Euphorbia tithymaloides: Dual Role in Combatting Drug Resistance and Environmental Pollution through Nanoparticles Enhanced Dye Removal and Biochar-based Heavy Metal Remediation

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CANDIDATE'S DECLARATION

I **Ishika,** Roll Number: **2K22/MSCBIO/21** hereby certify that the work which is being presented in the thesis entitled "*Euphorbia* tithymaloides: Dual Role in Combatting Drug Resistance and Environmental Pollution through Nanparticles Enhanced Dye Removal and Biochar-based Heavy Metal Remediation" in partial fulfillment of the requirements for the award of the Degree of **Master of Biotechnology**, submitted in the **Department of Biotechnology**, Delhi Technological University is an authentic record of my own work carried out during the period from January 2024 to May 2024, under the supervision of Dr. Navneeta Bharadvaja.

The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other institute.

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Certified that **Ishika** has carried out their research work presented in this thesis entitled **"*Euphorbia tithymaloides*: Dual Role in Combatting Drug Resistance and Environmental Pollution through Nanoparticles Enhanced Dye Removal and Biochar-based Heavy Metal Remediation"** for the award of **Master of Biotechnology** from the Department of Biotechnology, Delhi Technological University, Delhi, under my supervision. The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/ Institution.

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***Euphorbia tithymaloides*: Dual Role in Combatting Drug Resistance and Environmental Pollution Through Nanoparticles Enhanced Dye Removal and Biochar-based Heavy Metal Remediation**

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ABSTRACT

Euphorbia tithymaloides is a plant with documented medicinal properties. The plant contains a diverse range of phytochemicals, including saponins, tannins, steroids, coumarins, triterpenes, etc. As the plant is rich in different phytochemicals leaves of this plant were employed in the manufacturing of ZnO nanoparticles. The absorption peak of manufactured ZnO nanoparticles was observed at 244nm. Antimicrobial resistance has become a substantial and urgent menace to public health. According to the Centers for Disease Control and Prevention, over two million people in the US acquire resistant pathogens annually, leading to a minimum of 23,000 fatalities. The need for an alternative antimicrobial agent is the demand of the current scenario. ZnO nanoparticles were employed as an antibacterial agent against a poly-antibiotic-resistant bacteria, *Bacillus clausii*. ZnO nanoparticles displayed remarkable microbicidal activity against drug-resistant *Bacillus clausii*. The zone of inhibition exhibited by ZnO nanoparticles was observed to be 22mm. friedelanol, ursolic acid, β-sitosterol, and epifriedelanyl acetate are the reported bioactive compounds that were evaluated through *In silico* analysis for their antibacterial activity against Lipoteichoic acid synthase enzyme synthesized by *Staphylococcus aureus* which is a crucial enzyme required in the synthesis of lipoteichoic acid. This enzyme was inhibited using these phytocompounds of *Euphorbia tithymaloides*. Lipoteichoic acid is crucial for the survival, development, and reproduction of cells. Mutants lacking LtaS are susceptible to bursting under osmotic pressure and can only multiply in environments that have a stable osmotic balance. Inhibiting the biosynthesis of teichoic acid is a potential

approach for therapeutic intervention. Further, the manufactured ZnO nanoparticles were employed as a photocatalyst in the removal of Eosin Yellow dye and Methylene Blue. It was observed that within 6 h 77.66% of methylene blue was degraded and 88.44% of Eosin Yellow was degraded by zinc oxide nanoparticles. the leaves of *Euphorbia tithymaloides* were also utilized in the synthesis of Biochar. Subsequently, the synthesized Biochar was utilized as an adsorbent to effectively eliminate Cd from the wastewater. Over time the efficiency of Cd removal from the solution improved. After 24 hr, the solution, which contained 250mg of biochar was able to remove 92.07% of cadmium from the solution by adsorption.

LIST OF PUBLICATIONS

1. A review paper entitled “Biogenic Nanoparticles from Phytochemicals: A New Frontier in Fighting Antimicrobial Resistance and Biofilm Formation” has been accepted in the Research Journal of Biotechnology.
2. A Paper entitled “Structure based Computational Investigation of Phytocompounds from *Vitex negundo* as Drug Candidate for Human Respiratory Syncytial Virus (hRSV)” has been accepted at International Conference on Emerging Technologies in Science and Engineering (ICETSE) – 2024.

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CHAPTER 1
INTRODUCTION

Euphorbia tithymaloides is a plant species that is most commonly found in Northern and Central parts of America and in some parts of South Asia. This plant is alternatively referred to as *Pedilanthus tithymaloides* in scientific terminology. In India, this plant is distributed in various parts including, Madhya Pradesh, Uttar Pradesh, Haryana, Bihar, Assam, Gujarat, Odhisa, and Maharashtra, and is known by different names such as naagfani and naagdon in Madhya Pradesh and Uttar Pradesh respectively. This plant typically grows to a height of 0.4-3 meters and has a width of 40 to 60 centimeters. It exhibits abundant branching from its base. The plant thrives in various types of soil, including well-drained, nutrient-rich, and sandy soil. It especially benefits from higher levels of boron, iron, copper, manganese, zinc, and molybdenum. However, it is not very tolerant of high levels of soil salinity, although it can withstand saline conditions if it is well-fertilized. The plant's stem exhibits a zigzag structure that closely resembles the spinal column, Hence commonly referred to as devil's-backbone. This is a plant with documented medicinal properties. In mushrooms, it is documented to minimize the damage caused by nematodes. Historically, earaches, calluses, ringworm, umbilical hernias, and toothaches have all been treated with sap. The plant contains a diverse range of phytochemicals, including saponins, tannins, steroids, coumarins, triterpenes, etc. The plant's abundance of phytochemicals makes them possess antibacterial, antioxidant, anti-diabetic, stomachic, antifungal, anti-helminthic, antimutagenic, anti-inflammatory, antiseptic, antitumor, and antiviral activity. As the plant is rich in different phytochemicals it can be successfully employed in the manufacturing of nanoparticles[1].

