ethnobotanical plants in modern day world.

Keywords: Traditional medicine, ethnobotanical plants, Ayurveda, Western Ghats.

I. INTRODUCTION

Medicinal Plants are major source for folklore medicine. From ages people are using the traditional Naaty vaidya practices. Folk medicine is not documented but, knowledge runs in family from one generation to next. National medicinal board estimate suggests that about 6000-7000 plant species are used among folk and documented system of ayurveda, Sidda, Unani & Homeopathy [1]. India is rich in biodiversity. One of the 8 major hot spot considered by UNESCO is Western Ghats in world. It is known for its richness and endemism of different species. Western Ghats hill ranges run parallel to west coast of India from 8ºN to 21ºN latitude, 73ºE to 77ºE longitude stretching along 1600km. This multifariousness region is considered as threat among 18 biodiversity hot spot of the world. Diversified climate factors such as annual rainfall, temperature, soil etc across ghats have contributed to heterogeneous biodiversity [2][3][4][5][6].

Dhakshin Kannada is a coastal district of Karnataka which is surrounded by Western Ghats at east and coastal region at west. It has area of 4,866sqkm, latitude and longitude at 12.6ºN 7.3ºE altitude of 69M. District can be divided into three belts-the coastal strip, middle belt and the western ghat section. The western ghat forms the eastern boundary of the district consisting of evergreen Shola forest. Climate of the district shares wider climatic pattern–coastal and Malnad region. Topography of district varies from plain to undulated terrains. District covers large track of tropical evergreen forest area of 12847 hectares.

Coorg or Kodagu situated southern western part of Karnataka in the malnad region. These regions are located on eastern slopes of Western Ghats. This hilly region lies at latitude longitude 12.4208ºN 75.7397ºE covering 4102sqkm, at elevation 3000ft above sea level and receiving annual rainfall of 3000-4000mm.Western ghats comprising of bhramagiri, talacauvery and pushpagiri wildlife sanchuries are known for their flora and fauna. The dense Shola forest, lush green valley are heart trove of medicinal plants [7][8]. Kodagu has

approximately 65%of geographical area under tree cover, making it one of the most densely forested districts in country.

II. METHODOLOGY

Traditional knowledge of medicinal plants and their uses in treating various diseases was collected through interviews with people involved in practicing traditional medical practitioners in Kannada they are called as Naati vaidyas. The two districts Coorg or Kodagu and Dakshina Kannada were surveyed for collecting traditional knowledge on medicinal plants. Information is collected by using the questionnaire covered about plants used for various disease and parts used. Sanskrit names identified from various literature [2][3][4][5]. The important plants and their uses in treating particular aliments are listed in Table [1].

III. APPLICATIONS OF SAME PLANT EXAMPLES

A. Strychnous nux-vomica

Seeds powder accelerated the neuronal function regeneration of mouse induced with nerve injury [9].

Tree fruit shell showed biosorption towards Cr(VI) from simulated aqueous solution [10].

Clinical studies showed effective treatment against sinusitis, insomnia and rhinitis [11].

Biomolecule-capped silver nanoparticles inhibit larval activity of potential Dengue, Chikungunya and Zika virus vectors [12].

B. Moringa oleifera

Silver nanoparticles synthesized from the leaf extracts shows antimicrobial potential [13].

Consumption of leaf powder showed decrease in blood glucose levels [14].

Promising prevention and treatment of hyperglycemia and hyperlipidemia [15].

Biosynthesis of Gold phytonanoparticles shows anticancer activity [16].

C. Nyctomthes arbos-tristis

Betulinic acid extracted shows anti-inflammatory, anticancer and antioxidant properties [17].

Presence of active compounds responsible for anti-diabetic and anti-hyperlipidemic activity [18].

Ethanolic extract of leaves of shows anti-inflammatory and anti-arthritic activity [19].

Biosynthesis of Zinc oxide by leaf extracts shows anticolon cancer ability [20].

D. Cynodon dactylon

Methanolic extract of rhizomes shows antibacterial activity and source antibiotics for treating bacterial infections [21].

Overexpression of cold-responsive ethylene responsive factor transcription factor (CdERF1) expressed in transgenic plants improved cold tolerance [22].

Ethanolic extract of shows antiviral activity for the prevention of White spot syndrome virus infections shrimp cultivation [23].

Hydoalcoholic extract showed neuroprotective activity by reducing the radiation-induced oxidative stress and cognitive dysfunction in mice [24].

E. Cassia fistula

Preparing seeds for production of activated carbon for removal of Ni(II) ion contaminants from aqueous solution [25].

Extracted natural dye from flower is used as photosensitizer to fabricate dye-sensitized solar cell [26].

Methanolic leaf extract showed antileishmanial potential [27].

Isolated rhein from flowers shows anti-inflammatory activity [28].

IV. RESULTS

Various plants available in both districts which are grown wild or cultivated about seventy-five plants mentioned above are used for treating various medicines. Traditional vaidhyas prepare medicine themselves through various methods given in the form of kashaya or leha with some mantras which is said to be important in curing the disease. The medicine may be in the form of powder or may be kashaya. The powder or kashaya is prepared from combination two or more plants/ their parts. The plant parts used varies from root, stem, bark, leaf, fruits and seeds etc.

Table1: List of medicinal Plants and their uses

Sl No	Local name	Sanskrit name	Scientific name	Family name	Uses
1	Kasaraka	Visha tindu/Tinduka	*Strychnous nux-vomica*	Loganiaceae	Leaves:Abscess,wounds Roots: after purification used as nervine tonic.
2	Nugge	Surajana	*Moringa oleifera*	Moringaceae	Leaves:Abscess,Wound Root:Snake bite,mild head ache, Barck:Sinusitis
3	Paarijaatha	Kharapatraka	*Nyctomthes arbos-tristis*	Oleaceae	Flowers: Periorbital puffiness (eye swelling)
4	Garikehullu	Druva	*Cynodon dactylon*	Poaceae	Leaves: eye swelling and snake bite,menorragia,gastritis,piles,antidandruff,herpies
5	Kakke	Rajavrksha	*Cassia fistula*	Caesalpinaceae	Leaves:Eye swelling
6	Kirathe/Nelabevu	Bhunimba	*Andrographis paniculata*	Acanthaceae	Leaves:Jaundice,viral infection,fever,hepatoprotective,Herpis,antidiabetic
7	Nirulli	Sukandara	*Allium cepa*	Amaryllidaceae	Bulb:Blood Pressure
8	Bellulli	Sitalazuana	*Allium sativum*	Amaryllidaceae	Bulb:Vermicidal
9	Karibevu	Alakavhayah	*Murraya koenigii*	Rutaceae	Leaves: Urinary infection, hair tonic
10	Bile dasavala	Ambasthaki	*Hibiscus sabdariffa*	Malvaceae	Flower & Leaves:Leucorrhoea
11	Panchavalli	Tamboolavalli	*Piper betle*	Piperaceae	Felon finger
12	Garudapathala beru	Sarpaghandha	*Rauvolfia serpentina*	Apocynaceae	Root:Snake bite, herpis ,Hypertension,Insomia Dementia
13	Sogade	Sariva	*Hemidesmus indicus*	Asclepidaceae	Root:Snake bite Blood purifier,gastritis,urinary infection
14	Nandibatalu	Nandeevriksha	*Tabernaemontana coronaria*	Apocynaceae	Root:Snake bite
15	Neerubrahmi	Indri	*Bacopa monnieri*	Scrophulariaceae	Memory tonic
16	Nithyapushpa	Shankhpushpi	*Convolvulus pluricalis*	Convlvulaceae	Used for Dementia
17	Kariganne	Jyotishmati	*Celastrus paniculatus*	Celastraceae	Used for Dementia
18	Budekumbalakaui	Kushmanda	*Benincasa hispida*	Cucurbitaceae	Used for Dementia
19	Baje	Vacha	*Acorus calamus*	Acoraceae	Used for Dementia
20	Jatamansi	Jatamansi	*Nardostachys jatamansi*	Valerianaceae	Used for Dementia
21	Nila Nelli	Bhumyamalki	*Phyllanthus amarus*	Phyllanthaceae	Leaves:Jaundice Roots& full plant: Renal Calculi,Constipation
22	Agnisikhe/ Gaurigedde	Langali	*Gloriosa superbha*	Colchicaceae	Tubers:Analgesic & abortive
23	Madarangi	Madayanthika	*Lawsonia inermis*	Lythraceae	Leaves:Hepatoprotective & hair tonic

ISBN: 979-85-27243-61-1

24	Garagadasappu	Bhringaraj	*Eclipta alba*	Asteraceae	Hepatoprotective,carminative,,memory improvement,Iron rich,Hair tonic
25	Madalahannu/ Madiphala	Matulunga	*Citrus medica*	Rutaceae	Gastritis. Hyperemesis gravidarum
26	Anne chagathe	Dadrughna	*Cassia alata*	Fabaceae	Skin problems,vitiligo,post pregnengy healing tonic
27	Nelatangedi	Markandika	*Cassia angustifolia*	Fabaceae	Skin problems
28	Sanna tagache	Cakramarda	*Cassia tora*	Fabaceae	Skin problems
29	Chillu	Kataka	*Strychnous potatorum*	Loganiaceae	Water purifier
30	Punarpuli	Vrikshamia	*Garcinia indica*	Clusiaceae	Fat Reduction
31	Kachampuli/Panpuli	Tintidika	*Garcinia Morella*	Clusiaceae	Fat Reduction
32	Adumuttada soppu	Adusa	*Justicia adhatoda*	Acanthaceae	Haemostatic,asthama,Sputum,Vermicidal,Sinustis
33	Utharanee	Apamarga	*Achyranthes aspera*	Amaranthaceae	Leaves:Menorrhagia Root:Painless pregnancy
34	Muthuga	Kimsuka	*Butea monosperma*	Fabaceae	Leaves:Carbinative, Gastritis Seed:Vermicidal
35	Arreballi	Manjista	*Rubia cordifolia*	Rubiaceae	Fever,Vascular disorder,Blood purifier
36	Kempu Honne	Pitasara	*Pterocarpus marsupium*	Fabaceae	Bloosd purifier,Cosmetic
37	Datura	Datura	*Datura metel*	Solanaceae	Seed(Purifried):Nervine tonic,broncho dialater
38	Muttidare muni	Lajjalu	*Mimosa pudica*	Fabaceae	Piles
39	Honagone	Matsyaki	*Stirfried alternanthera*	Amarentheceae	Retinitis pigmentosa,Antioxidant,Iron rich
40	Madd toppu	SSveta-sahacarah	*Justicia wynaadensis*	Acanthaceae	Urinary tract infection
41	Nasagunni	Kapikachhu	*Mucuna pruriens*	Fabaceae	Deminentia.
42	Anipe balli	Dadhipushpi	*Mucuna monosperma*	Fabaceae	Seed:Oligospermia
43	Unmatthi	Mandara	*Datura stramonium*	Solonaceae	Mumps
44	Yekkada gida	Arka	*Calotrapis gigantea*	Asclepidaceae	Latex:Mumps Roots:Snake bite
45	Bevu	Nimba	*Azadirachta indica*	Meliaceae	Skin problems,vermicidal,antidandruff
46	Thulsi	Shri thulsi	*Ociumum tenuiflorum*	Lamiaceae	Antidandruff
47	Benga	Bijaka	*Pterocarpus marsupium*	Fabaceae	Antidiabetic
48	Dodda pathre	Pasanabedhi	*Coleus amboinicus*	Lamiaceae	Renal calculi, Urinary tract infection
49	Sannagerasehambu	Madhunasini	*Gymnema sylvestre*	Asclepidaceace	Antidandruff
50	Ondelaga	Mandukapami	*Centella asiatica*	Mackinlayaceae	Memory tonic
51	Tumbe	Dronapushpi	*Leucas aspera*	Lamiaceae	Leaves: Sinusitis
52	Ashoka	Sita- Ashoka	*Saraca indica*	Fabaceae	Menstrual problems
53	Neralle	Nilaphata	*Syzygium Jambolanum*	Myrtaceae	Antidandruff Seeds:Urinary infection
54	Hulli Kabbu	Pushkarmula	*Chelilocostus speciosus*	Costaceae	Leaves:Conjunctivitis

55	Kallu Menasu	Vellaja	*Piper nigrum*	Piperaceae	Sputum
56	Hippali	Magadhi	*Piper longum*	Piperaceae	Sputum
57	Koyamaruva/Nirmatti	Dhananjaya	*Terminalia arjuna*	Combretaceae	Heart tonic
58	Nekki /Lakki	Sindhuvara	*Vitex negundo*	Lamiaceae	Antimalarial
59	Vishambari	Duranta	*Duranta erecta*	Verbenaceae	Antimalarial
60	Sithaphala	Sudha	*Annona reticulata*	Annonaceae	Antidandruff
61	Bilva patra	Vilvah	*Aegle marmelos*	Rutaceae	Antidiabetic,Antidandruff
62	Arishina	Haridra	*Curcuma longa*	Zingiberaceae	Tuber: heals burnt,Wounds
63	Ashwatha	Bhodi	*Ficus religiosa*	Moraceae	Inner barck:Mumps
64	Honge	Karanja	*Pongamia pinnata*	Fabaceae	Bark: cancer treatment
65	Neggina mullu	Amrita	*Tinospora cordifolia*	Menispermaceae	Throns: anticancerous
66	Isvaberu	Eesvari	*Aristolochia indica*	Aristolochiocea e	Roots:Snake bite,skin disease
67	Vishaparihari	Kundali	*Cleodendron inerme*	Verbenaceae	Leaves:Snake bite
68	Batla hoo	Nityakalyani	*Catharanthus roseus*	Apocynaceae	Antidiabetic
69	Kaddu honaganne soppu	Rakthapamarga	*Cyathula prostrata*	Amaranthaceae	Body detoxifier
70	Hagala kayi	Karavella	*Momordica charantia*	Cucurbitaceae	Fruits & Leaves :Urinary infection,Antidiabetic
71	Koligottu	Nagakeshara	*Heterophragma quadriloculare*	Bigoniaceae	Roots: Snake poison
72	Lolisara	Grita -Kumari	*Aloe vera*	Xanthorrhoeaceae	Antidiabetics

V. DISCUSSION

India is known to have diverse climatic conditions. These climatic conditions are congenial for growing variety of plants. Many plants are known to have medicinal values. India has long history when it comes to medicine. The Indian traditional medicinal system is known as ayurveda. Ayurvedic system of medicine involves use of different natural sources such as plants and its parts. Ayurveda has medicine for most of the ailments of the humans as well as animals. In India, many local people practice ayurveda from ages. Though Ayurveda has answer for many diseases but the scientific evidence for the treatment effects is not elucidated and recorded. Present study was aimed at collecting the information from local medicinal practitioners in Kodagu and daksina Kannada districts of Karnataka about the medicinal plants used for different preparations of ayurvedic medicine and for particular disease.

Among 72 medicinal plants listed in the table belongs to 41 different families. Of which 7 family belong to monocot. Rest 64 medicinal plants belong to 35 families are dicots. All these medicinal plants are available in local area, usually collected from forest of surrounding environment. Also, the parts of plants used in traditional medicine includes from leaves, flowers, roots, fruits, stem, bark etc. The type of plants includes climbers, shrubs, herbs, bulbs, trees, vines etc. Traditional medicinal practices have positive impact on health with no side effects. Majority of people follow this, especially in village

areas. The information collected from Naati viadyas shows that medicinal plants are being used to treat small wound to serious illnesses such as cancer, diabetes, dementia, digestive disorders etc. The information collected also infers that medicinal plants can be used to treat external application for example for treatment of skin diseases (Strychnous potatorum, Cassia angustifolia and Cassia tora), to get rid of dandruff from head. Also, medicinal plants are also used as memory tonic to enhance memory (Centella asiatica). The evidences also found that medicinal plants can be used to reduce body fat and purify blood (Garcina indica and Garcina Morella;Pterocarpus marsupium).The information collected from local Naati vaidyas infers that there is wealth of medicines available in the nature to cure various ailments from less dangers fever to deadliest diseases such as cancer. It is up to us to discover the right medicine for particular disease and standardize the dosage for the maximum benefit.

CONCLUSION

Indian traditional plants have been intensively used in Ayurveda and Traditional medicines for treating common flues to complicated disorders which can be fatal. The preparation methodologies of folk medicine have been passed on the next generations as family secrets. These medicines are extensively used in native parts of the country without the knowledge of their mechanism of action. The medicinal plants have massive potential which have modern day applications from treating disorders of humans and animals to various other environmental application. It is important to spread awareness against Deforestation to preserve these endangered beneficial plants and their natural habitats. This paper contributes towards the knowledge towards the applications of these ethno-medicinal plants. The information gathered helps in further scientific evaluation of the medicinal value of these plants.

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EVALUATION OF PHYTOCHEMICAL ANALYSIS AND ANTI BACTERIAL ACTIVITY OF *MADHUCA LONGIFOLIA* FLOWER EXTRACT

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Abstract

Madhucalongifolia is economically necessary medicative tree growing throughout the subtropic region. The present work deals with the phytochemical analysis and antibacterial activity of the of *Madhucalongifolia*flower extract. The phytochemical analysis of extract of the dried flowers confirmed the presence of quinones, phenols, sterols. The antibacterial activity was evaluated against *E.Coli, P. aeruginosa and B. subtilis* by agar well diffusion technique, *P. aeruginosa* exhibit high inhibitory effect compared to other organism.

Keywords: Antimicrobial activity,*Madhucalongifolia*, Phytochemical

1. Introduction

Plants are the chief supply of natural product that qualify them as articles of medications and remedial specialists. The presence of bioactive compounds are responsible for medicinal properties, hence play a big role in maintaining human health and up the standard of human life for thousands of*years(Khond et.al,2003).Madhucalongifolia* is Indian tropical tree found for the most part within the central and north Indian plains and forests. It's a invasive tree that grows to just about twenty meters tall, possesses evergreen or semi-evergreen foliage, and belongs to the dicot family(Chirantan et al., 2011).It'ss convertible to arid environments, being a distinguished tree in tropical mixed deciduous forests in Asian country within the states of Odisha, Chhattisgarh, Jharkhand, state, Bihar, geographical area, Telangana, Kerala, Gujarat, state, Madhya Pradesh, and TamilNadu(Fig1.1)(Jyoti et al., 2017).Many components of the tree, as well as the bark, used for medicative properties(Alok Mishra et al., 2015).

The therapeutic properties credited to this plant are astringent, emollient and demulcent (Awashathi and Mitra, 1967). The flowers have been generally utilized as cooling specialist, tonic, demulcent and astringent for the therapy of helminthes, pharyngitis, chronic tonsillitis(Chandra, 2001). *Madhucalongifolia* leaves are expectorant and furthermore utilized for ongoing Cushing's sickness, dipsia, bronchitis, verminosis, gastropathy, bronchitis, cephalgia and hemorrhoids. The seeds fat has emuluscent property, utilized in skin illness, ailment, migraine, diuretic, heaps and in some cases as galactogogue. The bark is utilized for ailment, ongoing bronchitis, diabetes mellitus, decoction for stiffness, draining and springy gums. It is a decent solution for tingle, expanding, cracks and snake- bite poisoning, inside utilized in diabetes mellitus, natural products are astringent and generally utilized as a salve in ongoing ulcer, in intense and persistent tonsillitis and pharyngitis(Sunita and Sarojini 2003).Hence phytochemical screening of the plants is essentially a significant perspective in tracking down the chemical constituents in plant materials. Thus, the current examination is done to discover the phytochemicals present and antibacterial activity in the flower extract of *Madhucalongifolia.*


Fig 1.1: Madhucalongifolia tree

Fig 1.2: Madhucalongifolia flowers.

2. Materials and methods

2.1 Collection of Plant Material

Flowers of *Madhucalongifolia* were collected for the experimental purpose from Art of Living International Center, Bengaluru. The collected flowers of *Madhucalongifolia* was washed thoroughly with distilled water to remove impurities and shade dried at the room temperature for 7-10 days. Dried flowers was grounded into powdered using grinder and stored in the refrigerator for further uses (Khareet.al, 2018).

2.2 Aqueous extraction of *Madhucalongifolia* flower

The powdered flower material was extracted with aqueous solution by taking flower extract and water in ratio 1:4(K N Akshatha, 2019). The mixture was boiled for about 30 minutes at boiling point, followed by filtration using Whatman No. 1 filter paper. The filtrate obtained were poured in petriplates and kept in hot air oven for drying. The crystallized powder obtained was used for future analysis (Ahmad I , Beg AJ, 2017).

2.3Qualitative analysis of phytoconstituents of *M.longifolia* extract

Qualitative analysis helps in identification of the secondary metabolites present in the flower extract of *Madhucalongifolia.* The1% aqueous flower extract was prepared to carry out for identification of metabolites present by standard protocol method (Ashnagara A , AlirezaGhannadi, 2017).

- **Test for Tannins:** The appearance of blue or greenish black colour after adding 0.5% of 2ml $FeCl_3$ to 1ml of flower extract indicates the positive result for tannins.
- **Test for Carbohydrates:** The formation of the purple colour ring after addition of Conc. H_2SO_4 to a mixture of 2ml of flower extract and 1ml of Molish's reagent shows the existence of carbohydrates.
- **Test for Saponins:** The formation of froth after 15min vigorous shaking of a mixture of 2ml of flower extract and 2ml of distilled H_2O indicates the existence of saponins and the presence of same is confirmed by formation of an emulsion with olive oil after vigorous mixing of sample.
- **Test for Flavonoids**: The flavonoid was confirmed by the yellow colour appearance after addition of 1ml of 2N NaOH to 2ml of flower extract.

ISBN: 979-85-27243-61-1

- **Test for Alkaloids**: The presence of alkaloids was confirmed by the appearance of green colour or white precipitate after adding Mayer's reagent to the mixture of 2ml of flower extract and 2ml of Conc. HCl.
- **Test for Anthraquinone:** The anthraquinone was confirmed by the formation of pink precipitate after adding a few drops of 10% ammonia solution to 1ml of flower extract.
- **Test for Anthocyanosides**: The pale pink colour appears after adding 5ml of dilute HCl to 1ml of flower extract which shows anthocyanosidespresences.
- **Test for Protein**: The development of a purple colour after adding 2-3 drops of ninhydrin solution to flower extract indicates the presence of protein.

2 Antibacterial activity of flower extract

2.4.1 Collection of Test Organisms

Test microorganisms including Gram Positive *B. subtilis* and Gram Negative *E.coli,* Pseudomonas aeruginosa were used for the study. These strains were procured from department of Microbiology, Sapthagiri Medical College, Banglore. The bacterial strains were subcultured and maintained in refrigerator.

2.4.2 Preparation of Mueller Hinton nutrient agar medium

Mueller Hinton agar media was prepared by dissolving 9.5 grams of Himedia in 250ml of distilled water. The components were gently heated to dissolve completely. The media was sterilized in an autoclave at 121^0C for 15 minutes.(Ayyanar et al., 2014).

2.4.3 Well Diffusion method

The antibacterial activity of plant extract was assessed as per agar well diffusion method (Rao AV,Gurfinkel DM, 2013). The autoclaved Mueller Hinton agar was poured in petriplates and allowed for solidification in laminar air flow. Later the solidified media plates are inoculated by 0.1ml of bacterial subculture by spreader and allowed to stand for 5 minutes.

Four wells equidistant to each other were created using cork borer and 100µl different concentration of flower extract was loaded in well. Streptomycin was used as positive control. After loading, the plates were incubated for 24hr at 37° C, in order to record the zone of inhibition (Bandow JE, 2003).

3. Results and discussions

3.1**Phytochemical screening**

Phytochemicals are chemical compounds naturally gift within the plants attributing to

positive or negative health effects. Medicative plants employed in totally different diseases and ailments are the richest bio reservoirs of varied phytochemicals. The medicative properties of the plants are determined by the phytochemical constituents. The preliminary phytochemical screening result's shown in (Table 1), that represents the presence or absence secondary metabolites are in extract of the flowers.