Green synthesis of nanoparticles is a method with several advantages including a sustainable and economically friendly method[2,3]. The unique physical and chemical properties of nano metals are a result of the tiny size, interface, quantum, and surface effect which are not observed in non-nano metals[4]. Consequently, this may lead to undesirable and detrimental health effects due to the lack of assurance and uncertain

regarding their composition. Green synthesis is a method that involves using natural extracts, such as leaves or fruits, instead of costly chemical-reducing agents in the manufacturing of nanoparticles[5]. This approach is similar to chemical reduction, but it is more environmentally friendly. The nanoparticles synthesized via the green approach also possess antibacterial, antifungal, antioxidant, and other biomedical applications. Green synthesis involves the manufacturing of nanoparticles by plants, algae, bacteria, and fungi[6]. The manufacturing of nanoparticles by plants is considered more beneficial than microbial synthesis due to its various benefits, including increased durability, reduced time requirement, and minimal risk of contamination[7]. Scientific evidence has shown that plant bioactive compounds including, polyphenols, phenolic acids, alkaloids, and proteins, have a remarkable impact on the manufacturing of nanoparticles and maintaining their stability[8]. The plant-derived biomolecules contain functional groups, primarily polar groups like hydroxy, amine, thiol, carbonyl, and carboxylic acids groups. These groups react with metal ions and undergo reduction through the transfer of electrons from the extract to the metal. The reduction process activates nucleation. A solution containing metal salts is added to the plant extract which is extracted using water or other solvent. The reaction for the production of nanoparticles is then completed in a short period[9].

Antimicrobial resistance has become a substantial and urgent menace to public health. Despite the implementation of various measures, the global patterns of antimicrobial resistance continue to show no signs of slowing down. When microorganisms adapt themselves to live in the existence of antimicrobials that once inhibited their growth, it is called antimicrobial resistance[10]. According to the Centers for Disease Control and Prevention, over two million people in the US acquire resistant pathogens annually, leading to a minimum of 23,000 fatalities. An increase in the development of drug resistance will diminish the impact of antimicrobials that are currently in use. Consequently, physicians will have no choice but to resort to using the last-resort classes of medication, such as polymyxins and carbapenems. However, it is important to note that these medications may not be easily accessible in developing countries, have various side effects, and are expensive[11]. Different reports suggest that overuse of antibiotics, not completing the complete course of antibiotics, and their misuse, for

example, administration of antibiotics, when not needed leads to their misuse, are the major reasons for the development of drug resistance[12,13]. The need for an alternative antimicrobial agent is the demand of the current scenario.

ZnO nanoparticles are utilized in various industrial sectors such as synthetic textiles, food packaging, environmental, medical care, and healthcare[14]. Zinc oxide, like other metal oxide groups, has been found to possess antibacterial properties. One of the benefits of utilizing inorganic oxides like zinc oxide as biocidal agents because they possess essential mineral elements for humans and demonstrate remarkable biocidal activity even in low doses. Zinc oxide (ZnO) nanoparticles demonstrate potent antibacterial properties against a wide range of bacteria[15,16]. Various mechanisms are thought to be involved in the antimicrobial activity of Zinc oxide nanoparticles out of which the production of Reactive Oxygen Species is found to be the most crucial reason which explains the mechanism of exerting microbicidal effect by zinc oxide nanoparticles[17]. Thus, Utilizing ZnO NPs shows potential as a viable option for reducing microbial resistance.

The demand for synthetic dyes, particularly in the textile and clothing industries, has resulted in a rise in the manufacturing of dyes including, crystal violet, methylene blue, congo red, aniline blue, etc. These dyes are produced on a large scale, with thousands of tons being manufactured worldwide every year. Out of the various synthetic dyes, methylene blue is found to be carcinogenic, mutagenic, and toxic which is however largely used as a colorant in cotton, wool, and silk. Nevertheless, the discharge of untreated wastewater containing Methylene blue from various industries can result in significant health hazards. For instance, in humans, the administration of this dye can cause a range of health problems including tissue necrosis, cyanosis, vomiting, shock, increased heart rate, and jaundice[18]. In context to the plants, the presence of methylene blue has emerged as a significant obstacle, leading to a decrease in pigment and protein levels and growth inhibition in microalgae species such as *Chlorella vulgaris* and *Spirulina platensis*[19]. Hence, it is imperative to eliminate methylene blue available in the wastewater effectively before discharging into the environment so as to prevent adverse consequences. Another dye named Eosin Yellow is an anionic dye which is a water-soluble, pink-colored acidic dye that gives a yellow fluorescence that is why it

is named Eosin Yellow. The precise quantification of the dye's toxicity remains limited, however, its significant presence in living systems has been proven to be detrimental. Photocatalysis has been scientifically proven to be a highly promising method for removing harmful dyes from industrial wastewater.

When there is not enough oxygen, plant-derived biomass can be thermally decomposed through a process called pyrolysis. This process can be controlled to produce combustible gases mainly H_2, CH_4, CO, CO_2, tarry vapors, volatile oils, and a solid carbon-rich residue known as char[20]. Biochar is a quite porous substance, carbon-rich material is acquired through the process of pyrolysis of plant biomass. The final products of pyrolysis can be influenced by both the pyrolysis condition and the feedstock biomass[21]. Several types of pyrolysis processes can be distinguished based on the pyrolytic condition. The three main processes are fast pyrolysis, slow pyrolysis commonly also known as carbonization, and gasification. The three distinct end products including char, gas, and liquid-oil are produced in varying proportions depending on the specific type of pyrolysis chosen. It was documented that slow pyrolysis yields a comparatively high amount of biochar. The pyrolysis reaction proceeds majorly in three different steps: During the initial stage, there is a reduction in moisture and the release of certain volatile compounds. The primary char is formed in the secondary phase. Rapid response is subsequently accompanied by a slower process that entails additional chemical reactions in order to generate secondary char[22].

The accumulation of nonbiodegradable contaminants has made heavy metal pollution a significant environmental concern. Industrial wastewater containing elevated levels of heavy metals can lead to significant contamination of water and soil. According to the China National Soil Pollution Survey Bulletin, 4.8% of the sampling sites were found to be contaminated with Nickel (Ni), which ranked second in contamination levels after Cadmium (Cd)[23]. Cadmium is considered to be a poisonous transition metal, a non-essential metal, and can be harmful to the health of both humans and animals. Epidemiological data suggests that exposure to cadmium can lead to the development of different cancer types, such as lung, breast, nasopharynx, etc. Cd is a pollutant that occurs naturally in the environment, originating from agricultural and industrial sources. Cadmium has a prolonged $t_{1/2}$ of approximately 25-30 years, leading

to its accumulation in plants and animals. Furthermore, studies have shown that exposure to environmental cadmium can increase the likelihood of developing osteoporosis[24].