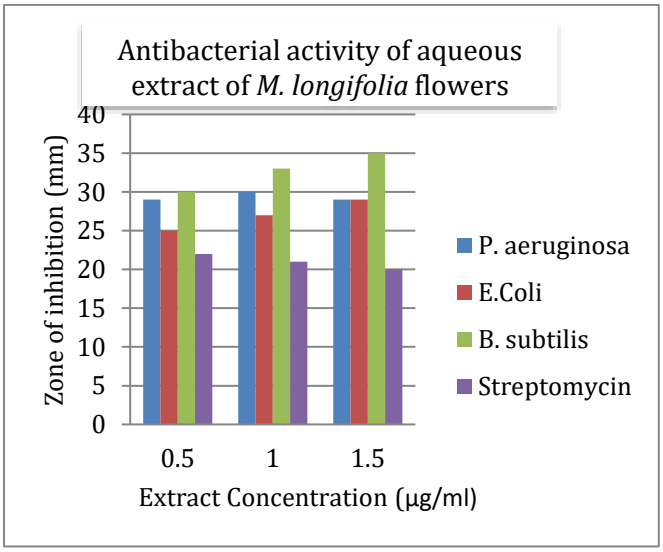
Table 1: Results of phytochemical analysis of *MadhucaLongifolia* flowers

Phytoconstituents	Tests	Results
Tannins	Braymer's test	+
Carbohydrates	Molish's test	+
Saponins	Foam test	_
Flavanoids	Lead Acetate test	+
Alkaloids	Mayer's test	+
Anthraquinone	Born Trager's test	_
Anthocyanosides	HCl test	_
Proteins	Ninhydrin test	_

3.2 **Antibacterial activity of flower extract**

Antibacterial efficacy of aqueous extracts of *M.longifolia* flowers were studied against bacterial pathogens. The zone formation takes place in agar well diffusion method determines if a particular bacteria is susceptible or resistant to the extract and applied antibiotic. If an antibiotic stops the bacteria from growing, one can see circular areas around the wafers where bacteria have not grown. If the observed zone of inhibition is greater than or equal to the size of the standard, the microorganism is considered to be sensitive to the antibiotic. Conversely, if the observed zone of inhibition is smaller than the standard size, the micro organism is considered to be resistant. The results revealed that, aqueous extract showed inhibitory effect against all the bacterial pathogens tested. Among that, *P. aeruginosa* and *B. subtilis* were found to be more susceptible to aqueous extract followed by *E.coli* (Table 2.).

Table 2: Antibacterial activity of aqueous extract of *M. longifolia* flowers



4. CONCLUSION

Medicinal plants are very important in our daily life as these are used for treatment of many diseases. *Madhucalongifolia* is highly regarded as a universal remedy in the ayurvedic medicine. The dried flowers are considered rich in various phytochemicals and secondary metabolites. The present study revealed the presence of bioactive compounds through phytochemical analysis that could be exploited for further human welfare(Table 1). The study also demonstrated that the aqueous extract of dried Madhuca flowers had antibacterial activities against significant bacterial isolates. This antibacterial activity is mainly due to the vital secondary metabolites in the flowers. This property can be further utilized for preparation of antimicrobial drugs. The findings of this study could provide an impetus for further research on active constituents of this plant extracts. Its antimicrobial property can be further exploited which could prove to be highly effective and potential against rapidly emerging microbial pathogens. Also further research needs to be carried out on flower extract in order to study the mode of action of action of the bioactive compounds individually and in combination.

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NATURAL ANTI-VIRAL COMPOUNDS AGAINST RE-EMERGED VIRUS: NOVEL CORONA VIRUS

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Abstract

Nature is the best medicine for every problem. This sentence holds a great value and we can experience it in everyday life. In the same way if we think whether there is any solution in the nature for the ongoing Coronavirus pandemic in the world, we can find a numerous natural products which are boon for fighting against the coronavirus. Natural products with good antiviral and nutraceutical properties can provide relief from viral infections. When a pathogen or a foreign particle enters into the host body the immune system recognizes the pathogen and fight against it leading to its destruction. In the same way there are many antiviral compounds which are found in many of the natural plants which can encourage the immune response towards the invading virus. World health organization reported that 80% of the population depends on plants to meet their basic health requirements as plants possess bioactive compounds among which phytochemicals are the large group synthesized. Phytochemicals are reported to exhibit antiviral and antioxidant activities. The mechanism involved in antiviral activity of phytochemicals is that it inhibits the synthesis of RNA or obstructs the reproduction of virus by blocking cellular receptors and enzymatic function. This work aims to analyze the mechanism of immune response against invaded pathogens and to survey the role of natural compounds in immune response which are obtained from the natural products.

Keywords: Coronavirus, anti-viral, immunomodulatory, Phytochemicals, mechanism.

Introduction

Viruses are responsible for a number of human pathogeneses which includes cancer.Several Hard to cure disease and complex syndrome disease like Alzheimer's disease, type1 diabetes and hepatocellular carcinoma have been associated with viral infections [1]. This epidemics outbreaks caused by emerging and re-emerging viruses represent a critical threats to human health, particularly when preventive vaccines and Anti-viral therapies are unavailable.However so many viruses remain without effective immunization only few anti-viral drugs get license for a clinical practice[2]. Hence there is an urgent obligation to discover novel Anti-viral that are highly efficacious and cost effective for the management. Herbal medicine and purified natural products provides a rich source for novel antiviral drug development. Identification of the antiviral mechanism from these natural agents has enlightened on where they interact with the viral life cycle such as viral entry, replication, assembly and releases as well as on the targeting virus-host-specific interaction. In this we summarize the anti-viralactivities from several natural products and herbal medicine against some notable viral pathogens which includes corona virus (CoV).

The disease is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which originated in China in December 2019. From there, it has spread to over 192 countries and territories. The pathogen is a coronavirus, a type of virus that typically causes respiratory illness.

Coronaviruses are a large family of viruses that can infect both animals and humans, first identified in the mid-1960s. They are a respiratory virus named for the crown-like spikes on their surface. These viruses are offender inseveral

outbreaks across the globe. Usually this virus spread in animals but it can jump to human, fromthere the pathogens spread from person to person.

There are seven known types of human Coronaviruses, wherein four types, namely KHU1, OC43, NL63, and 229E, they cause mild to moderate respiratory infections, such as the common cold. Two types, however, cause severe respiratory infections – the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East Respiratory Syndrome coronavirus (MERS-CoV). The seventh coronavirus type is the novel coronavirus SARS-CoV-2 that has spread from China to the rest world.

They demonstrate the importance of the coronaviridae as emerging Human pathogens by the emergences of SARS-CoV-2, SARS-CoV, and MERS-CoV. The Coronaviridae family comprises enveloped positive-sense single-stranded RNA viruses of the order Nidovirales with a viral genome is 26–32 Kb in length. The particles are typically decorated with large (~20 nm), club- or petal-shaped surface projections (the "peplomers" or "spikes"), which in electron micrographs of spherical particles create an image similar of the solar corona.

The family is divided into Coronavirinae and Torovirinae sub-families, which are further divided into six genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, Deltacoronavirus, Torovirus, and Bafinivirus. While viruses in the genera Alphacoronaviruses and Betacoronaviruses infect mostly mammals, the Gammacoronavirus infect avian species, and members of the Deltacoronavirus genus have been found in both mammalian and avian hosts[3].

Coronavirus is an enveloped, positive-sense single-stranded RNA (ssRNA) virus belonging to the Coronaviridae family. This family consists of several species and causes upper respiratory tract and gastrointestinal infections in mammals and birds. In humans, it mainly causes common cold, but complications including pneumonia and SARS can occur[4].

In 2012, the World Health Organization (WHO) designated a sixth type of HCoV infection identified as the Middle East respiratory syndrome coronavirus (MERS-CoV) which is associated with high fatality rate [5].There are no specific treatments for CoV infection and preventive vaccines are still being explored. Thus, the situation reflects the need to develop effective antivirals for prophylaxis and treatment of CoV infection. It is been previously reported that saikosaponins (A, B2, C, and D), which are naturally occurring triterpene glycosides isolated from medicinal plants such as Bupleurum spp., Heteromorpha spp., and Scrophularia scorodonia , exert antiviral activity against HCoV-22E9. Upon co-challenge with the virus, these natural compounds effectively prevent the early stage of HCoV-22E9 infection, which includes viral attachment and penetration. Extracts from Lycoris radiata, Artemisia annual, Pyrrosia lingua and Lindera aggregata have also been documented to display anti–SARS-CoV effect from a screening analysis using hundreds of Chinese medicinal herbs[6, 7].

Natural inhibitors against the SARS-CoV enzymes, such as the nsP13 helicase and 3CL protease, have been identified as well and include myricetin, scutellarein, and phenolic compounds from Isatis indigotica and Torreya nucifera.Other anti-CoV natural medicines include the water extract from Houttuynia cordata, which has been observed to laid out several antiviral mechanisms against SARS-CoV, such as inhibiting the viral 3CL protease and blocking the viral RNA-dependent RNA polymerase activity[8, 9].

Phytochemicals as Anti Virals

In phytochemicals mainly flavonoids, alkaloids terpenoids, lignins, and coumarins are having anti-viral activity.World Health Organization (WHO) reported that the 80% of world's population depends on plants to meet their basic health requirements as plants possess the bioactive compounds among which phytochemicals are the largest group synthesized. Further, these phytochemicals are reported to possess the antioxidant and antiviral activity [10, 11]

The mechanism involved in antiviral activity of phytochemicals is that it inhibits the synthesis of RNA or hinders the reproduction of virus by blocking cellular receptors and enzymatic function subsequently, number of studies have been reported about the antiviral activity of various phytochemicals present in natural food products and herbal plants.

Natural food product which derives antivirals have been used for two previous coronavirus outbreaks of SARS-CoV and MERS-CoV which suggests it's potential to provide treatment for the on-going epidemic of COVID-19. The anti-coronavirus activity of some flavonoids (Herbacetin, rhoifolin and pectolinarin) was reported due to the inhibition of 3C-like protease (3CLpro). Other flavonoids

such as Herbacetin, quercetin, and helichrysetin were reported to inhibit the enzymatic activity of MERS-CoV/3CLpro. Flavonoids are among the principal group of phytochemicals that are widely distributed in plant kingdom. Their vast structural makes them available for antiviral research. Flavonoids such as chalcone, flavonone, iso-flavonone are commonly known for its antiviral [12].The mechanism behind the antiviral activity of flavonoids might be attributed to its antioxidant activity, scavenging ability and DNA inhibition, inhibition of entry of virus or its reproduction. Furthermore flavonoids also play a role as an immunomodulator as they have the capacity to direct macrophages from pro inflammatory to anti-inflammatory phenotypes.This anti-inflammatory activity of flavonoids have been attributed to various mechanisms which involves activation of nuclear factor κ-light chain amplifier of activated B cells (NF-κB), modulation of mitogen –activated protein kinase, cytokine synthesis and inhibition of reactive oxygen species. Various types of flavonoids are also reported to have Immunomodulatory activities like that of apigenin, oligomeric proanthocyanidin, isoflavonoids, flavones and anthocyanidin. Also, biflavonoids from Torreya nucifera were testified to inhibit SARS-CoV/3CL (pro) .Moreover, the antiviral activity of terpeonids against (SARS-CoV) was also reported. Deng etal reported the antiviral activity of chalcones. A bioflavonoid myricetin has been reported to possess excellent antiviral activity against hepatitis B virus, influenza virus, and SARS-CoV. The mechanism of antiviral activity of myricetin against SARS-CoV is it inhibits SARS-CoV protease .The polyphenols epicatechin, epigallocatechin, and epigallocatechin gallate, delphinidin, cyanidin, were found to be more effectiveagainst West Nile Virus (WNV), hepatitis C &B virus, herpes simplex virus, influenza a virus, dengue virus, adenovirus, reovirus, and ZIKV. The catechin compound was found to inhibit viral entry and attachment. Other polyphenols such as honokiol, baicalein and naringin have also been described to have antiviral activity. Also, polyphenolic compounds from plant extracts have been known to possess wide spectrum antiviral activities.

Mechanism of Action of Natural Antiviral on Human Health

Natural products which have good nutraceutical and antiviral properties can be a relief from viral infections. They can decline the viral infections such has COVID-19 as they have been proven effective against two earlier coronavirus outbreaks of SARS-CoV and MERS-CoV which suggests the potential use of natural antivirals in treatment of on-going epidemic of Covid-19. Natural antivirals do a great work by stimulating immune system and then immune system plays a key role in suppressing the viruses. There are various immune cells which are having the capability to recognize different foreign particles and provide protection against invading viruses, bacteria, fungi and these immune cells are the one which mediates immune responses. The innate immune responses are the first line of defense against invading pathogens. The cells of innate immunity system include monocytes, macrophages, natural killer (NK) cells, B cells, T cells and granulocytes. When a virus is invaded inside a host body, they are first recognized by macrophages/Dendritic cells. These cells possess specific receptors called Toll like receptors TLRs which recognizes the invaded foreign particle in the body. Some toll like receptors like TLR3, TLR7, TL9 are able to recognize the viral dsRNA and dsDNA. After recognition these receptors move towards nearby lymph nodes and alert the T helper cell. This T helper cell goes and binds to a B cell leading to the activation of the B cell. Some of the B cells are converted into plasma cells while some of the other B cells become B memory cells which help in combating second time of exposure. Now the plasma cells which are formed are released in the blood and these plasma cells secrete antibodies which bind to the antigens to fight the invading virus and prevent the entry of virus. Also, many of the macrophages and dendritic cells express Fc receptor which engulfs the virus and presents the pathogen's antigens by a process called Phagocytosis. T cells, mainly CD4+ and CD8+ cells also have an effective role as antivirals against the virus by inducing their effector function to stop further invasion and decreasing the risk of inflammation.

Main Source of Antiviral Derived From Plants

Garlic and Onion

Garlic and onion belongs to Allium family. They have an excellent antiviral activity [13, 14]. They contain allicin and quercetin which are of organosulfur compounds. Allicin and quercetin have a significant role in inhibiting the viral infections by obstructing the attachment of virus to the host cell [15]. Garlic and onion also contains some of the important bioactive compounds such as myricetin, kaempferol, ajoene and isorhamnetin that have remarkable antiviral effect against RNA viruses. Several studies have reported the antiviral activity of these bioactive compounds against different viruses such as Dengue virus hepatitis B virus, influenza virus A&B, and coronavirus [15-17].

ISBN: 979-85-27243-61-1

The two main bioactive compounds which are present in onion and broccoli are quercetin and kamferol [18]. They act as an anti-infective and anti-replicative [19]. A literature study revealed that quercetin has been successfully used as an anti-viral agent against RNA viruses [18]. Quercetin plays many active role such as, preventing the entry of polio virus and Hepatitis C and it was also found to inhibit the SARS-CoV-protease which is an important enzyme used for multiplication of SARS virus [20-23]. Furthermore, quercetin is an effective compound which enhances the immune response in host cell.

The important bioactive compound which is found in garlic is the Allicin, it has strong antiviral property. Allicin inhibits the virus multiplication and modulates immune system in response to viral infection. The antiviral role of garlic against influenza virus was reported [24]. Allicin also contains a compound called selenium which has an antiviral property [25]. Selenium was reported for inhibiting replication of Coxsackie virus [26]. Hence, Allicin, Kamferol, myricetin and quercetin can be used as a novel antiviral agent against COVID-19.

Fig.1: Garlic Plant

Fig.2: Onion Plant

Mushroom

Mushrooms are the good source of many bioactive compounds such as alcohols, terpenoids, peptides, nucleosides, glycoproteins, mineral elements and some of the antioxidants like phenolic compounds, tocopherols, ascorbic acid etc. [27]. These compounds possess antiviral activity in addition to that they also exhibit antimicrobial, anticancer, antioxidant and anti-inflammatory activities. The antiviral activity is found in both edible and nonedible mushrooms [27]. Certain studies reported the antiviral effect of bioactive compounds which were isolated from different variety of mushrooms against different RNA virus such as herpes simplex virus, human immunodeficiency virus, hepatitis B, influenza and C viruses [28]. Novel Polysaccharide-Peptide Complex (PPC) which is from a mushroom source (*Pleurotus abalonus* and *Russula paludos*) inhibited the reverse transcriptase activity of human immunodeficiency virus [29, 30]. Similarly the HIV antigen expression was inhibited by an extract obtained from mycelium of Lentinus edodes and thereby inhibiting HIV [31]. Also, the antiviral protein which was isolated from the fruiting body of Grifola frondosa was found to have an effective antiviral activity against Herpes Simplex Virus [32]. The antiviral activity exhibited by *Pleurotus ostreatus* extract against swine flu (H1N1) was also testified [33]. The Phenolic extracts obtained from *Inonotus hispidus* showed antiviral effect against influenza A and B [34]. Likewise, Hwang et al testified the antiviral effect of polyphenolic extract which were isolated from Phellinus baumii against influenza A, H1N1, H5N1, and H3N2 [35]. Saboulard et al reported basidiomycete Macrocystidia cucumis which showed antiviral response against herpes simplex virus in baby hamster kidney cells [36]. Apart from all this, bioactive compounds which are found in mushrooms helps in boosting the immune system by activating the cellular immune function and helps to defend against viral infections by inhibiting the entry of virus.

Fig.3: Mushroom

Blueberries, Grapes and Cranberries

Blueberries, grapes and cranberries contain an important polyphenol compound called Resveratrol which is responsible for antiviral activity [37]. Resveratrol also possess antioxidant property. The mechanism which is responsible for antiviral activity of resveratrol includes inhibition of viral replication, protein synthesis, gene expression and nucleic acid synthesis [37]. The antiviral effects of resveratrol has been proven against several viral infections including Epstein-Barr Virus(EBV), Herpes Simplex Virus (HSV), also respiratory viral infections which are caused by influenza, rhinovirus and MERS-CoV infections [38-42]. After getting the knowledge of resveratrol we can say that, it can be used as a therapeutic option for treatment of Covid-19. The extracts obtained from the cranberries found to possess antiviral activities against reovirus, enterovirus and influenza virus by preventing the attachment of virus to the target cells [43-47]. Also, blueberries are rich in anthocyanin which inhibited the replication of influenza virus A/H3N2, Human Respiratory Syncytial Virus A2 (HRSV-A2) and coxsackievirus [48]. Grape seed extract contain bioactive compounds such as catechin, epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate which possess antiviral, anticancerous, anti-inflammatory and antioxidant properties. The extract obtained from the grape showed antiviral effects against HIV, herpes virus and hepatitis by effecting the binding of the virus [49].

Fig.4: Blueberry Plant

Fig.5: Grape Plant

Fig.6: Cranberry Plant

Citrus Fruits

Citrus fruits are a good source of Vitamin C. Vitamin C is a powerful antioxidant which scavenges the free radical. Recent studies have shown that antioxidant plays a wonderful role in boosting immune response against bacteria and virus which invades the host body, thus helping the immune system to make it easier to fight off the virus. Vitamin C is also reported as aid in flu symptoms such as sneezing, swollen sinuses and a running nose [50]. When Vitamin C is supplemented it is found that it reduces the rate of pneumonia in human trails who are suffering from SARS coronavirus suggesting that Vitamin C may inhibit the lower respiratory tract infections caused by Covid-19 [51]. Hence, Vitamin C can be explored as an effective option for the treatment of Covid-19 [52].

Fig.7: Plant of Citrus Fruit

Antiviral Activity of β-glucan

β-glucan is a non-starch polysaccharide which is present is many sources such as cereals, bacteria, fungi and yeast. β-glucan possess various properties like antioxidant, anticancerous, antimicrobial and they also act as immunomodulant (modulates immune system) [53]. β-glucan boosts the immune system (innate and adaptive immune response) making it more effective.

It encourages the action of macrophages which swallow and destroy the invading pathogens and triggers the other immune cells to attack the invading pathogens. Furthermore, macrophages enable the communication of immune cells by releasing a chemical compound called cytokines. β-glucan also encourages lymphocytes (white blood cells) which goes and binds to viruses and releases a chemical which will destroy it. It was reported that the supplement of β-glucan reduces the upper respiratory symptoms in stressed woman and also helps in improving the mood swing [54]. Jesenak et al., reported the immunomodulatory effect of β-glucan in children with respiratory infections [55].

S.no	Natural Antiviral Food Sources	Antiviral Bioactive Agent	Reported Mechanism of Action	Effective Against Virus
1.	Garlic	Allicin Selenium	Inhibits virus multiplication	Influenza [26] Coxsackie [28]
2.	Onion	Quercetin Kaempferol Myricetin	Inhibits viral entry and translation	Ebola virus [22] Hepatitis C [24] Polio virus [23] Coronavirus [26]
3.	Mushroom	Polyphenol extract Peptides Glycosides Lignin Terpinoids	Inhibits entry, reverse transcriptase and spread ability of virus	H1N1 [35] H5N1 [37] HIV [35] HSV [34]
4.	Blueberries	Resveratrol	Inhibits replication, protein synthesis, gene expression, and nucleic acid synthesis of virus	HSV [41] Influenza [50] Rhinovirus [43] MERS-CoV [44] CA [40]
5.	Cranberries	Resveratrol	Inhibits viral attachment	Reovirus [45] Enterovirus [46] Influenza [47]
6.	Grapes	Resveratrol Catechin Epicatechin Epicatechin gallate	Inhibits viral binding	HIV [51] Herpes [51] Hepatitis [51]
7.	Cereals and yeast	Beta-glucan	Boosts immune system	Influenza [58]

Table.1: Anti-viral phytochemicals with its mechanism of action

Conclusion

Pathogenic infections have become a serious threat to the present life and finding a promising remedy against them is a demanding work. This can get the better of by exploring about the luxury of the natural source. There are many plants which are studied for their medicinal and nutraceutical effects. This study is a prompt attempt to bring out the rewarding compounds from natural source which shows an effective antiviral activity and it is a fuel for further medical research on the natural compounds which have a great medicinal benefit. This study explains the best thought "Nature itself is a physician".

Acknowledgment

Authors wish to duly acknowledge all the contributions of different authors who have worked on natural sources of antiviral compounds during their research work and have been cited in our paper.

Also, we thank Department of Biotechnology, Sapthagiri College of Engineering and management for all the support provided.

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Achyranthes aspera (Devil's horsewhip): An overview

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Abstract

***Achyranthes aspera* is also known as devil's horsewhip or chaff flower plant is a species of plant in the family *Amaranthaceae*. It is distributed throughout the tropical world. It can be found in many places growing as an introduced species and a common weed. It is erect or ascending herbs or shrubs; 0.8-4 m high, sometimes almost treelike; woody at the base. Leaves opposite, ovulate and simple, up to 10 cm long by 8 cm wide, tapering to a point at both ends and shortly stalked, the blades entire. It appears to be a promising herbal candidate to undergo further exploration as evident from its pharmacological profile.Though almost all of its parts are used in traditional systems of medicines, seeds, roots and shoots are the most important parts which are used medicinally. It is a powerful healing agent that is widely used in ayurvedic medicine in India.The herb has been used traditionally for ages, in the treatment ofleprosy, asthma, fistula, piles, gynaecological disorders, arthritis, wound, renal and cardiac dropsy, fever, cough, kidney stone, diabetes, skin disorders, gonorrhea, malaria, pneumonia, pyorrhoea, dysentery, rabies, insect and snake bite, hysteria, toothache, etc. This work is an attempt to explore and compile the different pharmacognostic aspects of the action plant *Achyranthes aspera* reported till date and make it available commercial medicine to humanity.**

Key words*: Achyranthes aspera*, devil's horsewhip, chaff flower, arthritis, diabetes, pneumonia, gynaecologic disorders.

Introduction

Achyranthus aspera or *A. aspera* Linn. belongs to the family Amaranthaceae, is an annual, stiff erect or procumbent, annual or perennial herb, 1-2m in height, often with a woody base, commonly found as a weed of waysides, on roadsides. It is well known as devil's horsewhip or chaff flower. *Achyranthes aspera* Linn. (Apamarga in sanskrit) is a well-known plant drug in Ayurvedic, Unani-Tibbi, Siddha, Allopathic, Homeopathic, Naturopathic & Home Remedies. This Plant is found to have antiarthritic, antifertility, abentifacient, anti-helminthic, aphrodisiac, antiviral, anti-plasmodic, antihypertensive, anticoagulant, laxative, ecbolic, diuretic and anti-tumor and many other medicinal properties. It is used in preparation of Ayurvedic medicines and also as traditional medicine in Asia and Africa to treat various diseases such as arthritis, leprosy, asthma, diabetics, piles, wound, pneumonia, fever, insect and snake bite, renal and cardiac dropsy, kidney stone, skin disorders, gynaecological disorders, fistula, gonorrhea, malaria, cough, pyorrhoea, dysentery, rabies, hysteria, toothache, etc. It is one of the 21 leaves used in the Ganesh Patra Pooja done regularly on Ganesh Chaturthi day, also Maasai people of Kenya use this plant medicinally to ease the symptoms of malaria [1]. It is reported to contain alkaloids, flavonoids, saponins, steroids and terpenoids. Flavonoids have shown to prevent or slows the development of some cancers and mostly act as an antioxidant and anti-inflammatory agents. It has adapted to a wide range of environments, the spiny bracts cause the fruits to stick to the hair of animals, clothing etc. There is evidence of dispersal by livestock and noted little seed dormancy in this species [2].It is known to improve the fertility of soil.

Biological Source

Scientific Classification

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonae

Order: Caryophyllales

Family: Amaranthaceae

Genus: Achyranthes

Species: *Achyranthes aspera* [3]

***Synonyms*:** *Achyranthes acuminata E.Mey, Achyranthes argentea var. virgata (Desf. ex Poir.) Moq, Achyranthes aspera f. subgrandifolia Suess, Cadelaria indica Raf, Centrostachys aspera (L.) Standl, Centrostachys canescens Standl, Centrostachys grandifolia Standl [4].*

Common name: Burweed, Chaff flower, Chaffbur, devil's horsewhip, Prickly chaff-flower, Rough Chaff-flower, achyranthes, crocus stuff, crokars staff [5]

Vernacular Names

language	***names***
Assam	Bonsoth, Obhat kata, Ubhot-kata
Bengali	Apang
English	Burweed, Chaff flower, Chaffbur, devil's horsewhip, Prickly chaff-flower, Rough Chaff-flower, achyranthes, crocus stuff, crokars staff
Gujarati	Agharo (અાધારો), Safed Aghedo, Anghadi, Andhedi, Agado
Hindi	Aghara (अघाड़ा), Chirchira (चिरचिरा), Tarun (तरुण), Durabhigrah (दुरअभिग्रह), Dhanushka (धानुष्का), Madhukar (मधुकर), Mayur (मयूर), Latjira (लटजीरा), Vashir (वशीर)
Kannada	Uttaraani (ಉತ್ತರಾಣಿ)
Malayalam	Katalaati (കടലാടി), Kadala
Marathi	Aghada (अघाडा), Apamarga (अपामार्ग), Kini (किणी), Kharamanjari (खरमंजरी), Pandhara-aghada
Punjabi	Kutri, puthakanda
Sanskrit	Akshara (अक्षर), Adhahaghanta (अधःघण्टा), Apamarga (अपामार्ग), Akrutihchhatra (आकृतिःच्छत्रा), Kharamanjari (खरमञ्जरी), Pratyanchapushpa (प्रत्यञ्चपुष्प), Mayur (मयूर), Vashir (वशीर)
Tamil	Naagarkaai Mullu, Apamarkkam (அபாமார்க்கம்), Akatam (ஆகாடம்), Nayuruvi (நாயுருவி), Shiru-kadaladi [6]
Telegu	Uttarenu (ఉత్తరేణు), Pratyak-pushpi (ప్రత్యక్పుష్పి)

Origin: tropical regions of India and tropical Asia .

Geographical Distribution

The plant is widespread in the world as a weed, in Baluchistan, Ceylon, Tropical Asia, Africa, Australia and America. It is found on road sides, field boundaries waste places as a weed throughout India up to an altitude of 2100 m and in South Andaman Islands. In the northern part of India it is known as a medicinal plant in different systems of folk medicine.[8]

Habitat

Erect or spreading long-lived perennial herbs or shrubs**.** Growing Climates are disturbed areas, hillsides, waste places, road sides, gardens, crops, grasslands, savanna, forest margins and riverbanks. [9]

Propagation

It is a widespread weedy pantropical species readily transported to a new habitat because its spiny fruits easily detach and stick to clothes, fur, and feathers. Consequently, seeds of this species may be easily transported to new habitats by birds, mammals, and humans and became a main reason for its massive propagation. [10]

Morphology

Stem: Angular, ribbed, pubescent, simple or branched from the base, often reddish purplish tinged.

Leaves: Opposite, thick, ovate elliptic or obovate rounded, but variable in shape and size, 4-12 cm long and up to 8 cm wide, velvety tomentose (Fig. 1).

Flowers: Greenish-white, numerous, in axillary or terminal spikes up to 75 cm long; bracts membranous, oblong, enclosed in the hardened parianth.(Fig. 2)

Fruits: Utricle, oblong-cylindric, truncate at apex, rounded at base.

Seeds: Reddish-brown, sub-cylindrical.

Parts of plants used are dried whole plant (Fig. 3), roots, seeds, leaves, flower.

Available forms are Decoction, powder, paste, oil, infusion, juice

Taste: Acrid, bitter, sour, pungent [11]

Fig. 1 *Achyranthes aspera* plant

Fig. 2 Devil's horsewhip flower

Fig.3 Dried whole plant of chaff flower plant

Chemical Constituents of Plant

The *Achyranthes aspera* is stated to contain the following major classes of compounds: Fatty acids; D-glucuronic, Betaine; Oleanolic acid, triacontanol; Spathulenol, alkaloids; Achyranthine, different amino acids; Ecdysterone; Oleonic acid; Bisdesmosidic; triterpenoid-based saponins; Spinasterol, dihydroxy ketones; n-hexacos-14- enoic which help maintain an individual's overall body and mental health. [12]

Medicinal Properties

Wound Healing and Anti-allergic activity

It is reported that the plant extracted using petroleum ether (200 mg/kg, i.p.) has shown significant antiallergic activity in both milk induced leukocytosis and milk induced eosinophilia in mice. So it give a clear picture due to presence of steroids it exhibits anti allergic property and ethanolic aqueous extracts of leaves of Achyranthes aspera for wound healing activity [13].

Anti-inflammatory

Alcoholic extract of the roots of Achyranthes aspera, was found to exhibit anti-inflammatory activity in Wistar rats using carrageenan-induced paw edema method and cotton pellet granuloma test [14].

Antioxidant

Antioxidant activities were determined for the plant extracts as a measure of radical scavenging, using the DPPH assay determined by brand willims. Various concentrations of ascorbic acid and/or Trolox were used instead of the plant extract as reference standard during the experiment and it showed the positive result for antioxidant [15]

Anti-depressant Activity

It is reported that Methanolic extract of the leaves of Achyranthes aspera shows anti-depressant effect in mice and rats using forced swimming test in mice and rats and tail suspension test in rats [16]

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Spermicidal and anti-fertility Activity

Researches have made and reported thatExtracts from roots of Achyranthes aspera have been reported to possess spermicidal activity in human and rat sperm. Study was made on hydroethanolic, n-hexane and chloroform extracts, which were found to be most effective for sperm immobilization, sperm viability, acrosome status, 5'-nucleotidase activity and nuclear chromatin decondensation. And ethanolic extract of the root of Achyranthes aspera shows post coital antifertility activity in female albino rats. According to the study, the extract exhibited 83.3% anti-implantation activity when given orally at 200 mg/kg body weight. [17]

Antiparasitic Activity

Ethyl acetate extracts of A. Aspera have been proved to contain anti parasitic activity. It has been studied that dried leaf, flower and seed extract of A. Aspera are active against the larvae of cattle tick *Rhipicephalus (Boophilus) microplus* (Acari:lxodidae), sheep internal parasite *Paramphistomum cervi.* [18]

Cardiovascular Activity

Achyranthine, a water-soluble alkaloid isolated from Achyranthes aspera, decreased blood pressure and heart rate, dilated blood vessels, and increased the rate and amplitude of respiration in dogs and frogs. The contractile effect of the alkaloid at 0.5 mg/ml on frog rectus abdominal muscle was less than that of acetylcholine (0.1 mg/ml), and its spasmogenic effect was not blocked by tubocurarine. [19]

Hypoglyceamic and Cancer chemo preventive Activity

Aqueous methanolic extract of the whole plant have been shown to possess hypoglycaemic activity. It is also reported Methanolic extracts from leaves of Achyranthes aspera have been proved to have cancer preventive action on Epstein-Barr virus early antigen activation induced by tumor promoter 12-O-tetradecanoylphorbol-13-acetate in Raji cells. [20]

Hepatoprotective Activity

It is reported that the methanolic extract of the aerial parts of Achyranthes aspera shows hepatoprotective activity on rifampicin induced hepatotoxicity in albino rats. Methanolic extract showed dose dependent decrease in the levels of SGPT, SGOT, ALKP and total bilirubin.

Anti- diabetic

Chaff flower was being used to treat diabetes mellitus from the ancient times but there was no scientific evidence for its anti-diabetic activity Hence, to evaluate the efficiency of chaff flower against diabetes mellitus, ethanol extract was prepared andtested against alloxan induced diabetic Swiss albino mice. The level of blood glucose level was found to be increased by 123% and 128% on fifteenth and thirteenth day of exposure. From the result it wasevident that ethanol extract of chaff flowershowssignificant anti-diabetic activity.[21]

Diuretic Activity

The acute toxicity profile and diuretic activity of chaff flower was determined by using Albino rats of either sex and were treated with the crude aqueous extract at different doses of 10, 30 and 50mg/kg by intra-peritoneal route respectively. The results of the experiments showed that chaff flower has significant diuretic effects. It also was evident from results that aqueous extract of chaff flower increase urine volume in a dose dependent manner [22].

Anti-Arthritic activity

Evaluation of the anti-arthritic activity of ethanolic extract of chaff flower, protein inhibition assay method was used. Different concentrations (10, 50, 100, 200, 400, 800, 1000 µg/ml) of ethanolic extract and diclofenac sodium were used in this experiment. The extracts showed significant anti-arthritic activity in comparison with standard drug diclofenac sodium at concentration of 800-1000 µg/ml. Findings of this investigation demonstrate that chaff flower show significant anti-arthritic activity. Tannins and flavonoids compounds are responsible for this activity [23]

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Skin cancer treatment

It has reported to be used in treatment of skin cancer. This plant is used in boils, scabies and eruptions of skin and other skin diseases. In this *in vitro* assay the non-alkaloid fraction containing mainly non-polar compounds showed the most significant inhibitory activity (96.9%; 60% viability). In the *in vivo* two-stage mouse skin carcinogenesis test the total methanolic extract possessed a pronounced ant carcinogenic effect (76%). The results revealed that leaf extract and the non-alkaloid fraction are valuable antitumor promotors in carcinogenesis. [24]

USES

Traditional Uses

- The pulp of fresh *Achyranthens aspera* leaves, and flowering spikes is an effective home remedy for scorpion bites when applied externally, which is believed to paralyze.
- For snake/reptile bites, the seeds are beneficial for ophthalmic diseases and other corneal infections.
- Flowering, elongated spikes mix with little sugar and used in mad dog bites to treat hydrophobia.
- *Achyranthens aspera* ash loaded with potash has been used for washing clothes.
- Flowers and fruits of *Achyranthens aspera* are useful for the treatment of menorrhagia.

Therapeutic uses [25]

Asthma

Devil's Horsewhip (Chaff Flower) open up for lungs making it wonderful for bronchitis and asthma. In India the flowers are made into a paste with equal amounts of garlic and black pepper to treat asthma and bronchitis. Take 1/2 teaspoon 3 to 4 times a day. It also works well for fevers associated with colds and flues.

Relieve nausea and Heal wounds

In case you are suffered from continued attacks of vomiting, you can use the herb to get relief. It will relieve your symptoms of nausea within a short time. The action of the herb on the superficial cuts, scrapes, and wounds helps to improve the healing time.

Weight loss

If you want to lose weight, take the decoction of the herb in morning and evening. Soon, you will experience a loss of weight. This is due to the scraping effect of the herb on the cholesterol. Also, it reduces fat deposition, so your body begins to lose weight.

Tooth Powder

Devil's Horsewhip (Chaff Flower) seeds can be ground with salt into a find powder, it cleans and whitens your teeth like nothing else and stops your gums from bleeding. And the dried stems of the plant can be used as a toothbrush.

Detoxify the body

It is considered a strong herb that produces effective detoxification for the whole body. You can remove all the overrunning kapha and vatadoshas from the body.

Get relief from piles and itching

You can get relief from hemorrhoids and piles by using the Devil's Horsewhip (Chaff Flower) herb. This is due to the balancing action of the doshas that prevents constipation and other conditions that lead to piles. The herb provides relief from external pain, scorpion bites, and itching. You can apply the paste on the site of the wound or bite locally to get relief.

Cure infections and Helps decrease sputum

It is useful for curing infections and getting rid of worms in the head and neck region. By having the herb regularly, you can break down and expel sputum.

Hunger control

Devil's Horsewhip (Chaff Flower) has the ability to control the vata and kaphadoshas in the body. In those diseases where uncontrollable hunger plays a part, one may use this herb for therapy. Make porridge with the Chaff Flower and feed this to the patient. They will soon recover from their ailment. Also, you can digest the 'ama' in the body which is the leftover undigested food in the GI tract.

Treat Ear pain and excellent diuretic action

People use the oil of the Devil's Horsewhip (Chaff Flower) to get relief from an earache. Put a few drops of the oil in the ear, and soon your earache will disappear. It helps people

suffering from dysuria, urinary retention, water retention, and urinary stones. You can use the herb to break down the stones in the bladder and kidney.

Improve appetite, Cure glandular growths and hiccups

The herb improves taste, and this is useful for relieving anorexia. If you have fibroid and growth in the glands, you can use the Devil's Horsewhip (Chaff Flower) herb to cure this condition. If you develop hiccups, have the decoction of the herb, and your hiccups will vanish.

Relieve gas, cure jaundice and Helpful in Asthma

When there is an accumulation of gas in the intestines, it can lead to bloating and distension of the abdomen. By adding the paste of Chaff Flower to the diet, one may get relief from the gas problem. When you have disorders of the liver and remain affected by diseases such as jaundice, you can use the herb to get a cure. Have the herb regularly morning and evening with tea. Your jaundice problem will soon disappear. The Chaff Flower helps relieve asthmatic problems. It helps enhance breathing by opening the air passages. You can use the decoction of the herb for this.

Treat Anemia and cure bleeding disorder

This herb can improve the blood count. Make a paste of the herb and add it to the diet. Have it at least two to three times a week to relieve the symptoms of anemia. The fruit of the Devil's Horsewhip (Chaff Flower) herb is difficult to digest. This is useful for controlling and curing bleeding disorders.

Strong purgative and antimicrobial action

Devil's Horsewhip (Chaff Flower) is quite beneficial for removing the intestinal microbes. It helps remove the worms in the gut through its purgative action. This plant fights microbes anywhere in the body including the skin. It helps you fight eczema and removes all infectious conditions.

Helpful women medicine and Help treat many conditions

Chaff Flower finds a use for gynecology and obstetrics for induction of labor and abortion. It helps in the termination of postpartum bleeding. One can use Chaff Flower plant to treat a variety of conditions such as migraines, convulsions, and epilepsy. It also finds a use for treating psychological conditions. This is one of the best herbs for treating conditions affecting the head region.

Endemic uses

- It is a diuretic in goats and is used in other veterinary preparations.
- It has been investigated for energy production.
- It is the host for many pests.
- It helps in soil improvement by increasing the fertility of the soil.

Toxicity and its Impact

- It is moderately toxic to humans and livestock incase not used in suggested quantity.
- Aspera is moderately resistant to 2,4-D and MCPA. In the young seedling stage, a reasonable kill can be obtained with rates of the order of 1.0 kg/ha but resistance increases rapidly with age and older plants require 2.0 kg/ha or more.
- Damaged ecosystem services
- Ecosystem change/ habitat alteration
- Negatively impacts agriculture
- Negatively impacts animal health
- Reduced native biodiversity

Limitations and Precautions

- *Achyranthens aspera* paste is used with caution since it may cause skin irritation, rashes over the skin. Therefore it is better to use it along with some cooling substances like ice.
- If taken in excess it may cause nausea, vomiting, extremes of diarrhea, convulsions, spasms in abdomen and dehydration.
- Do not use Devil's Horsewhip in pregnancy, it can cause abortion.
- During pregnancy and breastfeeding situations, it is very much advisable to take *Achyranthens aspera* under the supervision of a medical practitioner.
- For children's below 12 years, taking or giving Achyranthes Aspera supplements are not advisable. [24]
- If one is undergoing infertility treatment, then it is better to avoid its use.

- Seek suggestion of concerned doctors before use
- Due sperm killing properties it should be cautiously taken orally. [26]

Conclusion

Achyranthes aspera is a invasive weed, it has been a threat to agricultural crops, but the plant has prominent role in the texts of Ayurveda. This review represents the brief profile of Achyranthes aspera plant which has important role in allover improvement of health. The potency of this plant is reported in many recorded works but this review is a prompt attempt to create awareness amongst people and to ignite further medical researches on this plant, and to commercialize this herb. It is suggestive of greater benefits as it is easily available, economically viable and a reservoir of significant medicinal properties. It would be a beautiful example of "treasure from trash" by commercializing this plant products.

ACKNOWLEDGEMENT

Authors wish to duly acknowledge all the contributions of different authors who have worked on *Achyranthes aspera during their research work and have been cited in our paper.*

Also, we thank Department of Biotechnology, SCE and management for all the support provided.

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SYNTHESIS AND CHARACTERIZATION OF POLYPYRROLE BY CHEMICAL OXIDATION OF PYRROLE IN AQUEOUS $FeCl_3$ SOLUTION

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Abstract:

Conductive polymer, polypyrrole (PPy), was synthesized by chemical oxidative polymerization technique by using pyrrole monomer (mPPy) in aqueous solution with oxidizing solution of ferric chloride ($FeCl_3$) . Polymers with conjugated pi- electron (i.e.system have C=C conjugated bonds) backbones display unusual electronic properties such as low energy optical trasition,low ionization potentials, and high electron affinities.

The produced PPy samples were characterized by using different techniques such as the UV-VIS and IR spectroscopy, differential scanning calorimetry (DSC), X-ray diffraction (XRD), and scanning electron microscopy (SEM).

Introduction:

Polypyrrole, a chemical compound formed from a number of connected pyrrole ring structures is an inherently conductive polymer due to interchain hopping of electrons. Polypyrrole is easy to prepare and its surface charge characteristics can easily be modified by changing the dopant anion (X^-) that is incorporated during synthesis. Polypyrrole was the first of conducting polymers that shows relative high conductivity[2] .

Polymerization occurs readily in the presence of different oxidants, such as $FeCl_3$ and $K_2S_2O_8$. More studies have been reported about the formation of PPy films on solid surfaces by chemical polymerization of pyrrole . There are reports about the polymerization of pyrrole onto printed circuit boards and various textile composites . In the present work, the conducting polypyrrole (PPy) films were synthesized by chemical oxidation of pyrrole with $FeCl_3$ in aqueous methods by mixing a solution of pyrrole with an oxidizing solution of $FeCl_3$.[2]

ISBN: 979-85-27243-61-1

Experiment:

Materials used:

Pyrrole monomer (mPPy) 98% .Anhydrous iron (III) chloride, also called ferric chloride ($FeCl_3$) 98%, and ammonium persulphate ($N_2H_8S_2O_8$) 98% were used as the oxidizing agent.[1]

Synthesis:

Polypyrrole (PPy) was synthesized by chemical oxidative polymerization technique using pyrrole monomer and oxidants. The chemical polymerization was carried out in a beaker with 100 ml of distilled water by mixing given molar ratios of pyrrole monomer, an oxidant. Since this is an exothermic reaction, the addition of distilled water (cooled) was done slowly. A given volume of pyrrole monomer was quickly added to the distilled water with the required amount of oxidant; vigorous magnetic stirring was maintained to facilitate the dispersion of pyrrole. The polymerization reaction was carried out for a period of 4 hrs at a temperature of 25°C. The precipitation of fine black particles was nearly immediate. After the prescribed polymerization time, synthesized PPy was filtered from the solution with filter paper and thoroughly washed with distilled water and ethanol several times; PPy was dried in a vacuum oven at about 40°C overnight.[1]

Characterisation of product has been done by using following methods.

1.UV-VIS SPECTROSCOPY

The UV-VIS absorption diffuse-reflectance spectra were recorded on a spectrophotometer with double beam and microprocessor. These spectra (Fig. 1.) are typical spectra for polypyrrole, and a constant and progressive increase of the pyrrole bands is observed due to the radicalic polymerization, associated with the character of the chemical bond between

pyrrole rings in polymer chains and the chlorine anions from $FeCl_3$.

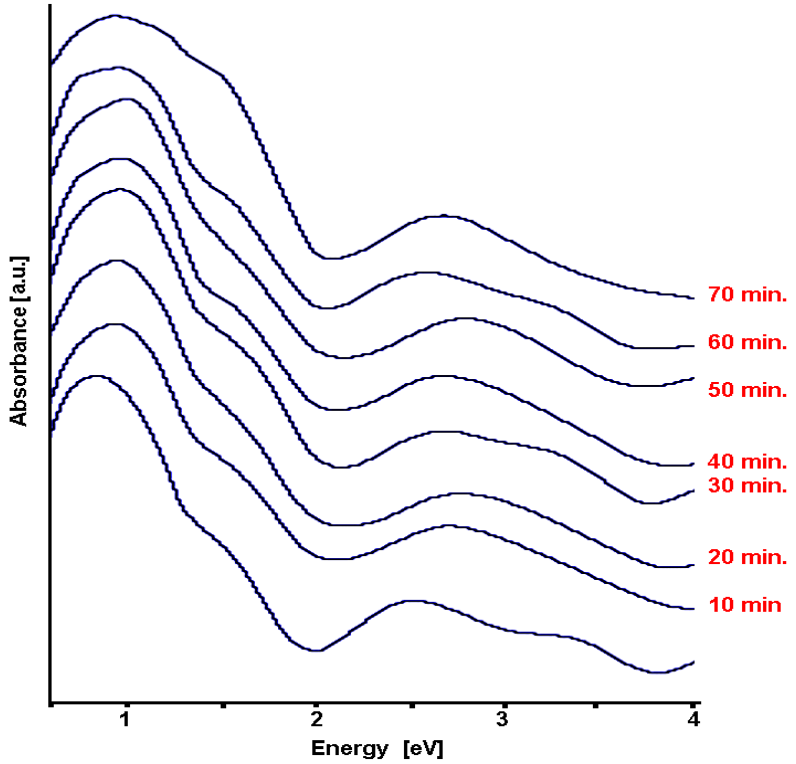


Fig 1; The UV-Vis spectra of PPy in the presence of $FeCl_3$ in time (⍰t= 10 minutes)[2]

2. IR SPECTROSCOPY:

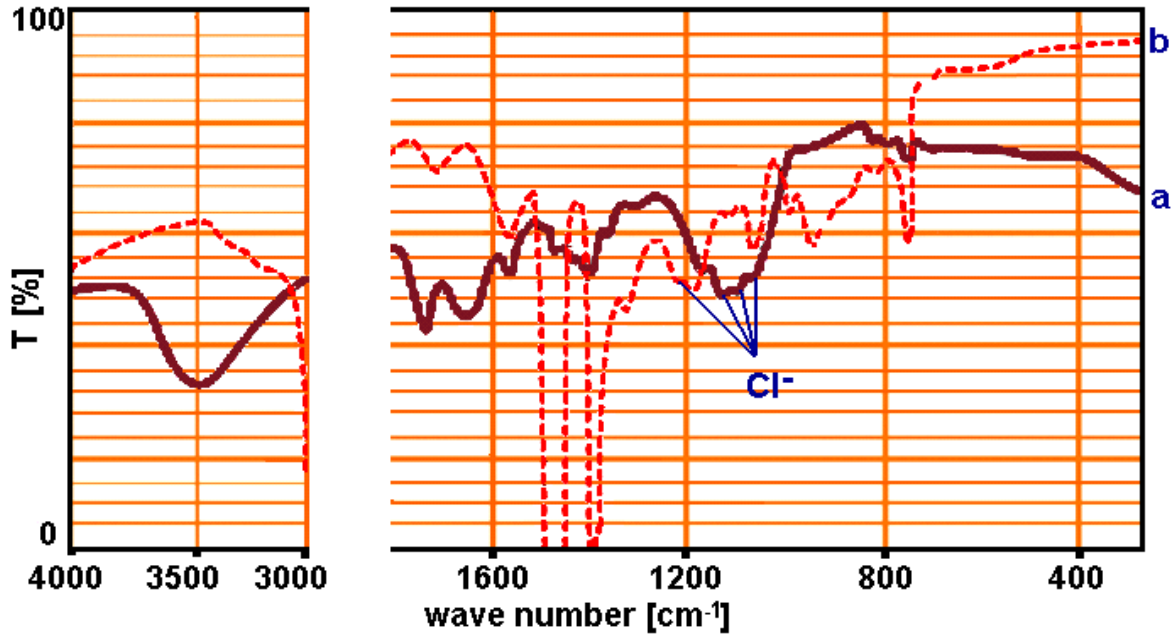
The mechanism and kinetics of the formation of polypyrrole (PPy) films were studied by IR spectroscopy, with a spectrophotometer. IR spectroscopy made it possible to conclude that the anion is linked with the links of a polymer chain with a charge transfer (Fig. 2.). The IR spectra for pyrrole in water display intensive narrow bands of plane vibrations of deformation $\delta_{pl}(CH^{-})$ at 1015, 1045, and 1075 cm^{-1}. Immediately, after adding $FeCl_3$ to the pyrrole solution, new weak bands at 1100, 1125, and 1150 cm^{-1} appear against the background of the pyrrole bands [10-12]. The intensity of the new bands increases with time, while that of the pyrrole vibrations simultaneously decreases[11] . The full width at half maximum and the mutual arrangement of these bands suggest that they refer to deformation vibrations of the pyrrole ring in a pyrrole complex.