The current research aims to utilize *Euphorbia tithymaloides* leaves in the manufacturing of ZnO nanoparticles. The photo-fabricated ZnO nanoparticles were then utilized as an antibacterial agent in inhibiting the poly-antibiotic-resistant bacterium *Bacillus clausii.* friedelanol, ursolic acid, β-sitosterol, and epifriedelanyl acetate are the reported bioactive compounds that were evaluated through *in silico* analysis for their antibacterial activity against Lipoteichoic acid synthase enzyme synthesized by *Staphylococcus aureus* which is a crucial enzyme required in the synthesis of lipoteichoic acid. This enzyme was inhibited using these phytocompounds of *Euphorbia tithymaloides*. Lipoteichoic acid is crucial for the survival, development, and reproduction of cells. Mutants lacking LtaS are susceptible to bursting under osmotic pressure and can only multiply in environments that have a stable osmotic balance. Inhibiting the biosynthesis of teichoic acid is a potential approach for therapeutic intervention. Further, these nanoparticles were employed as a photocatalyst for the elimination of two dyes Eosin Yellow and Methylene Blue. The leaves of *Euphorbia tithymaloides* were then utilized in the synthesis of biochar and the biochar produced was subsequently utilized in the removal of Cadmium from the wastewater.

CHAPTER 2

LITERATURE REVIEW

Euphorbia tithymaloides is a plant rich in various phytochemicals such as saponins, tannins, steroids, coumarins, and triterpenes, etc. The plant's abundance of phytochemicals makes them possess antibacterial, antioxidant, anti-diabetic, stomachic, antifungal, anti-helminthic, antimutagenic, anti-inflammatory, antiseptic, antitumor, and antiviral activity. A scientific study was executed to assess the *in vitro* anthelmintic activity of leaf extract of *Euphorbia tithymaloides* derived using ethanol as a solvent using *Pheretima posthuman* an adult earthworm. The presence of different bioactive compounds was confirmed in the extract of the leaves. Additionally, the extracts were effective in inhibiting *Candida albicans* with a MIC of 6 μg mL^{-1}. Thus, it proved to exhibit a potent antimicrobial activity[25]. The antiviral activity of *Euphorbia tithymaloides* was evaluated against HSV type 2. The leaf extract derived using methanol was used which contained a bioactive compound luteolin and was considered to be responsible for inhibiting the viral replication. It was observed that the extract was successfully able to suppress the activation of NF-κB. NF-κB activation is necessary for viral replication and suppressing its activation ultimately contributes to the prevention of viral replication. Additionally, the production of IFN-γ, (IL)-1β, IL-6, and TNF-α, directly responsible for regulating the NF-κB signaling pathway was all potently down-regulated by the leaf extract and luteolin[26]. The latex was obtained from different plant species that belong to the Euphorbiaceae family and has traditionally been used to stop bleeding on fresh cuts and promote wound healing. Clot-inducing properties of *Euphorbia tithymaloides* were studied. The stem latex of *Pedilanthus tithymaloides* demonstrates exceptional procoagulant activity. The blood sample from the ox exhibited a higher sensitivity to latex protease compared to the blood of other mammals. Simultaneously, the plant latex protease was able to significantly decrease the clotting time of whole blood samples from both humans and mice [27]. In another study, the extract of *Euphorbia tithymaloides* leaves derived using methanol and chloroform, along with its isolated constituents, were evaluated for their antinociceptive, anti-inflammatory, and antipyretic, properties in animal models. The

study found that chloroform and methanol-derived plant extract demonstrated significant effects that were tested in acute and chronic models. The bioactive compound screening of both the extracts made using chloroform and methanol resulted in the identification of five previously identified compounds: friedelanol, b-sitosterol, epifriedelanyl acetate, luteolin, and ursolic acid[28]. Phytochemicals available in plant extracts serve as electron donors to metal ions, causing their reduction into metal nanoparticles. Ag nanoparticles were manufactured utilizing leaf extract of *Euphorbia tithymaloides*. It was found that the silver nanoparticles exerted a potent larvicidal effect and could effectively be employed with other insecticides[29]. In another study, the biosynthesized silver nanoparticles from this plant were evaluated for biocidal properties against *Escherichia coli, Micrococcus luteus*, *Pseudomonas aeruginosa, Staphylococcus epidermis, and Staphylococcus aureus.* It was observed that the manufactured Ag nanoparticles were able to effectively inhibit the test microorganisms[30]. Manufacturing of nanoparticles involving the utilization of plant extracts is uncomplicated. A solution containing metal salts is mixed with the plant extract which is extracted using water or other solvent at room temperature. The process for manufacturing of nanoparticles is then completed in a short period[9].

ZnO nanoparticles are highly suitable for biomedical applications because of their convenient manufacturing process[31]. ZnO nanoparticles are currently being employed in various applications[32]. They are valued for their ability to absorb UV radiation while remaining transparent to visible light in sunscreen[33]. They are utilized in food packaging to preserve the quality of the food. Additionally, nanoparticles are incorporated into cosmetics because of their antibacterial and antifungal properties. Different mechanisms are reported to contribute to the microbicidal effect of nanoparticles on microorganisms. A scientific study was conducted to produce Ag NPs using the seed extract of Tectona grandis. Their findings demonstrated that the cell membrane's alteration in permeability was the cause of protein and reduced sugar leakage from the bacterial cell. As the incubation time increased, there was a corresponding increase in the leakage of proteins and reducing sugar when the Silver NPs were incubated with Bacteria, and eventually caused the death of microorganism[34]. DNA degradation of microorganisms by the nanoparticles can also