An additional proof for the viability of the assumed mechanism involving a discharge of a complex which includes a protonated molecule of pyrrole with the anion is the fact that

a strong quantum chemical interaction exists between anions and chains of pyrrole rings in the film, as will be shown below.

Thus, the discharge of pyrrole complexes with the anion and proton and their partial destruction yields radical-cation and radical species. When interacting with active ends of pyrrole links, these induce the growth and development of polymer chains. The formation of a polypyrrole film may probably be represented by the reactions shown in the Fig.3.[13]

Fig. 2. IR spectra of Py (a) and PPy (b) in the presence of chlorine ions



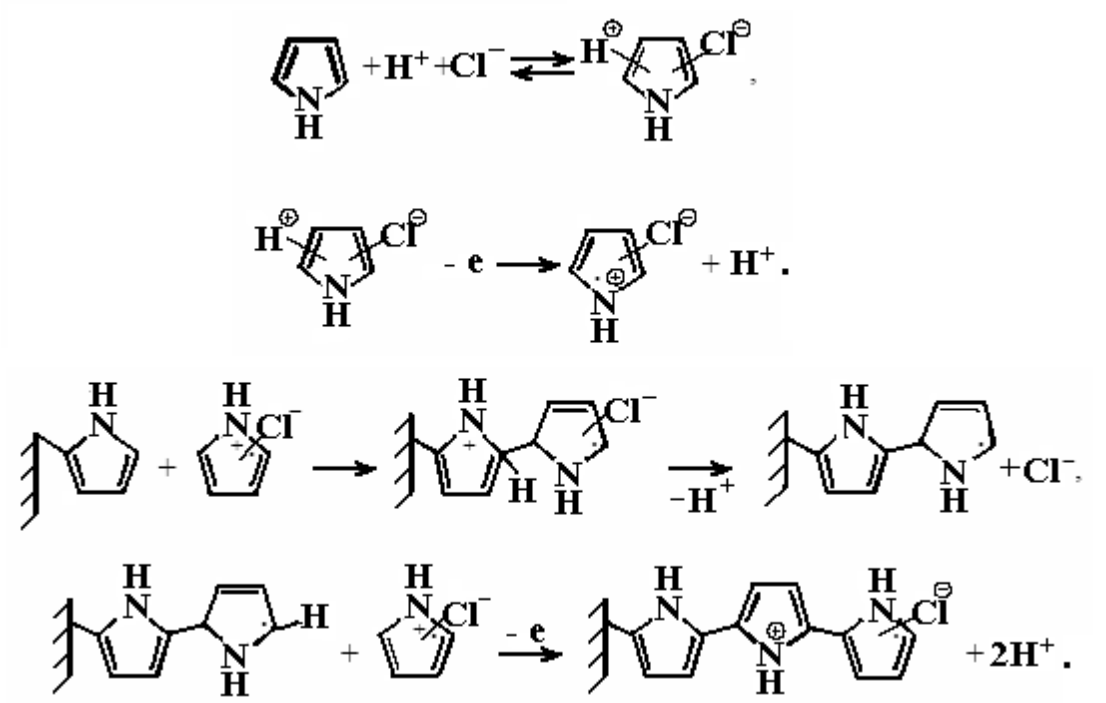


Fig.3 Reaction of pyrrole to form polypyrrole

3. SEM Image :

The morphology of the polypyrrole sample was examined using scanning electron microscope (SEM). Fig.4 represent the SEM image of PPy film in contact with the substrate surface and show the striations on the film surface and many pores and cavities. This result means the chemical synthesized deposition mechanism of the conducting polymer film is realized. The deposition mechanism mainly depends on the substrate material. [2]

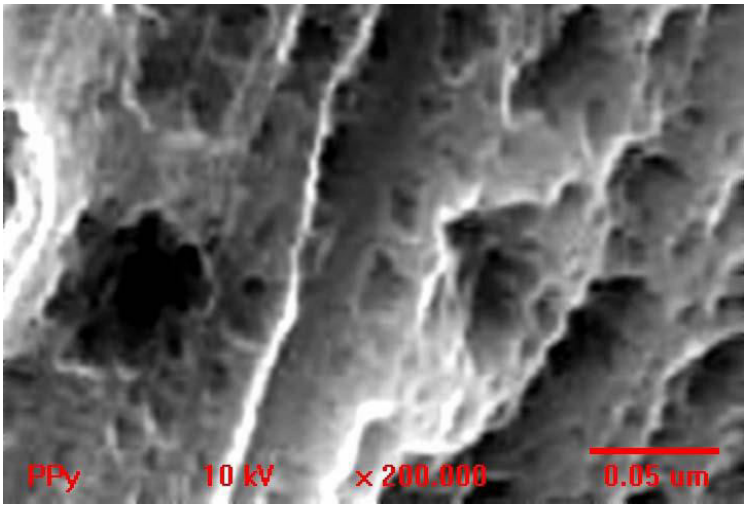


Fig 4. SEM image of the conducting PPy film surface in contacted with substrate (x200000)

4.X-ray diffraction method:

X-ray diffraction (XRD) studies were carried out by using a PAN Analytical, Rontgun Diffractometer System, with Cu-Kα (1.5418 Å) radiation operating at 30 kV and 20 mA. The XRD patterns were recorded in the range 10 to 80° with step width 0.02° and step time 1.25 sec.[3]

Applications:

1. Polypyrrole (PPy) with various morphologies were synthesized by chemical oxidative polymerization and further used as the counter electrode (CE) in **dye–sensitized solar cells** (DSSCs).[6]
2. Conducting polymers are used in **Chemiresistors** . These are simply formed by two electrodes as contact points with the conducting polymer put on to an insulate substrate (fig.5.).These capacitors have very significant role if an alternating current is used to excite those chemiresistors as well as if transient signals are involved.

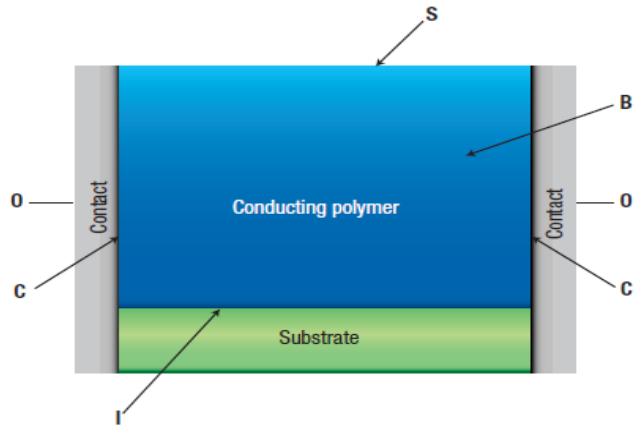


Fig.5."Chemiresistor; B: bulk of the CP, S: surface, I: interface with the insulating substrate. C: interface with the contacts".[4]

3. Polypyrrole is a potential vehicle for **drug delivery**. The polymer matrix serves as a container for proteins.[8]

4. Polypyrrole has been investigated as a **catalyst support** for fuel cells and to sensitize cathode electrocatalysts.[4]

5. Together with other conjugated polymers such as polyaniline, poly(ethylenedioxythiophene) etc., polypyrrole has been studied as a material for "**artificial muscles**", a technology that offers advantages relative to traditional motor actuating elements.[4]

6. Polypyrrole was used to coat silica and reverse phase silica to yield a material capable of anion exchange and exhibiting **hydrophobic interactions**.

7. Polypyrrole was used in the microwave fabrication of multiwalled **carbon nanotubes,** a rapid method to grow CNT's.[4]

8. A water-resistant **polyurethane sponge** coated with a thin layer of polypyrrole absorbs 20 times its weight in oil and is reusable.[14]

CONCLUSION

Conducting polypyrrole (PPy) films is prepared by chemical oxidation on of pyrrole with an oxidizing solution of $FeCl_3$. The rate of polypyrrole polymerization is determined by the rate of the initial electron-transfer reaction.

The mechanism and kinetics of the formation of polypyrrole (PPy) films were studied by UV-VIS and IR spectroscopy.IR spectroscopy made it possible to conclude that the anion is linked with the links of a polymer chain with a charge transfer. It was found that morphological property of the sample plays a significant role in achieving higher conductivity.

Now a days the applications of these conducting polymer is very wide in the field of biomedical, solar cells, chemiresistors, fuel cells, etc. They used as the counterelectrode in dye sensitized solar cells. Plays a significant role in alternating current where conducting polymers are used in chemiresistors. Potential vehicle for drug delivery polypyrrole matrix serves as a container of proteins, microwave fabrication of multiwalled carbon nano tubes, etc.

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INSILICO DRUG DESIGN AGAINST LUNG CANCER

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ABSTRACT

Lung cancer is the leading cause of cancer-related death world-wide, with non–small-cell lung cancer (NSCLC) being the predominant form of the disease. Lung adenocarcinomas also harbor activating mutations in the downstream GTPase, v-Ki-ras2 Kirsten rat sarcoma viral oncogene (KRAS), and mutations in EGFR and KRAS appear to be mutually exclusive. Recent studies indicate that patients with mutant KRAS tumors fail to benefit from adjuvant chemo-therapy and do not respond to EGFR inhibitors. Single Nucleotide Polymorphisms (SNPs) as unique genetic variations are widely used as biomarkers for drug designing and development for individualized therapy. In this study we are extracting natural compounds from plants that would act as a natural therapy against lung cancer. Here we are concentrating on docking studies to solve the mutation causing lung cancer in G12R position using various Bioinformatic tools such as Pubchem, Pymol, Autodockvena etc.

Keywords: Lung Cancer, Adenocarcinomas, Biomarkers, Bioinformatic tools.

Introduction

Lung cancer is one of the most common types of cancer which is of malignant type, the causes of lung cancer are Chronic smoking, occupational exposure, air pollution, and other factors. Smoking causes over 80% of lung cancer cases (Salim et al. 2011). The reasons for varied cancer susceptibility among smokers are still unknown. The carcinogens present in the cigarette smoke such as numerous carcinogens, including tar and benzopyrene activate signaling pathways that affect cell growth, cell differentiation, and apoptosis. Out of all the compounds present, nicotine is the primary component responsible for the addiction toward tobacco consumption, and this addiction greatly exacerbates the cumulative health dangers of tobacco (Shen et al. 2012).

KRAS (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) is an oncogenic driver with mutations in 30% of non-small cell lung cancer (NSCLC). The incidence of non-small cell lung cancer (NSCLC) has become the highest death rate of cancer worldwide (Jing et al., 2018).

Non-small cell lung cancer (NSCLC) is the most common histological type of lung cancer, approximately, about 80-85% of total cases of lung cancer (Jing et al., 2018; Zappa & Mousa, 2016). Thus, molecular assays of Rat Sarcoma (RAS) family are widely used to guide treatment

ISBN: 979-85-27243-61-1

in NSCLC patients, it represents about 30% of all human cancers have mutations in RAS genes (Kodaz, 2017).

Moreover, RAS oncogene has many cellular functions including cellular proliferation, apoptosis, migration, division, and differentiation of the cells. RAS has three known isoforms as Harvey RAS (HRAS), Kirsten RAS (KRAS) and Neuroblastoma RAS (NRAS). Mutations in KRAS account about 85% for all RAS mutations in human tumors, NRAS between 11–15%, and 1% in the HRAS gene (Kodaz, 2017; Dang, Reddy, Shokat, & Soucek, 2017).

The KRAS mutations are more frequent in lung adenocarcinoma, which seems to indicate that KRAS mutations will be a more important target in the coming years, in addition to being an indicator in the selection of drug treatment (McCormick, 2015; Westcott & To, 2013).

Cancer-associated mutations in KRAS cluster in one of three hotspots, with a majority (84%) of mutations causing single amino acid substitutions at G12 (Nature reviews Drug discovery 2014). By comparing, of the six possible single-base missense mutations that can occur at G12, G12D is the most predominant (42%), followed by G12V, G12C, G12A, G12S and G12R, the last of which occurs infrequently in most cancers. However, while the KRAS G12R mutation is rare in lung and colorectal cancers (~1%), it is the third most common KRAS mutation in PDAC (. Cold Spring Harb Perspect Med 2017).

Therpenoids present in most of the plants that are active against to lung cancer are taken for our studies.

In this study the work is carried out on G12R mutation that occurs infrequently. Although several strategies to inhibit KRAS have been explored, the quest for therapeutic inhibitors of RAS has fallen short of expectations. An obstacle to the development of specific RAS inhibitors is that mutated RAS proteins have lost their normal enzymatic function and such loss-of-function mutated enzymes are much more difficult to inhibit.

To now, there are no specific treatments available, but many novel drugs are currently under investigation in phase II and III trials. In terms of the design of clinical trials, clinicians may need to revise the endpoints used to determine drug efficacy, if reduction of the tumor mass by single agents is the sole endpoint, then many useful drugs could be discarded before they have a chance to demonstrate their potential in combination with others. With regard to this, development of appropriate surrogate markers to monitor drug action will be crucial for identifying patients most likely to benefit, thus sparing them from ineffective treatment and unnecessary adverse drug reactions (Niki et al 2012).

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METHODOLOGY.

1. **Data retrieval:** retrieval of protein and ligand.
   Two proteins- wild type and mutant proteins are retrieved from protein data bank. Ligands are retrieved from pubchem and ligand information is retrieved from uniprot.
2. **Ligand preparation:** The collected ligands are converted into 2D and then to 3D structure for docking.
3. **Protein preparatio :** Phymol tool is used where proteins are visualized to check whether they are correct or wrong arrangement and also to find missing residues.
4. **Screening:** Since proteins are visualized in phymol, and we have a set of multiple compounds derived from plants, 1st screening of the compounds are done. The compound that has highest binding affinity are taken (binding affinity is shown in minus sign) and are docked by using tool autodock vena.
   In screening, the first step is protein preparation in autodock - Protein 6CU6 is selected and then the water molecules are removed along with sidechains B and C, only A chain is docked.
   In A chain, polar charges are added (indicated by plus charge) as all the ligands docked are in minus charge. In polar water molecule, H+ and OH- are added once. Next we move forward to go Grid box where active site present in protein are observed, in active site choosing dimensions a theoretical box is created, it is called unit cell. In this unit only ligand is binded in that position.
5. The data saved earlier is used in autodock vena., where docking command is used to dock. After docking the results obtained are analysed to select componds that have maximum binding affinity and these componds are again docked and the results are saved.
6. **ADME studies:** The obtained results are undertaken for ADME studies by swiss ADME server to check toxicity.

RESULTS:

System	docking score (kcal/ mol)
KRAS G12R	-6.00

ISBN: 979-85-27243-61-1

DISCUSSION;

The compounds predicted showed ADMET properties such as good pharmacokinetic properties with the acceptable absorption, good metabolism transformation, and are found to be neither toxic nor carcinogenic.

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ISBN: 979-85-27243-61-1

A comprehensive review on chemically induced animal models for Jaundice study

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Abstract

Basic knowledge of disorder pathogenesis, anatomy and research investigation can be understood by using animal models. Deposition of excess bilirubin leads to yellow discoloration of body tissues resulting to a condition called hyperbilirubinemia. In this review, we will be summing up about chemically induced jaundice models for further research investigation on jaundice.

Keywords: jaundice, animal models, bilirubin, pathogenesis.

1. Introduction

Jaundice is one of the life-threatening, wide spread disease observed throughout the world. Jaundice is a condition in which there is increase in the concentration of serum bilirubin in the body leading to yellow discoloration of the skin hence it is also called hyperbilirubinemia.

Jaundice is broadly classified into three types based on its causes

1. Pre-hepatic Jaundice.
2. Hepatic Jaundice.
3. Post-Hepatic Jaundice.

1. Pre-Hepatic Jaundice (Increased bilirubin production)

   Pre-hepatic jaundice is also called as Hemolytic Jaundice because it is caused due to hemolysis. Increase in the hemolysis is due to poor or defective plasma membrane of Red blood cells. Patients with hemolytic jaundice are presented with Anemia, Yellowing of sclera, dark yellow-brown colored urine, yellowish skin and high bilirubin levels.

2. Hepatic Jaundice (Liver dysfunction)

   Hepatic jaundice is caused due to defect in the liver mainly hepatocytes.

   Any pathology of the liver leading to defect in capture, conjugation and excretion of bilirubin can cause hepatic jaundice.

   Hepatic jaundice symptoms include abdominal pain, fever, vomiting and nausea, gastrointestinal bleeding, diarrhea, anemia, edema, weight-loss and if unchecked leading to mental disturbances like kernicterus, coma or even death.

3. Post- Hepatic Jaundice (Duct Obstruction)

   Post-Hepatic Jaundice is also called as Obstructive jaundice. The major cause of post hepatic jaundice is extra-hepatic biliary obstruction.

   Symptoms obstructive jaundice are dark urine, pale stools and pruritus, fever biliary colic, weight loss, abdominal pain and abdominal mass. Obstructive

   Jaundice may lead to various complications including cholangitis, pancreatitis, renal and hepatic failure.

Use of natural products such as mineral plant and animal products having therapeutic properties are being used as drugs from ages, at recent times it is observed that many studies and researches are growing in alternative therapies and therapeutic use of natural products specially obtained from plants and these products are first treated either on animal or animal cell lines.**[1,2,3,4]**

2. *In-vitro* studies and *In-vivo* studies

2.1. *In vitro studies*

In-vitro studies involves the growth of cells outside the body in a laboratory environment. Cells and tissues from the liver, kidney, brain, skin etc. are isolated from an animal body and is stored in a suitable growth medium outside the animal body, it can be stored for few months or even for few years. Various types of cultures like cell culture, callus culture, tissue culture and organ culture are cultured for various purposes. These studies are continuously being used for preliminary screening of potential drug molecules to check their toxicological effect and efficacy of herbal medicines.

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2.2. *In-vivo* studies

In-vivo study refers to research or work is done with or within an entire living organism. It mainly human clinical trials and animal studies.

Human Clinical Trials- In a clinical trial, participants that is human volunteers receives specific interventions according to the research protocol created by the investigators. These interventions may be medical products, such as drugs or devices, procedures, or changes to participant's diet.

Animal studies- Various animals such as rats, mice, hamster, rabbits, fishes (zebra fish, trout), birds (mainly chicken), guinea pigs, amphibians (xenopus frogs), primates etc. are being used and practiced in biological research and medicine from a long time.

The main purpose of animal studies is to discover and develop new drugs and treatments for both infectious and non-infectious diseases. Animals also helps in understanding the effects of medical process, physiological, patho-physiological conditions, surgical experiments, assessments of novel vaccines and therapies.

A good animal model should be good, practical and useful. They should possess some characteristics that resemble to the desired correlation example, Insulin was tested in rabbits because it showed that insulin decreases blood glucose in rabbits similar to humans.

Animals are chosen by its lowest phylogenic scale, similar to both human physiology and anatomy, low cost and also life span of animal must be considered.**[6,7,8,9]**

3. Bilirubin Metabolism

Bilirubin is a yellow-colored pigment, with molecular formula of $C_{33}H_{36}N_4O_6$which is a product of heme catabolism and destruction of premature erythroid cells in bone marrow as shown in the figure 1

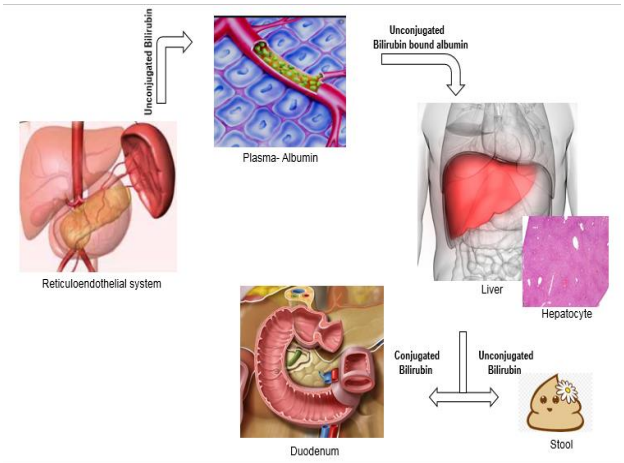

Fig 1: Depicts Bilirubin metabolism and transportation

Hemolysis of Red blood cells (RBCs) releases Hemoglobin which is broken down into heme and globin. Heme ring consists of four pyrroles linked by carbon bridges with central iron atom, heme is takenup by spleen, phagocytes or kupffer cells of liver where further heme oxygenase cleaves heme to iron ion and this ion catalyzes oxidation of Carbon Bridge which in turn results in release of carbon monoxide and green pigment called biliverdin.

Heme oxygenase is the rate limiting factor and is present in high concentration in Kupffer cells of liver. Biliverdin is further converted to unconjugated bilirubin yellowish-orange pigment by NADPH dependent biliverdin reductase. Bilirubin produced is highly compact due to hydrogen bonding and is insoluble in water hence it is called unconjugated bilirubin. This unconjugated bilirubin binds to albumin due to high binding affinity with albumin thus no free bilirubin is found in the plasma and is transported to the liver by two mechanisms- passive diffusion and receptor mediated endocytosis, where it is conjugated to hepatocyte by uridine diphosphate-glucuronyl transferase(UGT) with glucuronic acid, product synthesized is bilirubin diglucuronide and excreted into bile in the duodenum. Conjugation of bilirubin to water soluble form is important in order to eliminate from body through liver and kidney.

Bilirubin excretion in colon, colonic bacteria deconjugate bilirubin into urobilinogen is colorless. Further urobilinogen oxidized by intestinal bacteria and converted to stercobilinorange-yellow colour and excreted through faeces.

20% of the total urobilinogen is reabsorbed into the bloodstream as part of the enterohepatic circulation and carried to the liver where some is recycled for bile production and a small percentage reaches the kidneys where it is oxidized further into urobilinogen and excreted out of body through urine.

This Bilirubin metabolism is different in new born compared to adults.The rate of neonatal RBC destruction is higher compared to adults resulting in greater quantity of hemoglobin release. The level of glucuronyl transferase is initially low in the newborn and increase in the rate of bilirubin formation can affect the capacity to conjugate resulting in increase in bilirubin concentration. Studies shows approximately

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80% of newborns have some form of hyperbilirubinemia.

When bilirubin level exceeds 34.2 µmol/L or 2 mg/dL symptoms of jaundice appears. On a daily basis 250-300 mg bilirubin is produced in normal adults. The amount of bilirubin produced in neonates is higher than adults.**[2,3,4,5]**

4.Animal models on Hyperbilirubinemia

Chemically Induced Jaundice models- Jaundice induced by means of chemical agents.

4.1 Phenylhydrazine

Phenylhydrazine appears to induce jaundice because of its hemolytic activity. Jaundice was induced in Wister adult rats weighing 180-200g both male and female rats by treating them with phenylhydrazine 5mg/kg body weight for five days intraperitoneally. Phenylhydrazine solution is prepared by adding 100mg of phenylhdrazine with 10ml of 0.01 M sodium phosphate buffer. After 5 doses increase in the serum bilirubin is observed by measuring the concentration of total serum bilirubin. Histopathological studies of liver samples can throw some light on mechanism of action of drug.**[10]**

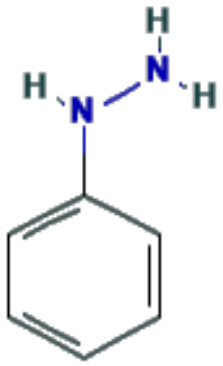

Fig 2: Structure of Phenylhydrazine

4.2 Phenylhydrazine and ursodeoxycholic acid

Has reported phenyl hydrazine induce hemolytic anemia causing hyperbilirubinemia and ursodeoxycholic acid is a bile acid inhibits UDP-glucoronosyl transferase in liver preventing excretion of bilirubin.White male rabbits of age 4 to 6 months weighing 1kg to 1.5kg was treated orally with phenylhydrazine solution i.e. 10% of phenylhydrazine in 20% of ethanolfollowed by immediate intraperitoneal injection of ursodeoxycholic acid. Progress of hyperbilirubinemia is determined by drawing the blood after 24 hours and measuring its total serum bilirubin and direct bilirubin**.[11]**

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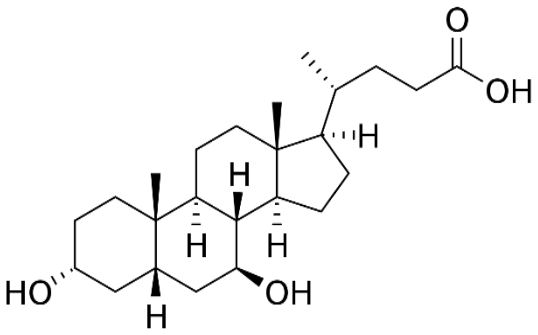

Fig 3: Ursodeeoxycholic acid

4.3 Alpha- aminolevulinic acid (ALA)

In this model hyperbilirubinemia is developed in rats by the use of heme precursor delta-aminolevulinic acid. ALA 100µmol/100g body weight was given intraperitoneally to male rats weighing 175-225g between 24, 20 and 16 hours before sacrifice for examination Increase in the serum bilirubin is calculated by measuring total serum bilirubin.**[12]**

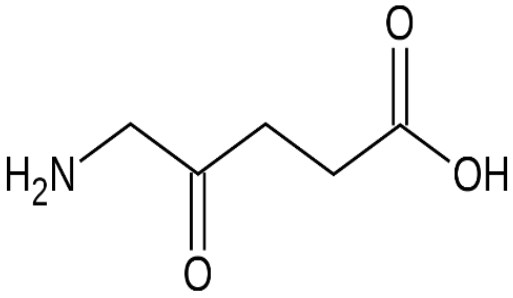

Fig 4: Alpha-aminolevulinic acid

4.4 Alpha-Naphthyl Isothiocyanate (ANIT)

Many studies shows that alpha-naphthyl-isothiocyanate intoxication are being very useful experimental model in research and especially in histochemical study of liver.In case of short term experiment alpha-naphthyl-isothiocyanate is proceeded with high dosage (150-200 mg/kg body weight) whereas for long term experiment alpha-naphthyl-isothiocyanate is provided in low dosage (45 mg/kg body weight). Here in this model albino rats weighing 240-350gm was given orally 0.1ml oil i.e. alpha-naphthyl-isothiocyanate which was dissolved in sufficient olive oil and it is observed that icteric urine, yellowish discoloration of the ears symptoms of jaundice appeared after 36 hours.**[13]**

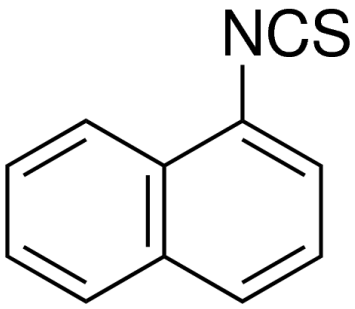

Fig 5: Alpha-Naphthyl Isothiocyanate

CONCLUSION:

Present paper concludes with Jaundice is a self - evident exposition of high level of bilirubin in the body. Bilirubin metabolism plays a very important role in jaundice. Rats, mice, rabbits are few of the animal models used in the research work carried out by many researchers and investigators. Different chemicals can induce jaundice in various animal models. Chemicals like phenylhydrazin, ursodeoxycholic acid, ALA, ANIT induce jaundice in the animal models which shows all the symptoms of jaundice, high levels of bilirubin and hyperbilirubinemia.

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Nanotechnology based mimic of natural red blood cells

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ABSTRACT

Artificial RBCs are the systems that has bio-mimic nature cellular functions to recapitulate either function or structure of red blood cells present in the blood. The development of artificial RBC as blood substitute is mainly for the use of oxygen carrier's and removal of carbon dioxide throughout the body. In recent decades' nanotechnology has the wide application in the field of medicine and it makes use of materials engineering strategies to create two types of RBC substitutes such as semi-synthetic RBC and synthetic RBC. The poly-haemoglobin (Semi synthetic) is the first artificial RBC substitute that has been used for the function of oxygen transport. In addition to that, the poly-haemoglobin catalase superoxide dismutase has been used for oxygen supply and antioxidant activity. Moreover, the artificial RBCs are conjugated with PEG-lipid membrane or with PEG-PLA polymer membrane to enhance the controlled sustained release of RBCs in the blood streams. Likewise, the other synthetic RBC substitute is respirocyte (Perfluro carbons) has the capability for the controlled release of oxygen and absorption of carbon dioxide based on selective transport mechanism. Artificial RBC have tried to overcome the limitations such as insufficient blood supply, blood typing, shelf-life, risk of disease in natural blood. Additionally, it can be used to meet the oxygen demands after the traumatic brain injury and injured brain tissues. The artificial RBCs are even used for specific functions like suppression of growth of melanoma (polyhaemoglobin-tyrosinase) and platelets like function (polyhaemoglobin-fibrinogen). Nanobots with application of sensors that can provide the scanning of entire body treating the specific tissue damage and perform minor operational functions, these strategies of artificial RBC's can be used in exhalation of toxic gases from the body. In this review article, we foresee the future applications of artificial RBC for the treatment of Thrombocytopenia.

Keywords: **Artificial RBC, Poly haemoglobin, Conjugate haemoglobin, Nanobots, Respirocytes, Perfluro carbons, Super oxide dismutase, PEG-PLA.**

1. INTRODUCTION

Every year blood transfusion saves millions of lives in the world. According to data given by World Health Organisation, in south east asian region there is a requirement of 15 million units of blood but the blood collected is only 9.3million units with a huge gap of 5.7 million units of blood [1]. In India the required amount of blood is around 9-9.5 million units per year but only 5-5.5 million of units are collected per year. In addition to that, the underdeveloped countries have more shortage of blood comparatively than developed countries [2]. Therefore, to meet the needs of the blood, the scientific community developed artificial RBCs. These artificial RBC's must be free from toxic side-effects, antigenicity, pathogenicity caused by bacteria or viruses. Artificial RBC's are of 2 types that is semi synthetic RBC and synthetic RBC. Semi synthetic RBC is haemoglobin based where the haemoglobin is obtained from the expired blood or the waste blood, synthetic haemoglobin involves use of perfluorocarbons along with emulsifiers. The first work based on Nano biotechnological approach of artificial RBC's reported was cross linking of haemoglobin into polyhaemoglobin membranes [3,4], then the artificial cells were made much smaller by cross linking into poly haemoglobin of Nano dimensions. Glutaraldehyde was used to crosslink haemoglobin to poly haemoglobin of Nano dimensions [5]. Further, the advancements included assembly of haemoglobin, catalase (CAT) and super oxide dismutase (SOD) in soluble Nano dimension complex (Hb-CAT-SOD) by using Nano biotechnological approach.

2. HAEMOGLOBIN BASED OXYGEN CARRIERS (HBOC'S)

Haemoglobin-based substitutes use haemoglobin derived from several different sources: human, animal, and recombinant DNA technology developed haemoglobin. Human haemoglobin is obtained from donated blood that has reached its expiration date and from the small amount of red cells collected as a by-product during plasma donation. HBOCs from expired human blood or fresh bovine blood have to undergo numerous modifications to make them safe and effective oxygen carriers. The RBCs are first lysed to release their haemoglobin, and then the stroma is removed by several methods, including centrifugation, filtration and chemical extraction. The stroma-free

haemoglobin is then purified and undergoes modifications to cross-link, polymerize or conjugate it to other compounds. Without these modifications, the oxygen affinity of the stroma-free haemoglobin is too great to facilitate oxygen release in the tissues. The genetic or chemical modifications of haemoglobin and four different HBOCs have been considered: cross-linked haemoglobin, polymerized haemoglobin, haemoglobin conjugated to macromolecules and encapsulated haemoglobin. Although there is an increasing concern that the worsening shortage of blood donors will eventually limit the availability of human haemoglobin for processing [6].

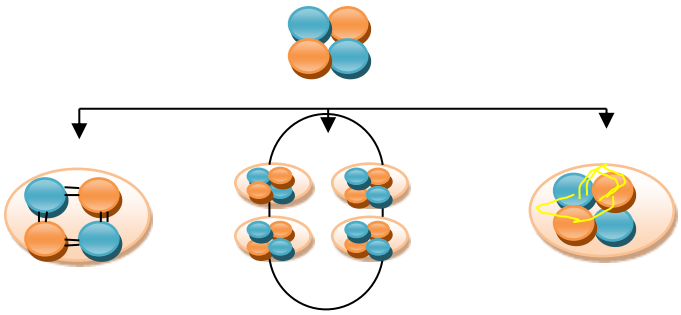

Fig 1. Depicting the cross linked Hb, polymerized Hb, conjugated Hb [21].

2.1 Cross-Linked Haemoglobin

The cross-linked tetrameric haemoglobin was developed by Bunn and Jandl by cross linking haemoglobin intramolecular in the year 1968 [7]. One of the effects observed with the infusion of this blood substitute is Vaso activity. Later a Diaspirin cross-linked haemoglobin (DCLHb) was developed by Baxter Healthcare ; it was the most widely studied of the haemoglobin-based blood substitutes, used in more than a dozen animal and clinical studies [8]. In the category of blood substitutes, diaspirin cross-linked haemoglobin (DCLHb) is the prototype molecule. It consists of cross-linking between the two a- chains that lend stability to the molecule. The cross-linking agent is bis (di-bromosalicyl) fumarate (DBBF). This process forms a solution which is chemically stable, has low viscosity, a longer intravascular retention time than haemoglobin monomers or dimers, and an oxygen carrying capacity similar to whole blood. DCLHb made from outdated human blood has a shelf life of approximately 9 months when frozen and 24 h when refrigerated. The intravascular half-life is 2–12 h and is dose dependant. By Clinical trials it was proved that this compound did indeed raise blood oxygen content, but it also caused intense vasoconstriction resulting in

increased systemic pressure, reduced cardiac output, and increased vascular resistance. Hence, no net benefit was derived from this product. Initially the US Army performed a great deal of work on DCLHb. But the army discontinued further development of this molecule, due to significant adverse effects associated with it. Subsequently, in 1998 after the product failed trials in patients with stroke and trauma, Baxter Healthcare halted further development of DCLHb [9].

2.2 Polymerized Haemoglobin

Polymerized haemoglobin (PolyHb) is currently undergoing development as a red blood cell substitute and is a promising haemoglobin (Hb)-based oxygen carrier [10]. In the process multiple Hb proteins are linked together through the use of di--aldehydes, such as glutaraldehyde and glycoaldeyde. The size of molecules is increased through the formation of Hb oligomers by the polymerization of Hb through intermolecular cross-linking. The increase in size as a result of polymerization prevents the rapid excretion of the molecule, prolonging the Hb plasma half-life. PolyHeme is a prototype glutaraldehyde cross-linked polymer of human Hb in which two or more tetramers are covalently linked. To facilitate oxygen offloading a pyridoxal molecule is incorporated into each tetramer of PolyHeme. It has a half-life of 24 h, a shelf life longer than 12 months when refrigerated. Currently, PolyHeme is being tested in phase III prehospital trauma study. Results of a trial showed that 75 % of patients with red cell haemoglobin levels less than 1 gm% survived traumatic injury after receiving PolyHeme as compared to 16 % of historical controls at the same haemoglobin level, which was published in the year 2002 [11]. Another potential source of haemoglobin is a Bovine haemoglobin, which after modification can be used in human subjects. Its production is based on reacting pure bovine haemoglobin with three chemicals: o-adenosine 50 - triphosphate (o-ATP), o-adenosine, and reduced glutathione (GSH). This process chemically modifies the bovine haemoglobin to generate ‘‘beneficial activities’’. The o-adenosine when counteracts with the haemoglobin properties causes narrowing of blood vessels as previously seen with other substitutes. The inflammation that is often caused by free haemoglobin can be reduced by the GSH with adenosine attached to it. Hemopure (inter-molecular cross-linked cow haemoglobin) is smaller in size (up to 1,000 times smaller than a typical red blood cell) and has less viscosity than human red blood cells. It can carry more oxygen at a lower

blood pressure than red blood cells and can also carry oxygen through partially obstructed or restricted blood vessels, where RBCs cannot reach. In South Africa since 2001, Hemopure was licensed for compassionate use in humans, but US Food and Drug Administration has imposed a ban on further clinical testing of the product on human test subjects in the United States [11].

2.3 Recombinant Haemoglobin

The use of recombinant technology to produce a genetically altered haemoglobin from *E. coli* results in a functional haemoglobin which avoids infectious risks [12]. It is cross-linked haemoglobin made by genetically altered organisms such as the bacteria Escherichia coli or Saccharomyces cerevisiae yeast. By inserting the gene for human haemoglobin into bacteria and then isolating the haemoglobin from the culture we can obtain Recombinant haemoglobin. To prevent dissociation into dimers and to help maintain adequate oxygen affinity, a few parts of the amino acid sequence of haemoglobin produced are replaced. This altered human haemoglobin coding segment DNA can be inserted into the bacteria or yeast DNA allowing for the manipulation of the gene itself to create variant forms of haemoglobin. From 750 l of E. coli culture 1 unit of haemoglobin solution can be produced. The trials have been discontinued as this recombinant haemoglobin leads to adverse effects causing vasoconstriction, GI distress, fever, chills, and backaches [10].

2.4 Conjugate haemoglobin

Chang in 1964 reported that conjugated haemoglobin can be formed in presence of diamine, sebacyl chloride cross linking haemoglobin with polyamide this will be in the form of Hb nan spheres [13,14] later in 1968 Wong extended the basic work to form haemoglobin dextran conjugates, furthermore in 1970 Davis group and Iwashita et al in 1980 extended to produce conjugate haemoglobin using polyethylene glycol [15,16].

2.5 Dextran haemoglobin

Dextran haemoglobin is a type of conjugate haemoglobin which involves conjugation of haemoglobin with dextran, as dextran is a hydrophilic polymer it is recently employed to create the Hb-dextran conjugates which will serve the purpose of haemoglobin oxygen carriers. Wang et al [17] has developed Hb-dextran conjugate of 30nm diameter which has a lower toxicity when compared to other Hb polymer conjugate. As one of the problem for development of Hb conjugate is that the Hb can be easily

oxidized to meet Hb which causes vasoconstriction when artificial RBC's are administered into the body. Hb-dextran conjugate was prepared by protecting the moieties of heme group, which was done by modifying the thiol moiety of amino acid residue Cys-93(β) thereby preventing the oxidation of Hb to meet Hb [18].

2.6 Encapsulation Strategies

Haemoglobin is encapsulated by membrane of RBC this encapsulation will entrap multienzyme and avoid conversion of Hb to metHb with no modifications in enzyme's secondary structure. If there are any alterations in enzymes secondary structure, then the oxygen binding and releasing properties are altered. Therefore, to prevent the chemical modifications of enzymes secondary structure and to increase the circulation time of artificial RBC's in blood encapsulation is done. Nanotechnology helps in encapsulating many compounds inside a delivery vehicle, which will help in protecting compounds from plasma induced effects, to enhance controlled sustained release of RBC inside the body, in addition to all these encapsulations also helps to decrease high viscosity of Hb [19]. Hb encapsulation was introduced few decades ago by Chang. It was done using double-emulsion-solvent evaporation technique, where encapsulation of haemoglobin was done using nanoparticles which are amphiphilic in nature such as polylactic acid(PLA), polylactic acid co-glycoside (PLGA), polylactic acid-polyethelene glycol(PLA-PEG) as matrix. Based on the required vesicle size, thickness of membrane and efficiency of drug encapsulation a proper selection of polymers molecular weight and concentration should be done [18,20].

3. APPLICATION:

Blood supply demand is increasing as compared to blood donations in the world. The application is to provide metabolic support in the event of impaired circulation, poor blood flow, that can result in serious tissue damage. These are blood substitutes belong to the universal blood group O negative, they can be given to patients regardless of their blood type, used during kidney transplantation, accidents, aplastic anemia and swollen tissues in sickle-cell anemia. When blood substitutes are manufactured they can be sterilized to destroy bacteria and viruses. This eliminates the risk for infectious diseases in a blood transfusion – a major issue in many parts of sub-Saharan Africa. With a longer shelf life, it can be stored for one to three years without

ISBN: 979-85-27243-61-1

refrigeration. Also, patients whose religious beliefs prevent them from accepting blood from donors would benefit from blood substitutes such as PFCs that are not derived from blood products [21, 22, 23, 24].

3.1 Thrombocytopenia

Immune thrombocytopenia (ITP) is an autoimmune bleeding disorder characterized by abnormal low levels of blood cells called platelets, a disease which is referred to as thrombocytopenia A normal platelet counts ranges from approximately 150,000 to 400,000 per microliter of blood. If someone has a platelet count lower than 100,000 per microliter of blood with no other reason for low platelets, than that person might have ITP [25]. During the primary hemostasis, platelets bind rapidly to specific proteins (e.g., von Willebrand Factor) and collagen at the bleeding site, followed by inter-platelet crosslinking via fibrinogen, which is recognized by the active GPIIb-IIIa on the surface of platelet molecules; on the phosphoserine-rich surface of active platelets, fibrin is formed and deposited in the process of coagulation cascade. Altogether, such coagulation process includes activation, adhesion and aggregation of platelets, as well as deposition and maturation of fibrin [26]. People diagnosed with ITP in USA is estimated to be 3.3 per 100,000 adults/year. Worldwide, it is estimated that there are almost 200,000 people affected by ITP and also 5% of pregnant women develop mild thrombocytopenia right before childbirth [25]. It can be diagnosed by physical exam, blood count, blood clot test, bone marrow biopsy, imaging tests and many other. There are some methods to treat, which includes medication, blood transfusion, splenectomy and using steroids [27]. Natural platelets offered during blood transfusion cases, suffer from short shelf-life, contamination, risks of infection/immunoreaction (unless prior serological testing was conducted). Artificial platelet-like Nano-biomaterials attract increasing attention to overcome such issues [26].

3.2 Approach to thrombocytopenia using artificial RBC's

Artificial RBC approach to treat thrombocytopenia is achieved by polyhaemoglobin-fibrinogen which deliver oxygen to tissues and also reduce the risk of excessive bleeding by performing similar functions to that of platelets. Polyhaemoglobin fibrinogen can be formed by cross linking fibrinogen to polyhaemoglobin. Polyhaemoglobin and polyhaemoglobin fibrinogen was compared

to measure the coagulation time, polyhaemoglobin fibrinogen showed similar coagulation time as that of blood. Brief procedure of poly haemoglobin fibrinogen: fibrinogen of 40mg is dissolved in 4ml of lactate and was added to polymerizing haemoglobin solution after 4 hours of polymerization. After 24 hours of polymerization reaction was stopped using 2.0M lysine solution in molar ratio of 200:1 lysine to haemoglobin. The solution was then dialyzed against ringer's lactate overnight [28].

4. Conclusion

The first generation of modified Hb based on cross-linked Hb is already well into human clinical trials, but Phase III clinical trials have to be completed before their routine clinical usefulness can be determined. Meanwhile, new generations of modified Hb are being developed that can modulate the effects of nitric oxide. Other systems are also being developed, including modifying the antioxidant properties of Hb for clinical applications that might have potential problems with oxygen radicals. A further development is the use of lipids or biodegradable polymer membranes to prepare artificial RBCs containing Hb and their complex [29]. However, although many important steps have been taken to date, no oxygen-carrying blood substitutes are approved for use by the US FDA. Currently, the only clinical product used are saline solutions to expand the volume of blood, which maintains blood pressure and allows red cells to keep working and regenerating.

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POLYPYRROLE PREPARATION AND CHARACTERIZATION BY CHEMICAL OXIDATION OF PYRROLE IN AQUEOUS FERRIC CHLORIDE SOLUTION

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ABSTRACT:

Polypyrrole (PPy) a conducting polymer was synthesized by chemical oxidation method using pyrrole monomer (mPPy) in aqueous solution with oxidizing solution of ferric chloride ($FeCl_3$) . Polymers having conjugated pi- electron (i.e. the system is having C=C conjugated bonds) these shows unusual electronic properties such as low ionization potential,low energy optical transition and high electron affinities.

The prepared Polypyrrole product were characterized by different techniques. They are UV-VIS and IR spectroscopy, differential scanning calorimetry (DSC), X-ray diffraction (XRD), and scanning electron microscopy (SEM).

INTRODUCTION:

Polypyrrole compound was formed by a number of pyrrole ring monomers is an immanent conductive polymer this is due to interchain jumping of electrons. Polypyrrole is easy to prepare and its surface charge characteristics can easily be modified by changing the dopant anion (X^-) that is incorporated during synthesis. Polypyrrole was the first of conducting polymers that shows relative high conductivity [2].

Polymerization occurs readily in the presence of different oxidants, such as $FeCl_3$ and $K_2S_2O_8$. More studies have been reported about the formation of PPy films on solid surfaces by chemical polymerization of pyrrole. There are reports about the polymerization of pyrrole onto printed circuit boards and various textile composites. In the present work, the conducting polypyrrole (PPy) films were synthesized by chemical oxidation of pyrrole with $FeCl_3$ in aqueous methods by mixing a solution of pyrrole with an oxidizing solution of $FeCl_3$.[2]

EXPERIMENT:

Materials used:

Pyrrole monomer (mPPy) 98%. Anhydrous ferric chloride solution ($FeCl_3$) 98%, and Ammonium persulphate ($N_2H_8S_2O_8$) these two were used as oxidizing agent.

Synthesis:

Polypyrrole (PPy) was synthesized by the method called chemical oxidative polymerization using pyrrole monomer and oxidants. This is carried out in a beaker containing 100ml distilled water and given volume of pyrrole monomer then quickly added required amount of ferric chloride solution. Magnetic stirring was maintained vigorously for the dispersion of pyrrole. Addition of cold distilled water was done slowly since this was an exothermic reaction. This experiment was carried out for a period of four hours at a temperature of 25^0C. Fine black particles were appeared after the prescribed time of polymerization. Filtered the product by using filter paper and washed with distilled water and ethanol for several times. Polypyrrole was dried in a vaccum oven for overnight at 40^0C.

Characterisation of product has been done by using following methods.

1.UV-VIS SPECTROSCOPY

The UV-VIS absorption diffuse-reflectance spectra were recorded on a spectrophotometer with double beam and microprocessor (fig.1) [2]. These spectra are the typical spectra of polypyrrole and constant progressive increase of pyrrole bands. These bands are observed due to radicalic polymerization, correlated with chemical bond between pyrrole rings in polymer and chloride anions from ferric chloride.

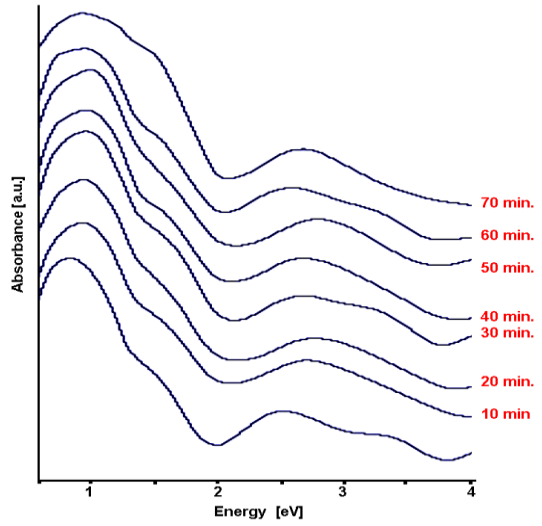

Fig.1 The UV-VIS spectra of PPy in the presence of ferric chloride in time (t=10 minutes)

2. IR SPECTROSCOPY:

The kinetics and mechanism of the product Polypyrrole films were studied by this IR spectroscopy by using spectrophotometer. IR spectroscopy made it possible to conclude that the anion is linked with the links of a polymer chain with a charge transfer (Fig. 2.).