be another reason for the demise of microorganisms[35]. In a study, Zinc oxide nanoparticles were manufactured utilizing *Eucalyptus globulus* essential oil. Hydro distillation was performed to extract the essential oil from their leaves. Zinc acetate dihydrate was utilized as a metal salt and was mixed in the extracted essential oil to produce ZnO nanoparticles. The dimension of the manufactured nanoparticles was 24nm. They were further evaluated for their antibacterial activity. The maximum inhibition zone was observed to be 19.35 ± 0.45 mm against K. pneumoniae[36]. *Mentha pulegium* aqueous leave extract was utilized during the manufacturing of ZnO nanoparticles. Their result indicated that the manufactured ZnO nanoparticles exhibited more biocidal effects on gram-positive bacteria. Additionally, the nanoparticles were also employed as a photocatalyst and effectively eliminated MB dye from the wastewater, within the first 30 min 50 percent of MB dye was eliminated from the solution by Zinc oxide nanoparticles[37]. *Punica granatum* was employed in the creation of ZnO nanoparticles. The dimension of ZnO nanoparticles was observed to be 57.75 and 52.50 nm for leaves and flower-mediated synthesized ZnO respectively. The biocidal activity of ZnO nanoparticles was assessed on different types of bacteria and at 5000µg/ml of ZnO nanoparticles maximum inhibition zone was observed against *Enterococcus faecium* with 19.33 ± 1.15mm inhibition[38]. Photocatalysis is a distinct technique that can be employed for multiple purposes, including breaking down different organic pollutants in wastewater, antibacterial activity, generating hydrogen, and purifying air. Wastewater treatment is increasingly focusing on the photocatalytic process Because of its ability to eliminate dyes under moderate conditions, surpassing other methods. The energy difference between the valence and conduction band of ZnO nanoparticles is very close thus it exhibits the efficiency of photodegradation[39]. Extracts of onion, garlic, and parsley were employed during the manufacturing of ZnO nanoparticles. Based on the type of extract used the size of produced nanoparticle varies from 14-70 nm. Under UV irradiation the methylene blue dye degradation by photocatalysis was carried out utilizing ZnO nanoparticle as photocatalyst. Their results concluded that the ZnO nanoparticles synthesized utilizing garlic extract exhibited the highest photodegradation capability compared to those synthesized using onion and parsley[40]. Leaves of *Salvia officinalis* were employed during the manufacturing of ZnO nanoparticles which were further

used in the breakdown of methyl orange dye through exposure to UV radiation. Under ideal experimental conditions, ZnONPs demonstrated a degradation rate of 92.47% for methyl orange[41]. The roots of a perennial herb, Codonopsis lanceolata have been documented to exhibit antioxidant and antimicrobial characteristics. The *Condonopsis lanceolata* plant contains saponins that are very reactive. These saponins can be used to cover the surface of ZnO nanoparticles, thereby enhancing its efficiency as a photocatalyst. One-pot manufacturing of ZnO nanoparticles was executed utilizing the extract obtained from the roots of *Codonopsis lanceolata.* Within 30 min ZnO nanoparticles degraded 90.3% methylene blue dye from the solution through photocatalysis[42]. Leaf extract of *Corymbia citriodora* employed in the biosynthesis of ZnO nanoparticles was evaluated for its photocatalytic efficiency and degraded 83.45% of methylene blue in 90 min. The synthesized nanoparticles were found in a range between 20-120nm and due to their small size, they proved themselves to be excellent for photocatalysis[43]. Adsorption is a commonly employed physical method of separation that effectively reduces the amount of pollutants in the water. This is achieved by using widely used adsorbents including activated carbon, silica gel, etc. Biochar is a material that can adsorb substances and is thus effectively utilized during the elimination of heavy metals in polluted water. There are numerous methods available for removing heavy metals, including both unconventional and conventional approaches. Their effectiveness is assessed based on the removal rate; however, adsorption is currently considered the most efficient approach because of its economical, simplicity, and potential for wider application[44,45]. Different properties such as a porous network and the ability to exchange cations make the biochar a suitable substance for detoxification of polluted water from heavy metals[46,47]. In a study, biochar was made using *Pinus sylvestris* and *Betula pendula* and was utilized during the elimination of heavy metals, copper, cadmium, lead, and zinc. The highest adsorption capacity for copper (128.7 $\mu g\ g^{-1}$) was achieved using biochar made using *Betula pendula* while for zinc (107.0 $\mu g\ g^{-1}$) biochar made from *Pinus sylvestris* demonstrated the highest adsorption capacity[48]. A batch study was conducted to observe the efficiency of biochar made from rise husk in the removal of Cadmium from the solution. Their result indicated that the biochar was able to remove 70% cadmium from the solution with 17.8 mg/g cadmium adsorption by biochar[49]. In

another study, biochar was made using rice husk, wheat straw, and corncob. The biochar made was studied for the adsorption of Pb and Cd from the polluted water. The biochar showed an excellent Pb and Cd adsorption capacity[50].

CHAPTER 3
METHODOLOGY

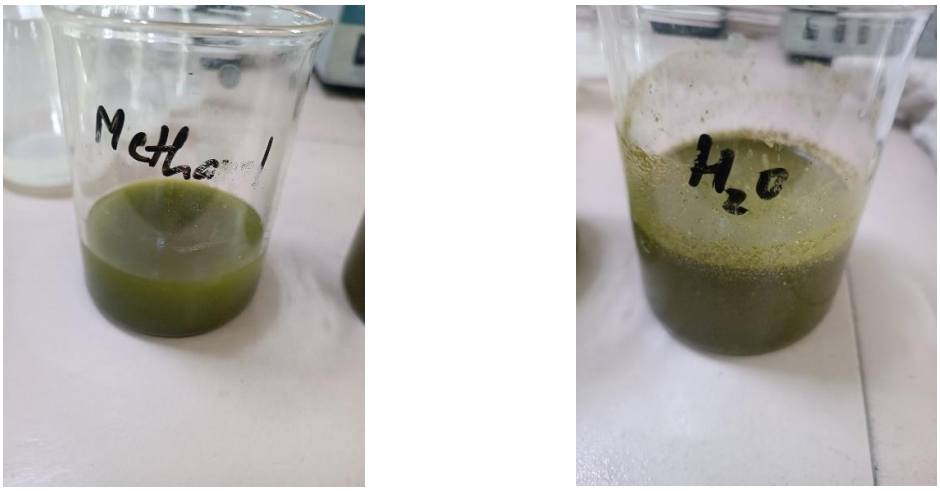
3.1 Biochemical assay to qualitatively determine the presence of bioactive compounds in *Euphorbia tithymaloides*.

3.1.1 Extract preparation:

- Weigh the collected leaves of *Euphorbia tithymaloides.*
- The leaves of *Euphorbia tithymaloides* were allowed to dry.
- Following the drying of leaves, weigh the dried leaves in order to identify their moisture content.
- After desiccating the leaves, the leaves were pulverized in a fine powder and weighed.
- 200 ml methanol and distilled water were taken in two different beakers and powder of crushed leaves was added to both the beakers in the same quantity.
- The solution contained in both the beaker was heated on the heating mantle at 80-90ºC.
- After a duration of 30 to 45 minutes, when approximately 50% of the original concentration of the solution remains, the mixture is subjected to filtration, and the extract obtained following the filtration was utilized for conducting a phytochemical analysis to ascertain whether or not various kind of bioactive compound was present.

Fig. 3.1 Collection of *Euphorbia tithymaloides* leaves.

Fig. 3.2 Powder of dried leaves of *Euphorbia tithymaloides*.



(a) (b)

Fig. 3.3 Extract preparation using (a) Methanol and (b) Distilled water.

3.1.2 Qualitative Test

3.1.2.1 Glucoside Test

In the plant extract which was obtained using methanol and H_2O, H_2SO_4 was added. Following the addition of sulfuric acid when the color of the solution changed to a yellow-orange color this is the positive indication of the glucoside test.

3.1.2.2 Saponins Test

Another name for the test used to identify saponins in plant extract is the frothing test. The 1 ml extract obtained using both methanol and H_2O was measured and introduced to the test tube. 5ml of H_2O was introduced to both the test tubes and shaken well. The Saponins are indicated by the development of a thick layer of foam [25].

3.1.2.3 Tannins Test

For the qualitative analysis of the Tannins $FeCl_3$ is added to the obtained extract that's why it is sometimes referred to as the ferric chloride test. $FeCl_3$ solution (5%) was prepared. The availability of tannins is confirmed when a blue-black color is produced when ferric chloride is added.

3.1.2.4 Cardiac Glycoside Test

glacial acetic acid to which 2% $FeCl_3$ solution was added and the solution was added to the extract. H_2SO_4 in a drop-wise manner was added. Brown ring formation is a positive result.

3.1.2.5 Terpenoids Test

The extracts were introduced to 2 ml of chloroform and then it was evaporated until they became completely dry. Subsequently, a volume of 2 ml H_2SO_4 was introduced and subjected to heating. The presence of terpenoids was indicated by a brownish color.

3.1.2.6 Steroids Test

The extract was mixed with 2 ml chloroform, and H_2SO_4 was added slowly and carefully. The presence of steroids was indicated by the production of red color in the lower chloroform layer.

3.1.2.7 Glycoside Test

Glacial acetic acid, $FeCl_3$, and a few drops of concentrated H_2SO_4 were added to the plant extract. Blue-green precipitates confirm the availability of glycosides in the plant extract.

3.1.2.8 Flavonoids Test

2ml of plant extract obtained using methanol and distilled water were taken in two separate test tubes. NaOH was introduced to both test tubes containing the plant extract obtained using methanol, and H_2O. The yellow color is proof of the availability of flavonoids.

3.2 Manufacturing of ZnO Nanoparticles employing the leaves of *Euphorbia tithymaloides*

3.2.1 Leaf Extract Preparation

- Leaves were gathered and cleaned using double-distilled water.
- The leaves were subsequently dried by exposure to ambient air until they were completely devoid of moisture.
- After the leaves had dried, they were crushed to produce a rough powder.
- A solution was prepared by combining 10 grams of powder with 200 milliliters of H_2O.
- The extract was acquired through the process of heating the mixture in a water bath at 70°C for 15 minutes.
- Subsequently, the mixture underwent filtration and the extracted substance was preserved at a temperature of -4°C until it was needed.

Fig. 3.4 Powder of the dried leaves of *Euphorbia tithymaloides*.

Fig. 3.5 Distilled water used as a solvent to extract bioactive compounds from leaves.

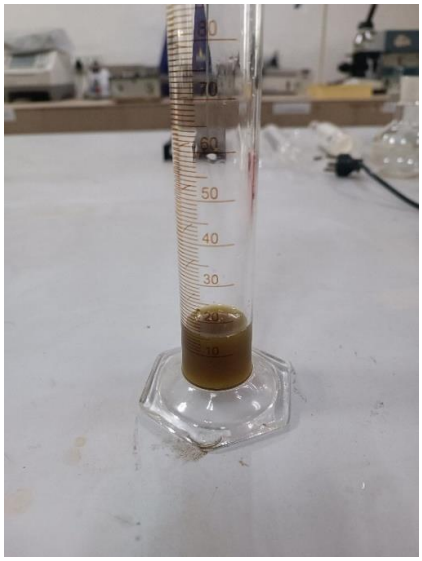


Fig. 3.6 Extract obtained used water as a solvent.

3.2.2 Manufacturing of ZnO nanoparticles

- The leaf extract obtained by heating was added to a 100 mL 100mM zinc sulfate heptahydrate solution in a dropwise manner. The zinc sulfate solution was kept on a magnetic stirrer and stirred at 700 rpm.

- The initial sign indicating nanoparticle synthesis was a visible color change in the solution.
- Subsequently, a 1 M sodium hydroxide (NaOH) solution was incrementally added to the mixture, and agitated at ambient temperature. At a pH of 12, we will obtain yellowish-white precipitates.
- The Solution underwent centrifugation and the resulting pellet was rinsed by dispersing them in sterile, double-distilled water and then centrifuging them three times at a speed of 5000rpm. The obtained ZnO powder was purified by washing it with ethanol to eliminate impurities. Subsequently, the nanoparticles were kept in a hot air oven for drying at 80°C for a duration of 5 hours to obtain the ultimate product [36].