The IR spectra for pyrrole in water display intensive narrow bands of plane vibrations of deformation $\delta_{pl}(CH^{-})$ at 1015, 1045, and 1075 cm^{-1}. Immediately, after adding $FeCl_3$ to the pyrrole solution, new weak bands at 1100, 1125, and 1150 cm^{-1} appear against the background of the pyrrole bands [10]. The intensity of the new bands increases with time, while that of the pyrrole vibrations simultaneously decreases [11] . The full width at half maximum and the mutual arrangement of these bands suggest that they refer to deformation vibrations of the pyrrole ring in a pyrrole complex.

An additional proof for the viability of the assumed mechanism involving a discharge of a complex which includes a protonated molecule of pyrrole with the anion is the fact that a strong quantum chemical interaction exists between anions and chains of pyrrole rings in the film, as will be shown below.

Thus, the discharge of pyrrole complexes with the anion and proton and their partial destruction yields radical-cation and radical species. When interacting with active ends of pyrrole links, these induce the growth and development of polymer chains. The formation of a polypyrrole film may probably be represented by the reactions shown in the Fig.3.[13]

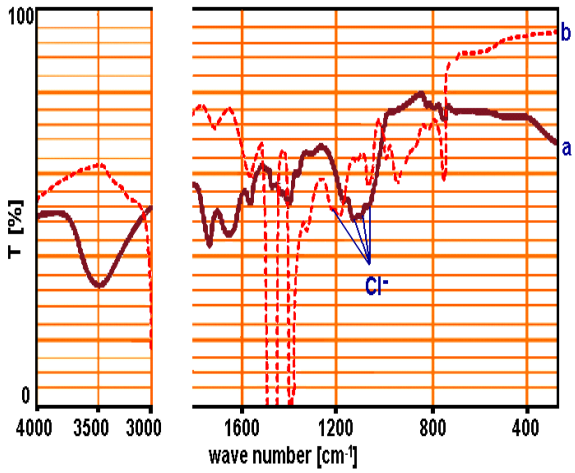

Fig. 2. IR spectra of Py (a) and PPy (b) in the presence of chlorine ions

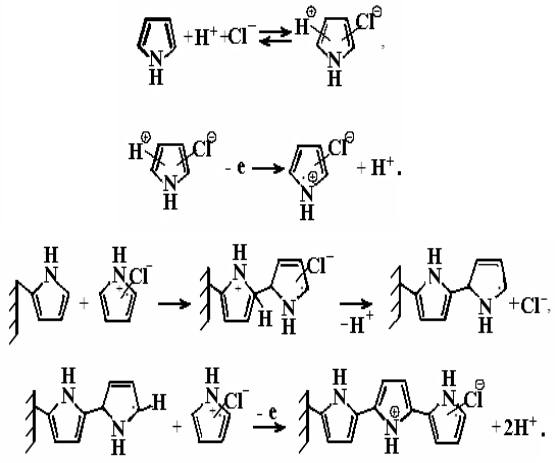

Fig.3 Reaction of pyrrole to form polypyrrole

3. SEM IMAGING:

The microstructure of Polypyrrole was examined by using scanning electron microscope (SEM). SEM image of the product which was in contact with the surface of substrate show grooves with many pores and cavities as shown in the Fig.4. This result means the chemical synthesized deposition mechanism of the conducting polymer film is realized. The deposition mechanism mainly depends on the substrate material. [2]

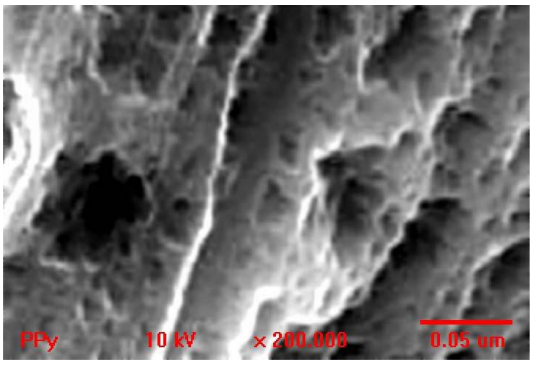

Fig 4. SEM image of the conducting PPy film surface in contacted with substrate (x200000)

4.X-RAYDIFFRACTION METHOD:

X-ray diffraction studies were carried out by using Diffractometer. The X-ray diffraction patterns of product were recorded in the range 10 to 80^0 with step width 0.02^o and step time 1.25 sec[3]

Applications:

1.Polypyrrole were synthesized by chemical oxidative polymerization and used as counter electrode in dye-sensitized solar cells (DSSCs).[6]

2.Conducting polymers are used in Chemiresistors . These are formed by two electrodes are in contact with conducting polymer which are placed on insulating substrate (fig.5.). These capacitors play a significant role to excite chemiresistors while alternating current is used and it involved transient signals.

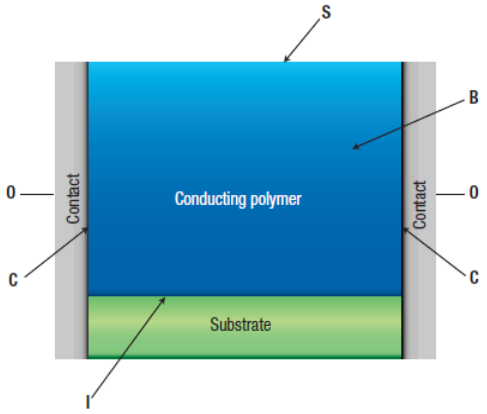

Fig 5.Chemiresistor, B: bulk of the CP, S: surface, I:interface with the insulating substrate. C: interface with the contacts. [4]

3. Polypyrrole is a potential vehicle for drug delivery. The polymer matrix serves as a container for proteins.[8]

4. Polypyrrole has been investigated as a catalyst support for fuel cells and to sensitize cathode electrocatalysts.[4]

5. Together with other conjugated polymers such as polyaniline, poly (ethylenedioxythiophene) etc., polypyrrole has been studied as a material for "artificial muscles", a technology that offers advantages relative to traditional motor actuating elements.[4]

6. Polypyrrole was used to coat silica and reverse phase silica to yield a material capable of anion exchange and exhibiting hydrophobic interactions.

7. Polypyrrole was used in the microwave fabrication of multiwalled carbon nanotubes, a rapid method to grow CNT's.[4]

8. A thin layer of polypyrrole which is coated on a water resistant polyurethane sponge absorbs 20 times its weight in oil and it can be reusable. [14]

CONCLUSION

Polypyrrole films which are conducting in nature were prepared by chemical oxidation of pyrrole with ferric chloride solution .The rate of polymerization is determined by the rate of initial electron-transfer reaction.

The prepared Polypyrrole films were characterized by many techniques as explained above. The mechanism and kinetics of formation can be achieved by UV-VIS and IR spectroscopy. Anion is linked with the links of a polymer chain with a charge transfer is concluded by IR spectroscopy. It was found that morphological property of the sample plays a significant role in achieving higher conductivity.

Now a day the applications of this conducting polymer are very wide in the field of biomedical, solar cells, chemiresistors, fuel cells, etc. They used as the counter electrode in dye sensitized solar cells. Plays a significant role in alternating current where conducting polymers are used in chemiresistors. Potential vehicle for drug delivery polypyrrole matrix serves as a container of proteins, microwave fabrication of multiwalled carbon nano tubes, etc.

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A CHEMICAL AND ELECTROCHEMICAL INVESTIGATION INTO THE USE OF CERIUM IONS TO MITIGATE ALKALINE CORROSION IN MILD STEEL STRIPS.

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Abstract - In this present work, the deterioration behaviour of Mild steel (MS) in a 3.5% NaCl solution was studied using Ce (IV) ammonium nitrate inhibitors. Rare earth metal (REM) organic compounds can deliver certain environmental secure as well as a safe alternative to chromates as corrosion inhibitory for few steel and aluminium applications. Cerium (IV) ammonium nitrate has been studied with electrochemical impedance spectroscopy (EIS) and potentiodynamic polarization, for the corrosion effect of steel in a solution of NaCl 3.5% at 25 ±1 °C. Tafel plots clearly show the corrosion inhibition property of a basic solution of a rare earth compound Cerium (IV) Ammonium Nitrate over an MS surface. According to the AC impedance spectroscopy technique, the electrical double layer values decrease as the concentration of the corrosion inhibitor increases.

Keywords - Rare earth compounds, Corrosion, AC Impedance, Polarization technique.

I. INTRODUCTION

Inhibitors are one way of corrosion hindrance. [1] Per annum, some several billion of dollars are spent on investment replacement and metal corrosion control methods for facilities.[2]As municipal and management guidelines for usage and removal of chemicals have become extra strict, current developments are seeing compounds that are little harmful to the atmosphere, substituting older, further poisonous methods.[3-4] Goldie and McCarroll first confirmed the success of rare-earth metal (REM) salts as corrosion inhibitors in a 1984 patent. [5] Aeronautical Research Laboratory (ARL) owner from Hinton's group, located at Australia's investigated the potential of REM salts of cerium, lanthanum, and yttrium as corrosion inhibitors while employed on metal cation inhibitors. Their findings confirmed that cerium salts inhibited the corrosion of aluminium alloys. [6, 7] zinc, [8] and mild steel [9]

The inhibitory effectiveness concerning a broad range of organic [10–13] and inorganic [14–16] materials have been considered. Amongst themselves, compounds containing RE elements have showed a notable effective inhibition effect. [17-19]. For example, cerium is nearly as distribute as copper also its harmfulness as slight as that of NaCl. It is important to note that the pioneering work had been taken in the field of corrosion inhibition by rare earth elements [20,21].

Based on Tafel plots and AC impedance spectroscopy techniques, the current study aims to investigate the anticorrosion property of cerium (IV) ammonium nitrate for the MS in 3.5 percent NaCl solution. External layer of MS was examined by Scanning electron microscopy (SEM).

Fig. 1 Cerium (IV) Ammonium Nitrate appearance like Red / Orange Crystals

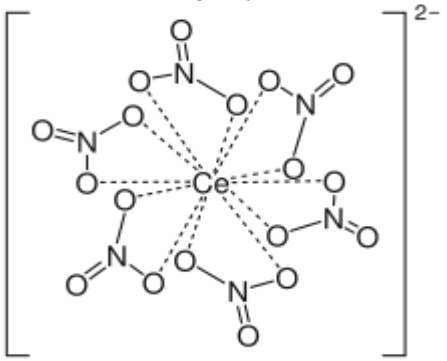

Fig. 2 Cerium (IV) Ammonium Nitrate molecular structure

Materials and method:

II. EXPERIMENTAL SET UP

Metal sample preparation: The MS belt has been polished with a SiC sheet of paper number 80 to 2500 up to a homogeneous surface. This analysis was carried out on commercially available steel strips (constitution 0.032% Manganese, 0.028% Phosphorous, 0.35% Carbon, 0.03% Sulphur and leftover Iron). Then a rubbed MS was degreased with acetone, cleaned and drained at ambient temperature with distillation water. These parts have been utilized for all corrosion protection.

Compound: Cerium (IV) Ammonium Nitrate used as an inhibitor (Star Earth Minerals Pvt Ltd) $(NH_4)^2.Ce\ (NO_3)_6$ were used as received

Corrosive media preparation: Prepared 3.5% NaCl solutions by diluting analytical-grade NaCl purchased from Merck using double-distilled water.

Inhibitor Preparation Different concentration of rare earth compound was prepared using the stock solution and used as a corrosion inhibitor. All the test solutions have been developed under AR level chemicals plus double distilled water.

Electrochemical studies: Electrochemical measuring have been performed at the electrochemical workstation CHI 608D at room temperature (302 ± 1 K). For polarization tests, the three-electrode cell consists of MS using as a working electrode. Silver-silver chloride electrode and platinum electrodes were used as reference and counter electrodes, accordingly. Before each electrochemical calculation, the continuous open-circuit (OCP) potential of the working electrode was determined by 30 minutes of immersion in the test solution. Current-potential curves estimated at a scan rate of 0.01 V s-1 in the potential range obtained by applying -0.2 and +0.2 V to the open-circuit potential (OCP) value. Impedance measurements were carried out using an AC signal with an amplitude of 5 mV at OCP in the frequency range from 0.1 Hz to 1 KHz. Using the ZSimp Win 3.21 software, the impedance data were fitted to the most appropriate equivalent circuit. The impedance parameters were determined using the Nyquist plot.

Surface analysis**:** SEM images analysis has now been used to investigate the exterior morphologies of unexposed and exposed MS (with the addition of RE compound as an inhibitor) in 3.5% NaCl solution for an effective 7-day period.

Table 2. Corrosion data obtained from potentiodynamic polarisation method

Inhibitor conc. (ppm)	**E_{corr} (V)**	***i_{corr}*** **(mA cm^{-2})**	**Corrosion Rate (mpy)**	**IE(ηp) (%)**
Blank	-0.471	9.92	4.55	----
10	-0.425	7.94	3.12	19.9
20	-0.402	5.99	2.73	39.6
30	-0.407	3.84	2.01	61.2
40	-0.410	2.75	1.27	72.2
50	-0.389	1.88	1.02	81.8

III. RESULTS AND DISCUSSION

Fig. 3 (A) Mild steel strips (B) Steel immersed into the solution at different concentration, Initial Time (C) Steel immersed into the solution at different concentration, After 7 days

IV. ELECTROCHEMICAL MEASUREMENTS

In an present study, the corrosion potential of MS in absence and in the inhibitor's presence by using Ce(IV) salts is investigated by following electrochemical methods.

1. Tafel polarization technique.
2. AC impedance technique.

Polarization measurements:

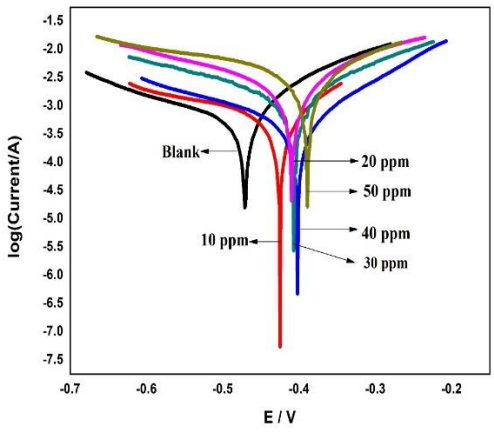

Fig. 4: Potentio-dynamic polarization Tafel curves for MS in the existence and non-existence Ceium (IV) Ammonium Nitrate

Table 2 summarizes the resulting corrosion variables like corrosion current (i_{corr}), corrosion potential (E_{corr}), corrosion rate (CR) also percentage of corrosion hindrnace [IE]**.**

The *%IE* was calculated by applying the following formula:

$$\%IE = 1 - \frac{I^1_{corr}}{I_{corr}} \text{ x } 100$$

Where, I_{corr} and I^1_{corr} are the corrosion current densities of MS in the existence and non-existence of Cerium (IV) Ammonium Nitrate in 3.5% NaCl medium.

Table 2, show the influence of inhibitor and its concentration, whereas the lowest positive potential E_{corr} (-0.425 V) and the highest corrosion current E_{corr} (-0.389 V) for the inhibition developed at 10 and 50 ppm respectively. The presence Cerium (IV) Ammonium Nitrate as inhibitor shifts both anodic and cathodic branches to the lower values of current densities and thus causes a notable reduction in the corrosion proportion. With increase in the concentration of Inhibitor from 10 pm to 50 ppm, I_{corr} values are decreased. Corroison rate (CR) shows similar results by decraesing the CR as inhibitors conecentaron increases. The corrosion rate was remains constant when the inhibitor concentration was increased to an optimum level of 100 ppm.

Electrochemical Impedance Spectroscopy:

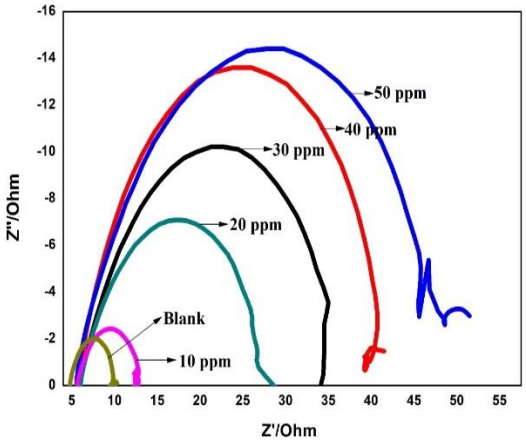

Fig. 5: Nyquist graphs developed for MS in the existence and non-existence of Cerium (IV) Ammonium Nitrate

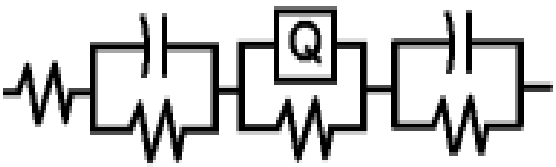

Fig. 6 Equivalent circuit

Inhibitor conc. (ppm)	R_p (Ωcm^2)	C_{dl} ($\mu F\ cm^{-2}$)	IE(η_z)
Empty	10.47	0.047	-
10	12.80	0.039	19.4
20	28.58	0.031	63.5
30	34.29	0.015	69.4
40	39.62	0.010	73.5
50	371.71	0.008	80.2

Table 3: Corrosion data obtained from Electrochemical Impedance Spectroscopy

Formula to calculate Inhibition efficiency (**ηz**)

$$\eta_p = \frac{R_p - R^o_p}{R_p} \times 100$$

Where R_p and R^o_p are polarization resistance values in the existence and non-existence of inhibitory.

Fig. 6 shows an example of a proposed equivalent circuit, and the corrosion data is shown in Table 3. Electrochemical Impedance Spectroscopy (EIS) becomes useful system for the evaluation of corrosion process at electrode and electrolyte interface. The plots having higher impedance and the semicircle having larger diameter reflects the higher corrosion resistance ability. **Fig.5** Shows Nyquist impedance plots of Cerium (IV) Ammonium Nitrate as inhibitor at various current density. The decrease of electrical double layer (C_{dl}) value indication of increasing the thickness of the electrical double layer, which retards the corrosion method Inhibition efficiency (η_z) was also increase with increase in the inhibition absorption. **Fig.7** shows how the experimental curve matched the electrical equivalent circuit curve perfectly.

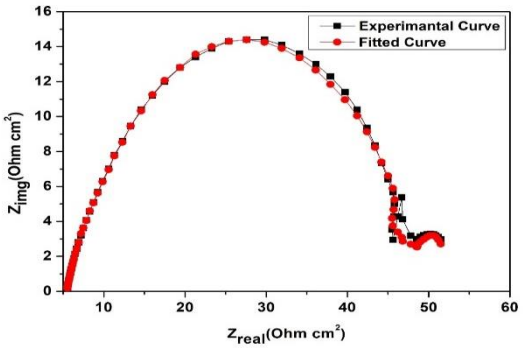

Fig. 7 Curve fitting diagram

V. CONCLUSIONS

Weight loss, Potentiodynamic polarization, impedance, and SEM techniques have been utilized to examine the corrosion inhibitory property of Cerium (IV) ammonium nitrate on MS in 3.5% NaCl. An increase in inhibitive efficiency was observed when the samples were exposed to the corrosive medium for up to 7 days forming a very good protective film on the MS surface. It has been accepted that Ce (IV) ions have a higher inhibitor efficiency than Ce (III) ions. The corrosion rate was achieved constant when the inhibitor concentration was increased to an optimum level of 100 ppm. With the increase of further cerium (IV) ammonium nitrate (>100 ppm), the stability of the surface layer of MS remains neutral because of the uniform layer of deposition. These corrosion results were well matched by the SEM image. AFM analysis also confirmed the inhibitor's strong adsorption on the mild steel surface. The results of the chemical and electrochemical tests agree well.

ACKNOWLEDGMENT

Researchers would like to convey our heartfelt appreciation to the Srinivas University, College of Engineering and Technology, Mangaluru for the laboratory facilities and assistance provided. We would also like to thank the Team Leader of Chemistry and Civil Department and the faculty for their support and guidance.

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Synthesis of yttria stabilized zirconia by combustion method and characterization

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Abstract

Nano-sized 8 mol% yttria stabilized zirconia (YSZ) powders were synthesized by the combustion method using two different fuels (urea and glycine). The effect of the nature and amount of the fuel was observed on the phase structure, particle size and microstructure of the resulted YSZ ceramics. This behavior is closely related to the combustion flame temperature. The elevated temperature during combustion synthesis with urea and glycine favored the formation of large aggregates, instead of loose and porous particles. As a consequence, the good result in terms of densification was formed for the pellets prepared by sintering of powders synthesized powder.

Keywords: A: Powders: chemical preparation; B: X-ray methods;

INTRODUCTION

Zirconia (Zr) nanoparticles have been reported to have unique properties such as excellent refractoriness, chemical resistance, good mechanical strength, highionic conductivity, low thermal conductivity at high temperature together with relatively high thermal expansion coefficient and good thermal stability [1,2]. A wide-rangingindustrial application including fabrication of dense ceramics, sensors, batteries, capacitors, corrosion resistant and thermal barrier coatings, solid electrolytes for fuel cells, catalysts, etc. have been established [3,4]. A high-quality starting powder is a prerequisite to obtain a high-performance zirconia material. There are various methods for synthesis of high-quality oxide powders; among them are precipitation techniques [5, 6], combustion techniques [7–9], sol-gel techniques [10, 11], and hydrothermal techniques [12]. All these deferent techniques are based on a solution type chemistry, where precursors of the various cations are dissolved in a solvent, commonly water, and then mixed in appropriate proportions.

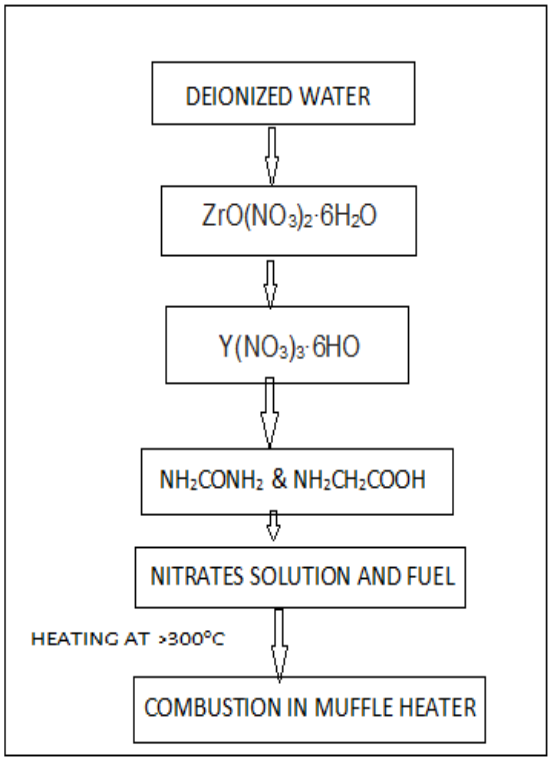
Combustion synthesis is another promising route for the synthesis of nano powder. This synthesis route is simple and faster method. This process starts at low temperature, with the help of an external heating source, followed by subsequent exothermic reaction between the oxidizer and fuel; this exothermic the reaction provides necessary heat to further carry out the reaction in the forward direction to produce nanocrystalline powders as the finalproduct. Several organic fuels such as urea, glycine, oxalyl dihydrazide, sucrose, glycine nitrate, urea-formaldehydehave beentried to synthesize nano-ceramic powders [13-17]. Solution combustion synthesis is a quick and easy process, with as main advantages the saving of time and energy. This process is used directly in the production of high purity, homogeneous ceramic oxide powders. This method is versatile for the synthesis of a wide size range of particles, including nanometer size alumina powders, as reported by Patil and Mimani [8].

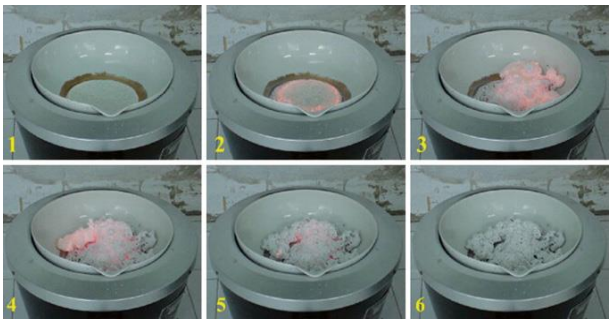
“Nanomaterials” possessing 1-100 nm grain sizes have unique chemical, physical, optical and mechanicalproperties. Because of these properties, they are useful as sensors, catalysts, coating materials (modifiers of surface properties) and miniaturization of devices (IC chips) [18]. For example, nanosized alumina or ceria having large surface/volume ratio are used as catalyst supports, t-ZrO-Al_2O_3 is a well-known toughened ceramic (ZTA) [19], yttria stabilized zirconia (YSZ) is a solid electrolyte [20] and CeO2-ZrO_2 oxygen storage capacitor (OSC) [21]. The dispersion of nanoparticles in various fluids allows the preparation of magnetic fluids (γ-Fe_2O_3), fabrication of thin film (sensors) and antireflection /

antifogging coatings (TiO_2) or improvements of optical properties [22].

Experimental

8 mole percent of yttria stabilized zirconia was prepared by using solution combustion synthesis. As for the stoichiometric ratio of $ZrO(NO_3)_2{\cdot}6H_2O$ (LOBA Practical grade), $Y(NO_3)_3{\cdot}6HO$ (HIMEDIA), urea(merck 98.5%) and glycine (merck, 98.5%) were used as metal nitrates as oxidizer and fuel, respectively. Metal nitrates was dissolved in deionized water with continuous stirring on a magnetic stirrer. After complete dissolution of metal nitrates, we can add required amount of urea and glycine. The clear solution was transferred into muffle heater for heating the solution. After water evaporation, the gels were obtained, when the temperature of the gel reaches to above 300^0c, the gel attack fire. Then the flacked power makes into a fine powder for further characterization.





Results and discussion

TGA curve shows a continuous weight loss from 70°C and it became faster up to 105 °C, which corresponds to endothermic reaction around 100 °C in the DSC curve (fig.3) This weight loss may be due to dehydration of nitrates and partial decomposition of urea. Sudden change in urea occurs, as evidenced by a strong exothermic peak at around 160 °C in the DSC curve. This reaction leads to the quick weight loss, as appeared in TGA curve.The exothermic peak appearing at 275 °C in DSC curve is due to the combustion of ureaby the nitrates. The continuous heat release, as showed by the exothermal broad peakabove 270 °C. This exothermal broad peak indicates that urea combustion occurs in a complex way. The strong exothermal combustion reaction raises the temperature more, thus for the formation of YSZ face centered cubic (FCC) phase during the combustion**.**

The XRD patterns are shown in Fig.2. The plots show the powder to be a single phase one with FCC structure. The lattice parameter of the FCC unit cell calculated by least squares method using XRD data was found to be 5.1175,5.1198 and 5.1219 Å. The increase in lattice parameter with increasing yttria concentration may due to the larger ionic size effect of Y^{3+}(1.159 Å) compared to smaller Zr (0.86Å); super saturation of vacancies in nano crystals may also distort unit cell lattice, thereby increasing lattice parameter. This Lattice distortion has also found that nano crystalline systems such as ceria. The crystallite size determined by measuring broadening of the peaks via Scherrer equation is 17 nm. it was also show that dopant concentration does not affect the crystalline size. The specific surface area analyzed by BET method is 2.66 m4+2/g.

ISBN: 979-85-27243-61-1

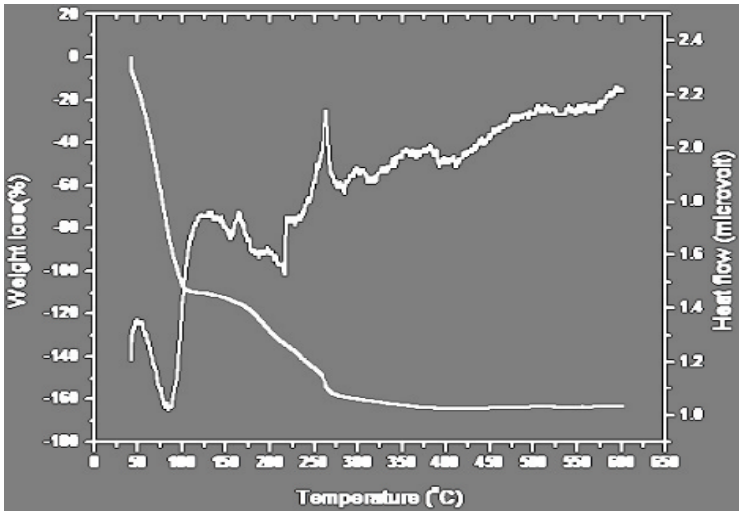


Figure 2. XRD Plots of YSZ Powders

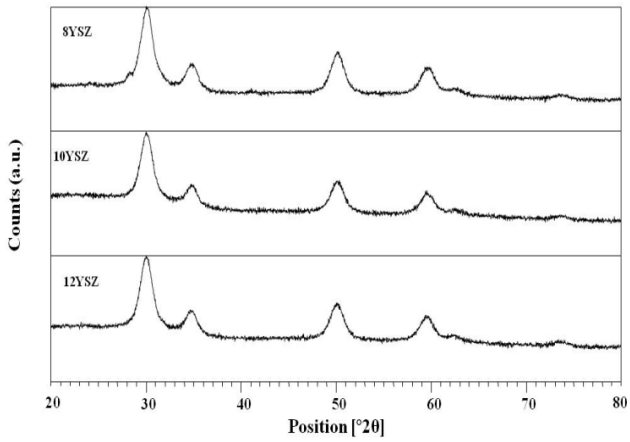


Figure 3. TGA DSC Plot of Gel

The TEM micrograph of 8 mole% yttria stabilized zi rconia powder is shown in Fig.4.The TEM images describe the particles are uniform size, with average size approximately 17 nm. The results confirmed the value of crystalline size determined from XRD data. Sintering kinetics of YSZ ceramics was measured as density vs. temperature for 30-minute soaking. It was observed that density increased with sintering temperature up to 1550°C due to removal of all unwanted particles. Maximum sintered density of 92.68% of x-ray density was achieved at 1550°C. The sintering behavior is described in fig.5 and the microstructure of maximum densesintered product is shown in fig.6.Poor sinter ability may be attributed to the pores Inside the YSZ grains**.** Therefore, this powder is not good for SOFC electrolyte. This powder can be suitable for the application as SOFC anode material since powder of lower surface area is required to Have better contact between metal particles to be at electronic conduction.

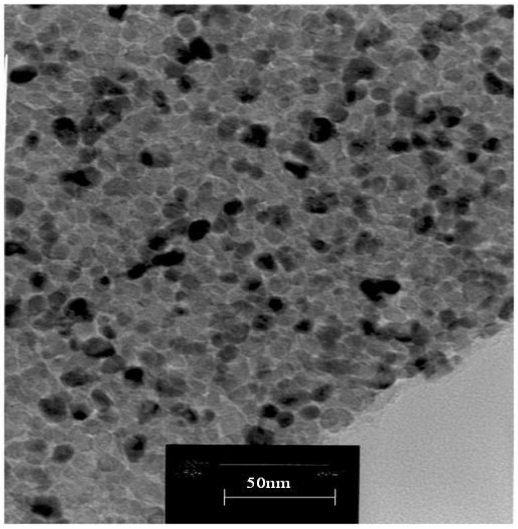


Fig.4c. TEM image of YSZ powder synthesized using urea as fuel

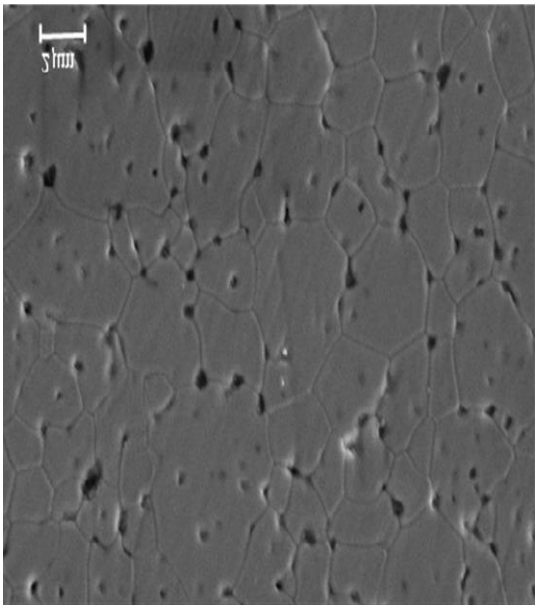


Figure 6. Sintered pellet of 10YSZ

Conclusion

Face centered cubic nanocrystalline YSZ powder can be prepared by urea solutioncombustion synthesis route. The DSC study states that the combustion occurred in a complex manner which shows in low surfacearea.

The average crystalline size of thepowder was17 nm. Lattice parameter was observed to increase with yttria concentration from 5.1175 to 5.1219 nm for 8 to 12 mol%yttria in zirconia. Study of sintering showed that maximum of 92.68% x-ray density can be achieved at 1550°C for 30 minutes.

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Phenothiazine as a class of Heterocyclic Pharmacophore and its Antimicrobial studies.

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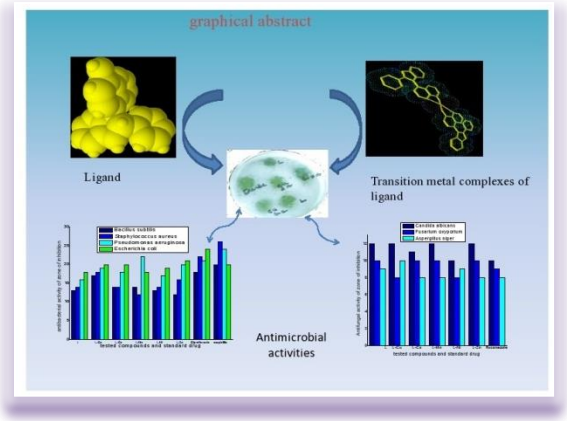
Abstract-Phenothiazine in different formulation as injection and tablet as an antipsychotic drug used in several biological treatment applications in this view some of the phenothiazine class of drugs quantitatively estimated and evaluated for their antimicrobial studies by selected bacterial and fungal strains by disc diffusion method and comparative activity studies made on the basis of structural reactivity.

Keywords: *phenothiazine, heterocyclic moiety gram positive bacteria, gram negative bacteria.*

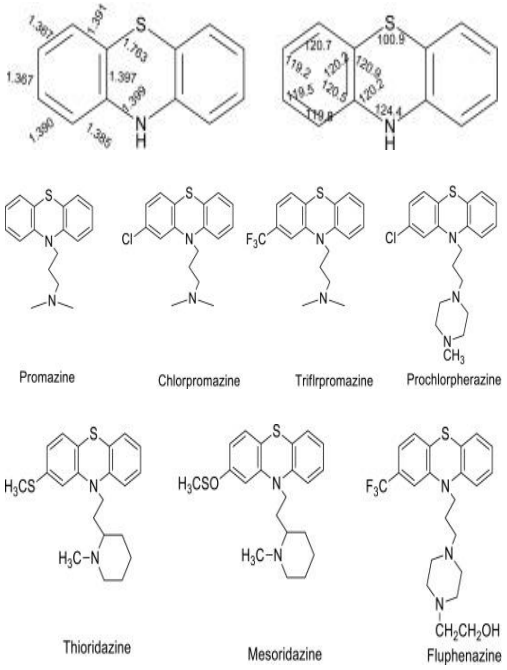


I.INTRODUCTION

Heterocyclic compounds are the important pharmacophore and their study of importance started 50 years ago, the important structural reactivity and their systematic studies also plays a important role in the field of medicine, biochemistry and agriculture[1]. Phenothiazine as a class of heterocyclic molecule also known as anti Psychotropic, anticholenirgic and antihistamine class of drug. The word drug derived from drogue which means a dry herb, a drug may be a single chemical substance or a combination of two or more different substances and Solubility of compounds to make the simplicity of the synthetic scheme helps to understand influence of oxidizing agent in the quantitative estimation of drugs [2]. Phenothiazine class of drug such as trifuoperazine hydrochloride plays an important role in chemotherapy and they are the part of aromatic ring system fused with imidazole five membered ring systems [3]. Phenothiazine and their derivatives have diverse application in coordination chemistry, medicinal chemistry.

II.STRUCTURE

Phenothiazine is a parental phenothiazine class of drugs and thiazine dyes, substituted phenothiazine class of drugs are the important class of psychiatric drugs owing to their pharmacological activity, they have been extensively studied for the application of chemical, biological and medical research.2- and 10- substituted class of phenothiazine drugs are particularly used in the treatment of anti psychotropic, anticholenirgic and antihistamine [4-8].



III.MATERIALSANDMETHODS

All chemicals used were of analytical reagent grade. Distilled water was used in the study.

Potassium Bromate solution (0.003M):- prepared by dissolving 0.501g of potassium Bromate (s.dfine-chem. Ltd. India.) in distilled water and diluting to one liter with the same solvent.

Sodium thiosulphate solution (0.05M):- prepared by dissolving about 8.26g of the salt (S.d fine-chem. Ltd. India) in distilled water and diluted to one liter with the same solvent and standardized using pure sample of dichromate.

Hydrochloric acid (3M): prepared by diluting about 265.5 ml of concentrated hydrochloric acid (s.d fine-chem. Ltd India.) to one liter with distilled water.

Indicator: - dissolved about 1g of soluble starch in 100ml distilled water.

Potassium Bromide solution (10%): dissolved about 100g of potassium bromide (s.d. fine-chem. Ltd India.) in 1000ml distilled water.

Potassium iodide solution (10%): dissolved about 100g of potassium iodide (s.d. fine-chem. Ltd India.) in 1000ml distilled water.

Standard solution of trifluoperazine hydrochloride

Pure drug sample of TFPH provided by Rhone-ponlenc, India Ltd, pharmaceutical company and used for the experiment. Stock standard solution containing 1mg/ml of drug was prepared by dissolving accurately weighed amount of trifluoperazine hydrochloride in water. Stock solutions were stored in amber colored bottle and kept in a refrigerator.

IV. Experimental procedure:

A 5 ml of aliquot containing 2-10mg of each drug was mixed with a known and excessive volume of potassium Bromate solutions (10ml of 0.003M) in the presence 5ml of 3M hydrochloric acid the content were set aside with occasional shaking. The time required for complete disappearance of orange color, was determined. After the complete disappearance of the color the un reacted oxidant titrated iodometrically using 4 drops of starch indicator. The reacting ratio (moles of Bromate per mole of drug) was taken and calculated for each test using the titration data and the results are finding in the table1.

iv. Result and discussions

The indirect titrimetric method is referred for the quantitative analysis of the drug based on the fact that the drug when reacted with known excess of potassium Bromate solution in presence of hydrochloric acid medium in the presence of KBr , are oxidized quantitatively first to colored radical cation and subsequently to colorless hydroxide, the untreated Bromate being determined iodometrically[9-12].

Optimization of experimental variables

In order to develop a back titration method, it is necessary to consider several factors like reaction medium and its strength, reaction time, temperature, effect of time, reagent concentration etc. these factors were optimized by study.

Effect of acid species and its strength

The oxidation of TFPH was found to proceed quantitatively and stochiometrically in acid media tried such as hydrochloric acid medium.

Optimum reaction time

At laboratory temperature (27 ± 5^0C) depending on the amount of drug the time required for the drug as indicated by the disappearance of the color due to the radical cation varied from 2 to 5 min.

Effect of time on the consumption of Bromate

The effect of time on the consumption of Bromate by the drug was studied tolerable time found to be 20 min. beyond this time very less amount of Bromate found without affecting the stochiometry.

Range of determination:

Under the experimental conditions, 2-10mg could be determined with reasonable degree of accuracy and precision.

Accuracy and precision of the method

The accuracy of the method was checked by determining different amounts of pure drugs by the proposed method. The precision was established by performing six replicate determinations on the same solution containing 3mg, 6mg and 9mg of drugs. The percent error, relative standard deviation and range of error for this study are summarized in table 1and with good accuracy and precision of the method [13, 14].

Usage of the drug:

Several classes of phenothiazine drugs are used in various treatments but they should be administered with precaution in the treatment of heart patient and pregnant women which may leads to adverse effect. Long term and continuous usage of drug causes various disorders such as itching, rash, hypertrophic papillae of the tongue, angioneurotic edema, erythema, allergic perpura, exfoliative dermitis, photosensitivity, nausea, vomiting, increase or decrease in appetite, gastric irritation, constipation, paralytic ileus, rarely diarrhea, urinary retention, anemia, cholestatic jaundice [15,16].

Application of the method to pharmaceutical formulations containing trifluoperazine hydrochloride:

Derivatives of phenothiazine can also be used as stabilizers, indicators, lubricants and in photography, displays, photovoltaic cells, organic photoreceptors and electronic glasses [17].

The convention practical procedure evaluated for the quantitative determination of trifuoperazine hydrochloride, in various formulations of phenothiazine classes of drugs. The table2 indicate the method gives good accuracy and precision with satisfactory agreement with the results obtained by official method [18].

Table1: Accuracy and Precision data

Amount taken, mg	Amount found, mg	Relative error %	RSD %
3.0	2.95	1.666	0.36
6.0	6.25	4.166	0.82
9.0	9.34	3.7778	1.04

formulations	Mg/ml of tablet	Proposed method	BP method	Student's t value	'F' value
Trazine	5	95.25±0.51	98.15±1.04	0.24	4.42
	10	98.55±1.25	101.25±0.85	0.42	3.02
Neocalm	5	100.05±0.44	102.38±0.75	1.6	1.15
	10	100.25±0.25	102.64±0.28	1.98	2.20
Espazine	5	94.25±0.45	1.03.44±0.46	1.65	2.90
	10	97.25±0.32	99.25±0.45	2.20	2.75

Antimicrobial studies on phenothiazine classes of drugs:

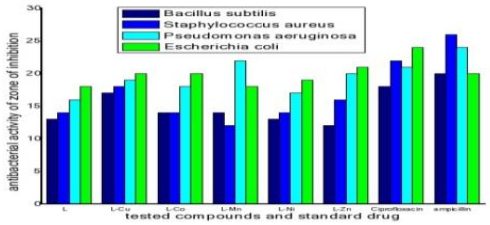
Validation and evaluation of the biological action of synthesized compounds are the prime importance for the biologically active pharmacophore, research on new antimicrobial agents and its pharmacological actions of small molecules major source of importance in the synthetic chemistry and heterocyclic chemistry, even today majority of antimicrobial agents are the natural source but the major issues lies in their systematic research and their biological application for the benefit of mankind, so in this view there is a vast importance for the synthesis of innovative and potential pharmacophore followed by simple convenient well known procedure. The synthesized ligand and copper, cobalt, manganese, nickel and zinc

complexes screened for preliminary antimicrobial studies, gram-ve and gram +ve bacterial cultures used for antibacterial studies and selected fungal cultures used for antifungal studies [19-21].

The antibacterial and antifungal activities were carried out by the cup plate disc diffusion method and the typical procedure is refereed, molten agar prepared and kept at 45^0C and then poured in to petridishes allowed to solidify. Holes of 6mm diameter were punched using sterile cork borer and then all the holes were completely filled with the prepared solution of the tested compounds of the concentration 50 and 100 mgmL^{-1} in DMSO solution followed by 24hrs of incubation at 37^0C.The diameter of the inhibition zone measured(in terms of millimeter) for all the compounds and compared with the standard drug ampicillin of the same concentration referred in the procedure under standard sets of condition as reported in the table3.

Antibacterial activity of ligands and complexes

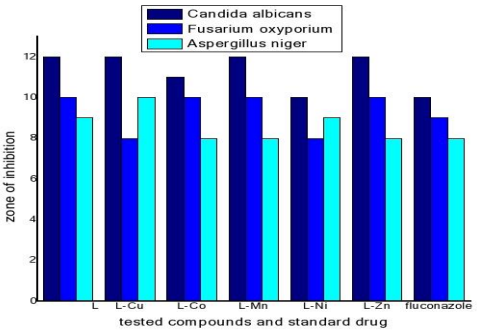
Tested compounds	Gram positive bacteria				Gram negative bacteria			
	Bacillus subtilis		Staphylococcus aureus		Pseudomonas aeruginosa		Escherichia Coli	
	50 mg/mL^{-1}	100 mg/mL^{-1}	50 mg/mL^{-1}	100 mg/mL-1	50mg/mL-1	100 mg/mL-1	50mg/mL-1	100 mg/mL^{-1}
Trazine	10	14	14	20	16	18	16	20
Neocalm	12	16	15	22	19	22	14	22
Espazine	12	18	12	18	18	20	18	23



Antifungal studies: antifungal activity of the ligand and complexes was carried out against Candida albicans, Fusarium oxyporium, aspergillus Niger by the typical cup plate disc diffusion method, potato dextrose agar medium prepared desired for the experiment. The plates were incubated at 370C for about 48hrs, the diameter of the inhibition zone measured in millimeter and compared with the standard drug fluconazole of the same concentration as referred in the procedure under identical condition [22-24]

Antifungal activity of ligand and complexes

Tested compounds	Candida albicans		Fusarium oxyporium		Aspergillus niger	
	50mg/mL^{-1}	100 mg/mL^{-1}	50 mg/mL^{-1}	100 mg/mL^{-1}	50 mg/mL-1	100 mg/mL^{-1}
Trazine	10	12	12	14	9	12
Neocalm	12	14	8	10	10	14
Espazine	11	12	10	12	8	13



Conclusion: the statistical and antimicrobial studies of phenothiazine classes of drugs proved to be effective antimicrobial agents with the antimicrobial studies of selected bacterial and fungal strains.

Acknowledgment

We would like to express our sincere gratitude to the Management, Principal Sapthagiri College of Engineering Bengaluru for the facilities provided and their support. Also, we would like to thank the Head of Department Chemistry and faculties for their encouragement and support.

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Survey on Material Science and Nanotechnology

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***Abstract*—The material of choice of a given time is often a defining point. Originally hailing from the manufacture of Ceramics and some of its derivatives, material science is one of the oldest forms of engineering and applied science. The right choice of material for the right task is very important. But some materials require their properties to be altered, say to increase mechanical strength while maintaining low weight. This requires altering properties of the materials at atomic level, which material science research looks into, while sometimes, the size of a particle must be reduced to nanoscale. Nanotechnology plays a very important role in changing various properties of any material like strength, conductivity and reactivity, color, etc. Richard Smalley, Noble laureate in chemistry, said in June 1999, "The impact of nanotechnology on health, wealth, and lives of people, will be at least the equivalent of the combined influences of microelectronics, medical imaging, computer-aided engineering and man-made polymers developed in this century." Study of nanostructures of materials, their atomic and molecular structures, bonding results in the advancement of available materials and invention of new materials with enhanced properties.**

Keywords- nanostructure, atoms, molecules, structures, materials.

I. INTRODUCTION

Materials science is an interdisciplinary field involving the properties of matter and its applications to various areas of science and engineering. It includes elements of applied physics and chemistry, as well as chemical, mechanical, civil and electrical engineering. With significant media attention to nano science and nanotechnology in the recent years, materials science has been propelled to the forefront at many universities, sometimes controversially.

In materials science, rather than haphazardly looking for and discovering materials and exploiting their properties, one instead aims to understand materials fundamentally so that new materials with the desired properties can be created. The basis of all materials science involves relating the desired properties and relative performance of a material in a certain application to the structure of the atoms and phases in that material through characterization.

Nanotechnology is a field of research and innovation concerned with building 'things' - generally, materials and devices - on the scale of atoms and molecules. A nanometre is one-billionth of a metre: ten times the diameter of a hydrogen atom. At such scales, the ordinary rules of physics and chemistry no longer apply. For instance, materials' characteristics, such as their colour, strength, conductivity and reactivity, can differ substantially between the nanoscale and the macro. Carbon 'nanotubes' are 100 times stronger than steel but six times lighter.

For example: Fullerenes reduced to nano size, show excellent superconducting properties. Also, Gold, which is catalytically inactive in macroscale, is catalytically very active at nanoscale.

Research and Development in Material Science

In order to completely understand a material, one must study about the fundamental properties of a material. Fundamentals in material science include the structure, bonding, atomic structure and nanostructure.

Structure is the main component of material science. Surveying the structure of a material from atomic level to the macro level, we can understand the materials composition, allowing us to select suitable materials for the task at hand. Many properties of materials depend upon how their atoms or molecules bond with each other, bond length, hybridization and how each ion is arranged or bonded to one another. Study at atomic level structure, arrangement of atoms in crystalline solids is done using Crystallography. In single crystals, the effects of the crystalline arrangement are visible at macro level as the arrangement reflects their atomic arrangement. But, as most crystal structures occur in polycrystalline form, material science techniques are used to study the arrangements at atomic level to understand varying degrees of crystallinity. Also, considering amorphous substances, studies are conducted to decipher the cause for their amorphous nature [1].