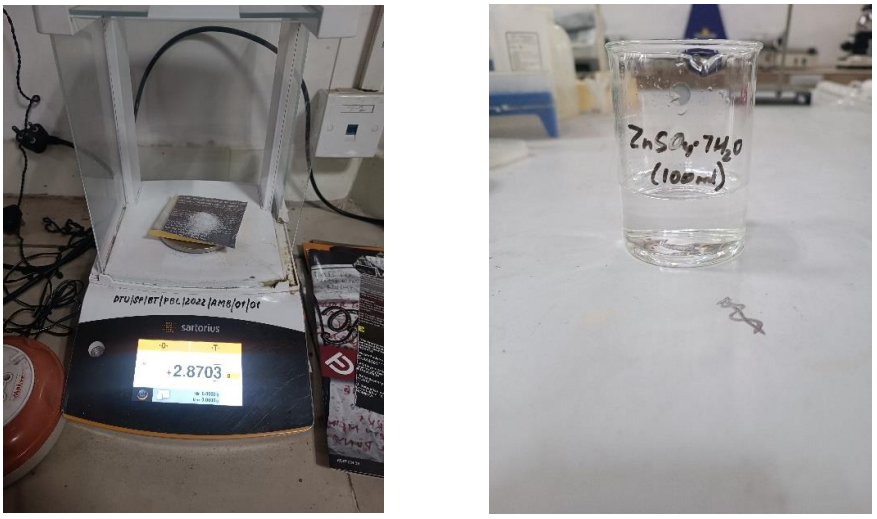


Fig. 3.7 100mM zinc sulfate heptahydrate weighed and added in 100 ml Distilled water.

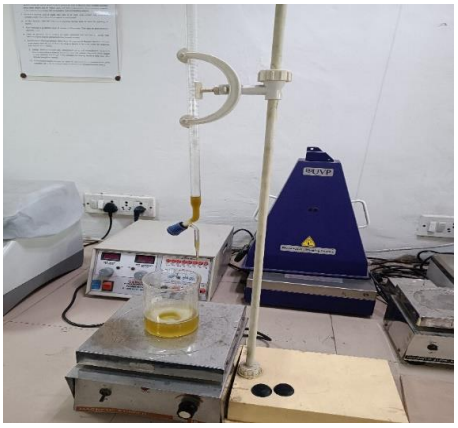


Fig. 3.8 Leaves Extract added in a dropwise manner in the solution of Zinc sulfate heptahydrate.

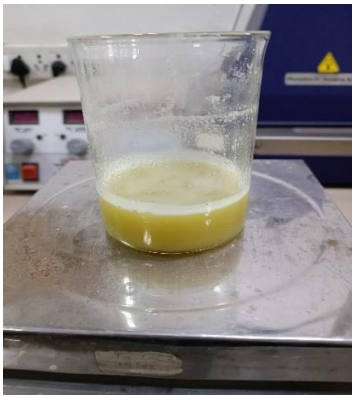


Fig. 3.9 An observable alteration in the color of the solution occurs upon the addition of NaOH to maintain a pH level of 12.

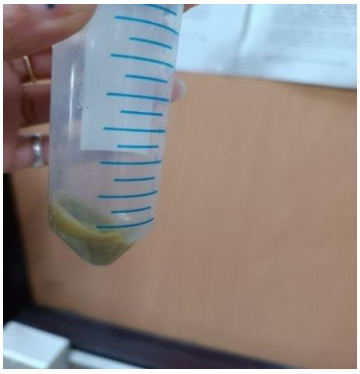


Fig. 3.10 Pellet obtained after washing the solution with water and ethanol by centrifugation.

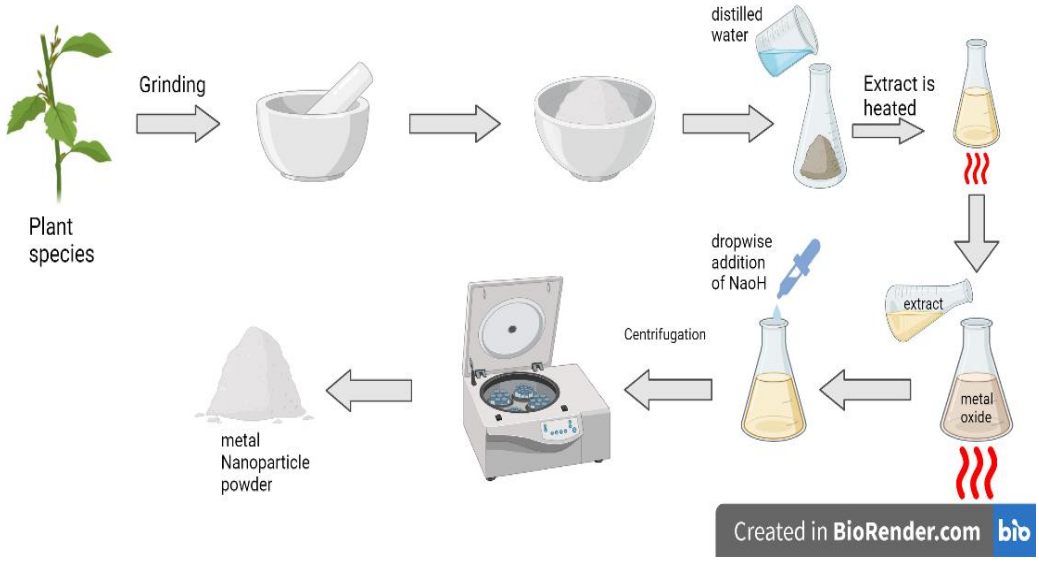


Fig. 3.11 Schematic illustration of manufacturing of Nanoparticles.

3.3 Analysis of ZnO nanoparticles

3.3.1 Colour change

The alteration in color of a solution is indeed one of the fundamental attributes of nanoparticles produced through the synthesis process involving plants. When plants are employed as bioreactors for the manufacturing of nanoparticles, the metal ions are reduced, creating the nanosized metals. The reduction process frequently causes a visible alteration in the color of the solution. When plant extract was introduced to zinc sulfate heptahydrate solution, alteration of the color was observed, indicating the manufacturing of ZnOnanoparticles.

3.3.2 UV-Vis Spectrometry

UV-Vis Spectrometer (PerkinElmer UV/Vis Lamda 365) was employed to analyze the spectral properties of ZnO nanoparticles.

The ZnO nanoparticles manufactured from *Euphorbia tithymaloides* were added into the distilled water, followed by sonication. Spectra of the solutions obtained were measured between 200–800 nm.