Nanostructures are materials whose atoms and molecules are at a nano scale. It deals with objects of the range 1-100 nm. Reduction of bulk materials to nano scale causes many electrical, magnetic, optical and mechanical property changes. Usage of nanomaterials in water purification systems, encapsulation systems for optimised drug delivery is under research and usage [2].

Biomaterial synthesis is another sub category in material science. Bio-material science is the study of biomaterials. These are the matter, surface that interacts with the biological systems.

Biomaterials can be derived from nature or be synthesized. They are utilized for medical purposes, some being benign,

Fig. 1. The iridescent nacre inside a nautilus shell

like being used for a heart valve and some being bioactive such as hydroxylapetite-coated hip implants [3].

Industrial application of material science includes incremental improvements and troubleshooting issues with existing materials, material design, and advancements in processing techniques such as rolling, welding, casting and analysis methods such as X-ray diffraction, electron microscopy. Further, it deals with material extraction and conversion of extracted materials into useful products.

Research in Nanotechnology and its Applications

In nutritional research, nanotechnology has enabled with obtaining the accurate information about the location of a nutrient or a bioactive food component in a cell or tissue. they may help to identify and characterize molecular targets and biomarkers of effect. Specific applications of nanotechnology in nutrition include modifying taste, color, enhancing nutritional quality of foods. For example, one food technology involves creating barriers or coatings that act as barriers to bacteria or contain additional nutrients.

DNA nanotechnology is the design and manufacture of artificial nucleic acids that serve mainly as non-biological engineering materials, rather than information carrying genetic material. Structural DNA nanotechnology, abbreviated as SDN, involves the synthesis and characterization of nucleic acids with a definitive, static endpoint. DNA nanotechnology is an example of bottom-up molecular self-assembly, where nano sized biological molecules spontaneously organise into stable structures, the structure being induced by physical and chemical properties of the material by the designers [4].

Manipulating the crops' genetic material to enable their growth in 'hostile' conditions, such as high salinity, low water levels has been achieved through nanotechnology. Using nano sensors on crops and usage of nanoparticles in fertilizers is also underway.

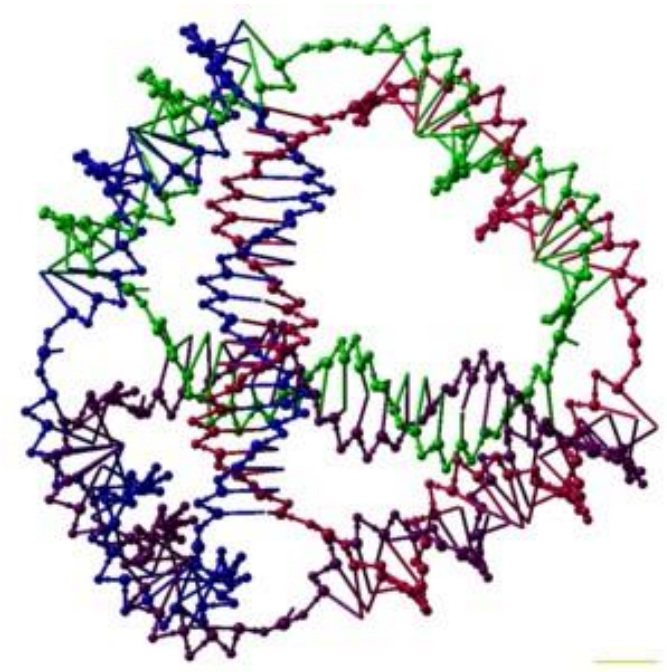

Fig. 2. Custom created nucleic acid using nanotechnology

II. MATERIALS AND METHODS

Artificial DNA Strand Synthesis

The sequences of DNA strands (template) making up the target structure are designed computationally, using molecular and thermodynamic modelling software .

The nucleic acid themselves are then synthesized using standard oligonucleotide synthesis method, preferably using oligonucleotide synthesizer. Purification is done using denaturating gel electrophoresis and precise concentration determined via nucleic acid quantitation using ultraviolet absorbance spectroscopy [5].

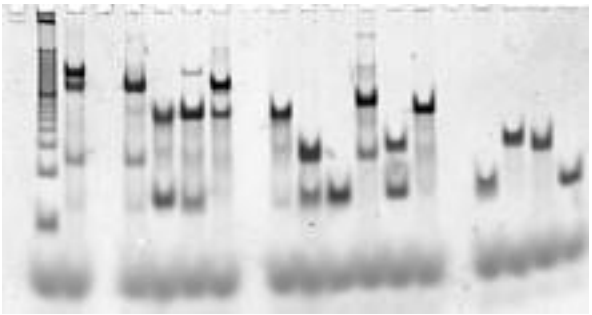

Fig. 3. Gel electrophoresis method

Fully formed target structures are verified using negative gel electrophoresis, which gives the size and shape information of the synthesized nucleic acid complexes [6].

III. CONCLUSION

The fields of Material science and Nanotechnology have contributed much to the science with new inventions, enforced materials, medical advancements and gene manipulation. This study involves research areas in fields of Material Science and Nanotechnology, their applications in daily life and more increasing utilization of their products in common mode.

ACKNOWLEDGMENT

I would like to express my sincere gratitude to the Principal, and faculties of Sapthagiri College of Engineering Bengaluru, for their encouragement and support.

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Ground water study of the Physico-Chemical parameters in the Kunigal Taluk.

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Abstract:

Current Study is deals with assessment of Waterquality of the Huttridurga (H) Kunigal (T)Tumkur (dist).The investigators collected 12 samples from different 12 villages in the Huttridurga (H) and measured pH, Electrical Conductivity, Total Dissolved Salt (TDS),Total Hardness,Alkalinity,Chloride,Calcium,Mg^{+2},Na^{+} K^{+} ion concentration which was analysis withreference to BIS.

Key Words: **Water quality, ground water, hardness, hobli, Karnataka**

I.INTRODUCTION

Water is essential for the existence of any form of life. Natural Water normally contains dissolved gases and solids. The quality of water that we consume as well as the quality of water in our lakes, river and streams occurs as an important variable in finding the overall quality of our lives. In urban and rural areas, the ground water is the chief source of drinking. Greater than 80% of diseases of mankind is due to the contaminated water (WHO 1984), Dev Burmanetal (1995) and Subba Rao (2003). The quality of water is a consequence of its natural, physical and chemical state of the water as well as consequence of human activities (S Venkateeswaran et.al 2011).

In the present investigation, the ground water samples were collected from 12 different villages and were examined for physico-chemical parameters like temperature pH,EC,TDS,Total Hardness,Ca^{2+}, Mg^{2+},Na^{+} K^{+},$Cl^{-}$and Total alkalinity.

II. STUDY AREA

Huttridurga hobli is located in the Kunigal (Taluk) of Tumkur (district), Karnataka, India at 13.02^{0} North latitude and 77.03^{0} East longitude. The hobli is in the region of Deccan plateau. It is situated 773meters above the sea level, it has an average rainfall of 680 mm per annum. The study area is surrounded by industries like Tiles, Brick, pharmaceutical and agriculture is the main occupation of the society.

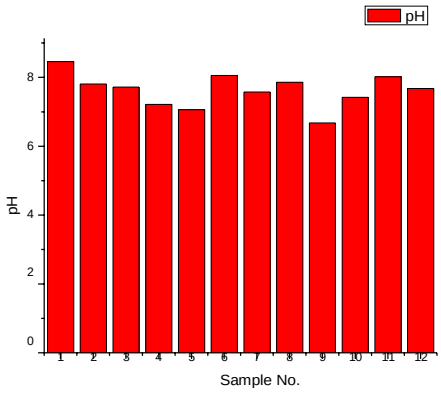
III. MATERIALS AND METHODS

During the post monsoon season, twelve ground water samples of different villages of Huttridurga hobli were collected for the study. The samples were collected using pre cleaned polyethene cans of 5 liters capacity. All the cans were rinsed with water to be sampled before the sample collected for analysis. All the samples were transported to the lab with precautionary condition as mentioned in the APHA (1998).The physical parameters like temperature, pH and EC were analysed on spot using water analyser 371 instrument of Systronics make. The TDS was analysed by the evaporation method. The chemical parameter such as total Hardness and Ca^{2+} is determined by EDTA method, Alkalinity and $Cl^{-}$was determined using methyl orange indicator and argentometric method respectively, Mg^{2+} wasdetermined using calculation method, the

Metals like Na^+& K^+ were analysed using micro control processor based Flame photometer FPM 128 instrument of Systronics make.

IV. RESULT AND DISCUSSIONS

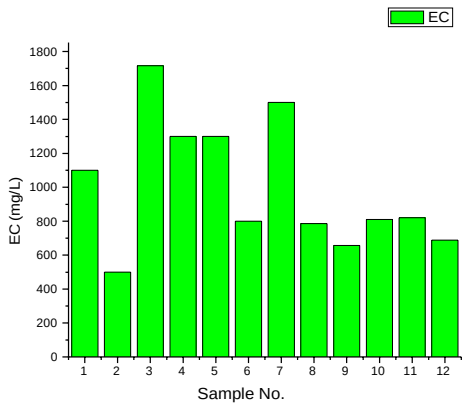
pH: Generally the pH of water is influenced by geology of catchment and buffering capacity of water (Achutha Nair et.al 2005), in the current study the pH values varied between 7.06 and 8.46, with an average value of pH 7.63 as shown in the Table – 1 and depicted in the Graph – 01, the pH values of all the study sites were within the permissible limits of BIS standards for drinking water.



Graph – 01 showing the pH values at different study site

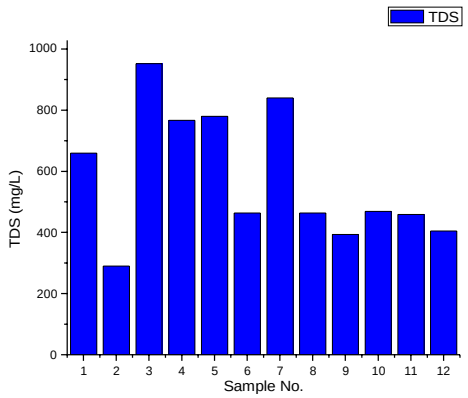
Electrical Conductance: The ability of water to conduct depends on the total dissolved solids, the conductivity is directly proportional to the dissolved salts (Abdul Jameeletal. 2002), the EC

Values in the current examination fluctuated from 500 micro S/cm to 1716 micro S/cm with an average value of EC 996.75 micro S/cm as showed in Table – 01 and depicted in graph - 02.



Graph – 02 showing the EC values at different study site

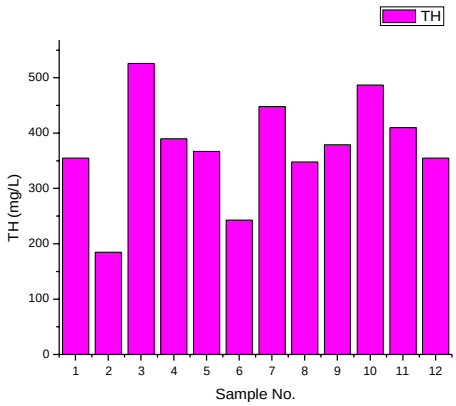
Total Dissolved Solids: The TDS value in this investigation is as high as 952 mg/L and a minimum value of 290 mg/L with an average TDS value of 578.82 mg/Las showed in Table – 01 and depicted in graph - 03. In these findings all the bore well samples were within the permissible values of BIS, the TDS values depend on the dissolved salts.



Graph – 03 showing the TDS values at different study site

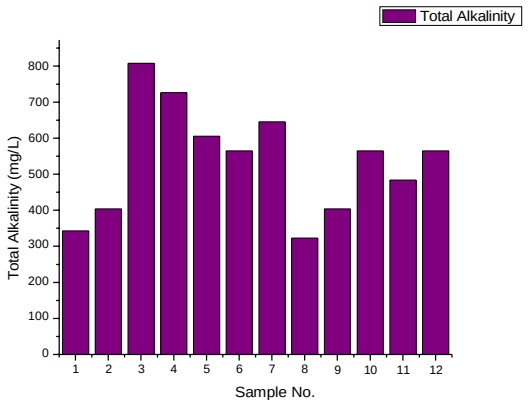
Total Hardness: The total hardness is an important parameter for the domestic and industrial use, it depends on the all the soluble bivalent metal ions in the water sample, thetotal hardness varied between a minimum of185mg/L and a maximum of 526 mg/L with an average value of 374.87 mg/Las showed in Table – 02 and depicted in graph - 04. In this study all the ground water samples examined

Were within the permissible limits of BIS (1991)for drinking water standards.



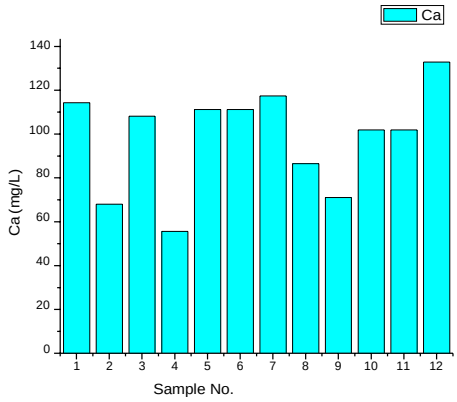
Graph – 04 showing the Total Hardness values at differentstudy site

Total Alkalinity: The total alkalinity refers to number of base component in the water sample, in the present investigation the alkalinity values ranged between a minimum of 323 mg/L and a maximum of 808 mg/L with an average alkalinity value of 536.98 mg/L, as showed in Table – 02 and depicted in graph -05.

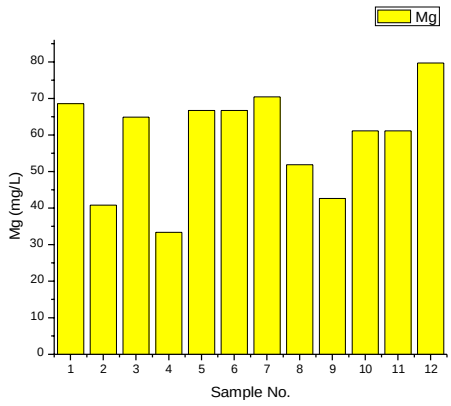


Graph – 05 showing the Total alkalinity values at differentstudy site

Calcium and Magnesium: The important bivalent ions in the determination of total hardness is calcium and magnesium apart from other soluble bivalents metal ions, the investigators recorded a minimum of 55.58 mg/L and a maximum of 132.78 mg/L, with an average of 98.30 mg/L of calcium, all the ground samples tested for calcium found within the permissible limits of BIS (1991) for drinking water standards. The Magnesium ion was found in the range between a minimum of 33.35 mg/L and a maximum of 79.67 mg/L with an average value of 58.98 mg/L, as showed in Table – 02 and depicted in graph – 05 and Graph - 06. The authors found the magnesium in the ground water samples within the permissible limits of BIS (1991) for drinking water standards.

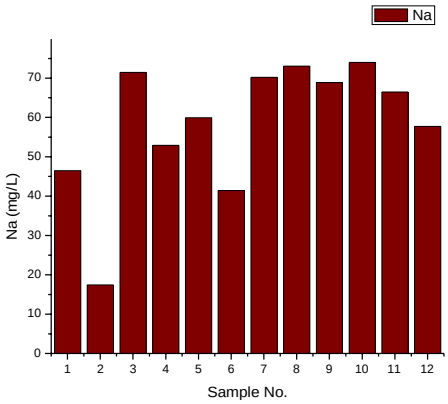


Graph – 06 showing the Ca^{2+} ion values at different studysite

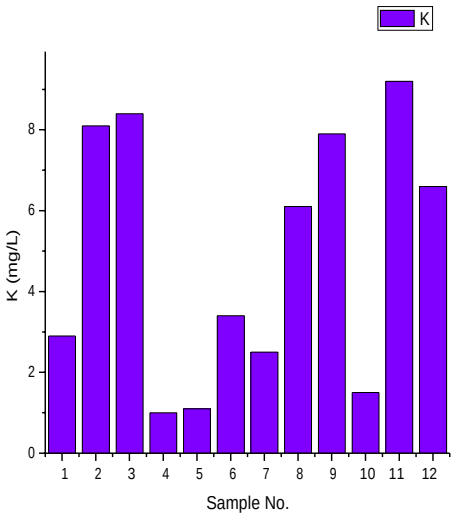


Graph – 07 showing the Mg^{2+} ion values at different studysite

Sodium and Potassium: It is an important constituent for determining the quality of water for irrigation. Most sodium salts are readily soluble in water, it is determined using flame photometer, in the present investigation of ground water samples the sodium varied between a minimum of 17.44 mg/L and maximum of 78.0 mg/L with an average of 58.67mg/L, and all the ground water samples were containing sodium within the permissible limits of BIS (1991) for drinking water standards. In igneous rocks, Potassium is nearly as abundant as Sodium but its concentration is very less as compared to Sodium, the authors recorded a minimum of 1.0 mg/L and a maximum of 9.2 mg/L with an average of 4.89 mg/L, as showed in Table – 02 and depicted in graph – 07 and Graph - 08.

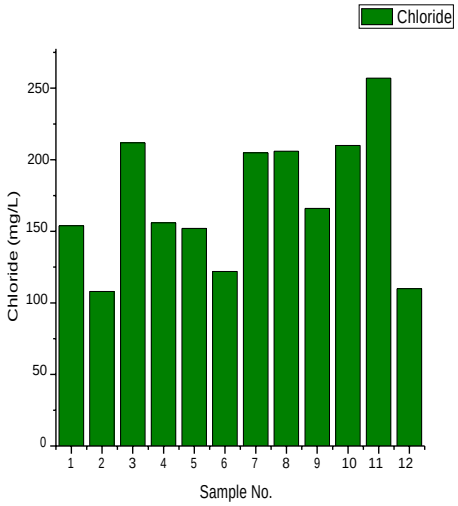


Graph – 08 showing the Na^+ ion values at different studysite



Graph – 09 showing the K^+ ion values at different study site

Chloride: Chloride is extremely soluble in water, the Sedimentary rocks and Igneous rocks arethe chief sources of chloride in ground water, inthe current study it fluctuated from a minimum of 108mg/L to a maximum of 257mg/L and with an average of 172.09 mg/L, all the ground water samples were within the permissible limits of (1000 mg/L) BIS (1991), as showed in Table – 02 and depicted in graph - 09.



Graph – 10 showing the Cl^- ion values at different studysite

Table: 1- showing the values of Physical properties of the different study sites.

Sl No.	Temp.	pH	EC	TDS
1	25.8	8.46	1100	660
2	25.1	7.81	500	290
3	25.6	7.72	1716	952
4	24.9	7.22	1300	767
5	24.4	7.06	1300	780
6	26.1	8.06	800	464
7	25.8	7.58	1500	840
8	24.7	7.86	786	464
9	24.1	6.68	657	394
10	23.6	7.42	810	469
11	24.5	8.02	820	459
12	23.7	7.68	688	405
BIS	-	6.5 – 8.5	-	2000

Table – 02 showing the values of Chemical properties of the different study sites.

Sl. NO	TH	Ca	Mg	Tot Alk	Na	K	Cl-
1	355	114	68.5	343	46.5	2.9	154
2	185	67.9	40.7	404	17.4	8.1	108
3	526	108	64.8	808	71.4	8.4	212
4	390	55.5	33.3	727	52.9	1	156
5	367	111	66.7	606	59.9	1.1	152
6	243	111	66.7	565	41.4	3.4	122
7	448	117	70.4	646	70.2	2.5	205
8	348	86.4	51.8	323	73	6.1	206
9	379	71	42.6	404	68.9	7.9	166
10	487	102	61.1	565	74	1.5	210
11	410	102	61.1	484	66.4	9.2	257
12	355	133	79.6	565	57.7	6.6	110
BIS	600	200	100	600	200	100	

ACKNOWLEDGMENT

We would like to express our sincere gratitude to the Management, Principal Sapthagiri College of Engineering Bengaluru for the facilities provided and their support. Also, we would like to thank the Head of Department Chemistry and faculties for their encouragement and support.

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Glimpses of ICGCP - 2021

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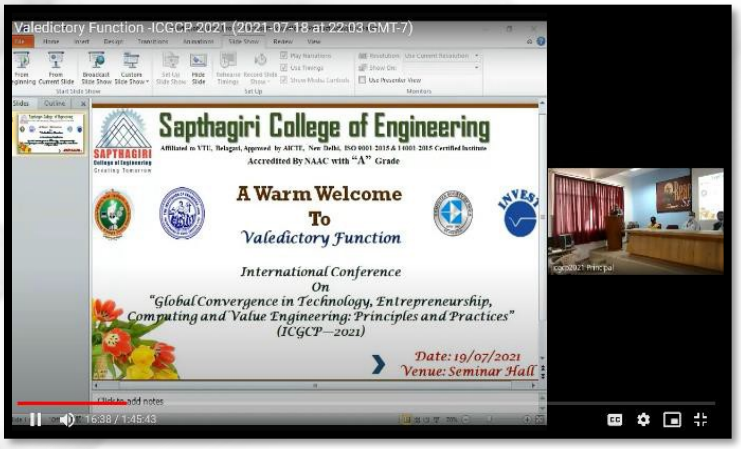

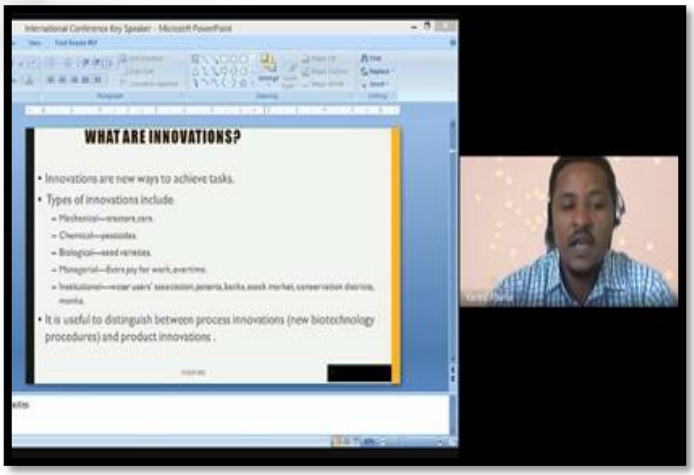

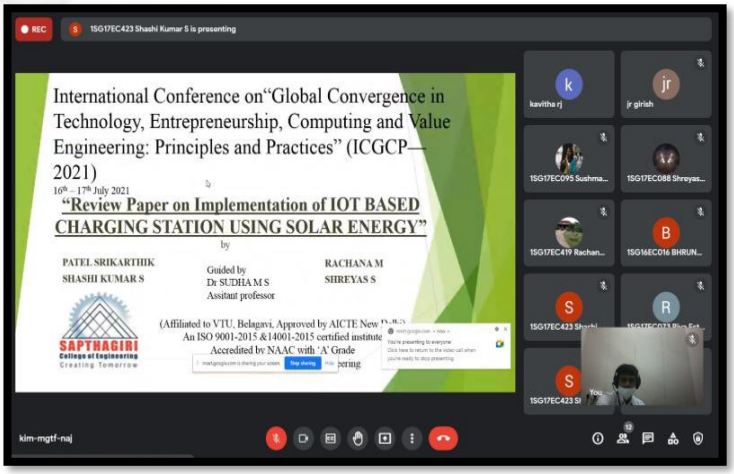

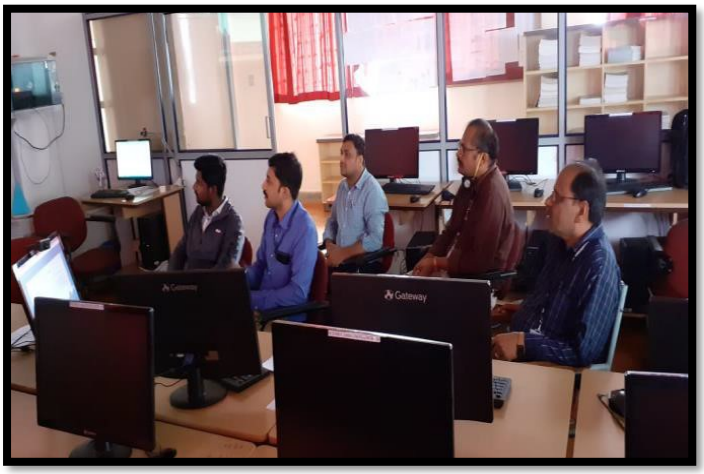

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