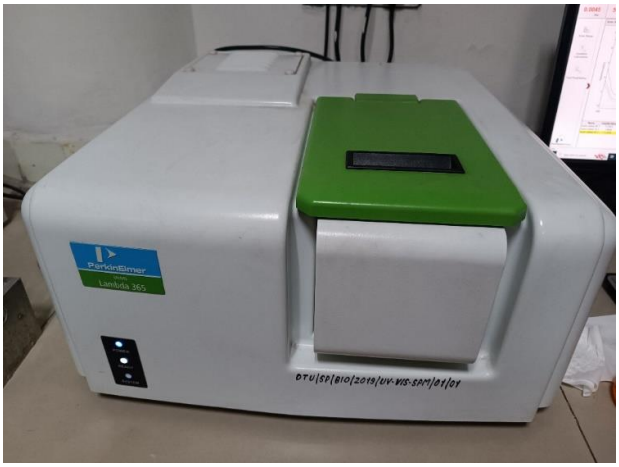


Fig.3.12 Analysis of ZnO nanoparticles using PerkinElmer UV/Vis Lamda 365.

3.4 Investigation of Antibacterial efficacy of ZnO nanoparticles manufactured using *Euphorbia tithymaloides.*

3.4.1 Isolation of Bacterial Species

A poly antibiotic-resistant spore suspension named Bactilus of *Bacillus clausii* was purchased. The spore suspension consists of 2 billion spores per 5 ml of *Bacillus clausii*. The bacteria were first serially diluted to get separated colonies on the nutrient agar plate.

Fig. 3.13 A poly antibiotic-resistant spore suspension of *Bacillus clausii*.

3.4.2 Serial Dilution

The objective of serial dilution was to quantify the amount of an unknown sample and to get countable colonies (between 30-300 colonies). A broad range (e.g., 10^{-3} to 10^{-10}) is typically used for plating as the precise quantity of viable bacteria in the sample is often uncertain. To achieve greater precision, it is recommended to plate duplicates or triplicates of each dilution.

CFU is determined by:

CFU / ml = No. of viable colonies / (Dilution X Volume of dilution plated)

Procedure:

- Ten test tubes were taken and 9 ml of saline solution added to each tube, cotton plugged, subjected to autoclaving.
- The dilution tubes were labeled as 10^{-1}, 10^{-2}, ..., 10^{-10} and arranged in the ascending order of the dilution.
- Under sterile conditions, one drop of spore suspension of *Bacillus clausii* was aseptically transferred to the tube labeled as 10^{-1}.
- The 10^{-1} dilution was thoroughly mixed by vortexing the tube. 1 ml of solution was taken from a 10^{-1} dilution to 10^{-2} dilution tube.
- In the same manner a series of dilutions were prepared, ranging from 10^{-1} to 10^{-10}.

3.4.3 Spread Plating

Microorganisms can be extracted from various environments. However, to analyze its specific traits, it is imperative that it exists in a state of pure culture. There are multiple techniques available for acquiring uncontaminated cultures. The Plating Method is a frequently employed technique. Spread Plate is one of the plating methods.

3.4.4 Composition of media:

Nutrient medium is a complex medium which is a routine medium and widely used to culture a wide range of bacteria including *Bacillus clausii*. Nutrient medium is a medium that is sufficient to meet all the nutritional requirements of various microorganisms. Thus, nutrient medium supports the culturing of a number of microorganisms this media is also called Supportive media.

Table 3.1 Composition of Nutrient medium.

Composition (for 200ml)	Amount
Agar	4g
Sodium Chloride	0.1g
Beef extract	0.6g
Peptone	1g
Distilled Water	200ml

Procedure:

Part 1: This can be done on benchtop

- Two clean conical flasks were taken.
- 200 ml distilled water was added to both the flasks.
- Using a filter paper, weigh 1g of peptone, 0.6g of beef extract, and 0.1g of NaCl and add them to both flasks.
- In a circular motion swirl the flask in order to mix the constituents.
- Weigh 4g agar powder and add it to one of the conical flasks.

Part 2: Autoclaving

- Both the flasks were sealed with cotton plugs and wrapped with aluminium foil.
- Before starting the autoclave, the water level was checked.

- The medium was then autoclaved at 121ºC, 15 psi pressure for 15-20 min.
- The nutrient medium was kept inside the autoclave until it cools around 50-55ºC.

Part 3: Pouring the plates for nutrient agar (done in Laminar airflow)

- Before the process of pouring for at least 15-20 min UV light was switched on in the laminar air flow in order to kill the microorganisms and spore to make the working area sterile.
- While working UV light was switched off and airflow was switched on along the tube light.
- The surface of the laminar was first sterilized using 70% Ethanol
- Bunsen burner was lighted up.
- The petri plates were labeled at the bottom with, the date, dilution factor, and name.
- The nutrient agar was poured about half of the plate when viewed from the side of the Petri plate. The agar was poured slowly in the Petri plates in order to prevent the bubble formation.
- After pouring the medium the lid was closed and the plates were swirled in a circular motion to evenly distribute the nutrient agar in the plate.
- The plates were kept still to allow the medium to get solidify.

Part 4: Spread plating

- After the solidification of nutrient agar, 0.1 ml of dilution from each test tube from 10^{-3} to 10^{-10} dilutions were taken separately and transferred to the plates containing nutrient agar. The liquid was evenly distributed on the plate's surface using a spreader while the plate was rotating continuously. The spreading process was continued until the surface of the plate was completely dry.
- The plates were placed inside an incubator at 37ºC overnight.
- The no. of colonies was enumerated and the colony-forming units per milliliter were calculated.

3.5 Characterization of the bacteria

3.5.1 Gram-Staining

Principle

It is the extensively used stain in the field of bacteriology to differentiate the bacteria. They are categorized into two distinct groups on the basis of staining technique: Gram-negative and Gram-positive. The fundamental dye crystal violet is first added for staining. This is the main or principal stain. Afterward, the bacterial cell is treated with an iodine solution. It enhances the interaction resulting in a stronger binding of the dye or a more intense staining of the cell. The smear was rinsed with 95% ethanol which works as a decoloriser. Gram-positive bacteria retain the primary stain even after being rinsed with the decolorizer, while gram-negative bacteria lose the primary stain and are devoid of color. Ultimately, the smear is stained again using a basic dye that has a distinct color from crystal violet. The typical counterstain employed is safranin. Safranin will impart a pink color to the colorless, gram-negative bacteria, while it does not affect gram-positive bacteria's dark purple color. This difference in acquiring different colors is because of the difference in the thickness of peptidoglycan. Gram-positive cells possess a substantial amount of peptidoglycan and a minimal amount of lipids, whereas Gram-negative bacteria exhibit a significant concentration of lipids and a low concentration of peptidoglycan. When exposed to alcohol, the lipids in the cell walls of Gram-positive bacteria undergo treatment. Alcohol causes the dissolution of negative bacteria, resulting in an enhanced permeability that allows the removal of crystal violet stain from the cell. Gram-positive bacteria lack sufficient lipid content to generate porosity, resulting in the entrapment of crystal violet molecules within a dense peptidoglycan meshwork.

Procedure

- A colony from the inoculated plate was picked and a smear was made on the slide, allowed to dry in the air, and then fixed by applying heat.
- Crystal violet stain was added onto the smear for 1 minute.
- Smear was washed with water until all surplus stain was eliminated.

- Smear was immersed in Gram's iodine solution for a duration of 1 minute. The smear was then subsequently washed with water.
- The smear was bleached using 95% ethanol for a duration of 20 seconds.
- A safranin counterstain was applied for a duration of 1 minute.
- Further washing with water, dried, and examination using an oil immersion lens was done.

3.5.2 Negative Staining

Negative staining differs from traditional staining methods in that it colors the background surrounding the specimen while leaving the specimen itself unstained with the stained background. A bright field compound microscope is used to visualize the sample.

Procedure:

- A colony from an inoculated plate was picked and a smear was made on the slide, and allowed to dry in the air.
- Negative stain, Nigrosin was added to the smear.
- Another slide was taken and was kept onto the stain at an angle of 45º spread along the other slide and dried.
- The slide was observed under a compound microscope.

3.5.3 Growth pattern in broth

The bacteria when inoculated in the broth can exhibit different growth patterns based on their nature whether aerobic or anaerobic. A tube with no growth indicates no bacterial growth. A tube with growth only on the surface of the broth with a clear bottom indicates that the bacterial species is obligate aerobe. While bacterial growth in the bottom of a tube indicates that it must be anaerobic. A tube with the growth of bacterial species all over the broth indicates the bacteria is a facultative anaerobe.

Procedure

- Nutrient broth was added to a test tube, cotton plugged, and autoclaved.
- After autoclaving the test tube was taken to the laminar airflow and a colony using an inoculating loop from the nutrient agar plate was picked and transferred to the nutrient broth.
- The test tube was followed by keeping in a growth chamber at 37ºC for a duration of 24 hours.
- After 24 hr the growth pattern was observed.

3.6 Microbicidal potency of ZnO NPs on drug-resistant bacteria

The microbicidal effect of ZnO nanoparticles was examined against a bacterium that is resistant to multiple drugs. The diffusion concept was utilized to examine the microbicidal activity of ZnO nanoparticles with poly antibiotic-resistant *Bacillus clausii* strain.

In this study, the bacterial strains were tested against standard antibiotics as a means of internal control. Pre-adjusted overnight cultures were evenly spread on nutrient agar plates and plates were left for 10 min. Rifampicin was taken as a control antibiotic. Small circular pieces of filter paper were taken and with the help of sterile forceps were placed on the inoculated nutrient agar plates. Plant extract obtained using water and methanol was added in a small amount onto the filter paper and in a similar manner, ZnO nanoparticles were also added. To compare the biocidal properties of biosynthesized ZnO nanoparticles, blank ZnO nanoparticles which were synthesized using chemicals were also added on one piece of filter paper placed on a medium. The plates remained in the biosafety cabinet undisturbed for 1 hour to make sure that the samples were evenly distributed on the agar. Afterward, they were retained in the incubator at 37ºC for a period of 24 hours. The diameter of the inhibition zone for bacterial growth was measured and recorded [38].

3.7 *In Silico* analysis of the antibacterial activity of phytocompounds of *Euphorbia tithymaloides* against *Staphylococcus aureus*

Lipoteichoic acid synthase enzyme (PDB ID: 2W5S) was downloaded in PDB format from RCSB PDB. Using Biovia Discovery Studio the water droplets and Heteroatoms were cleared from the framework of the enzyme as these molecules could inhibit the binding of specific ligands to their designated targets.

Three-dimensional structures of four phytocompounds, friedelanol, ursolic acid, β-sitosterol, and epifriedelanyl acetate [28] that are reported to be present in the leaves of *Euphorbia tithymaloides* were downloaded from PubChem.

Molecular Docking of these molecules was performed using PyRx- Python Prescription 0.8 and the binding energy between the ligand and protein was obtained and stored. After the docking procedure was finished, the resulting relationship between the target protein and ligand was saved in PDB format. The interaction was then analyzed using PyMOL and Biovia Discovery Studio. A 2D graphic illustrating the interaction between the protein and ligand was generated and saved as image files.

3.8 Photocatalytic performance of zinc oxide NPs

3.8.1 Decolorisation of Eosin Yellow using ZnO nanoparticles

The photocatalytic capability of ZnO nanoparticles observed, with examining the reduction in color intensity of eosin yellow solution.

- 50 milligrams of photocatalyst were weighed.
- 2.7 mg of eosin yellow is weighed and incorporated in 100 ml distilled water with a ultimate concentration i.e. 4×10^{-4} M.
- Prior to being exposed to the UV lamp, the suspension was kept on a magnetic stirrer at a rotation of 600/min for certain period of 1 hour in a dark environment.
- The reaction was conducted with continuous magnetic agitation throughout the process.

- At regular interval the sample from the suspension was taken in the cuvette for absorbance. The degradation of eosin yellow was examined with a ultraviolet-visible spectrum shows wavelength between 200 to 700 nanometer[51].
- Percentage decolorization was calculated and recorded [52].

$$\%\text{Decolourization} = (C_0 - C)/C_0 \times 100$$

C_0 - dye's starting concentration

C- after photocatalysis dye's ultimate concentration

3.8.2 Decolorisation of Methylene Blue using ZnO nanoparticles.

- A stock soln. was prepared via adding 10 mg MB dye in 1L double distilled water.
- Approximately 10 mg of ZnO nanoparticles that were produced through biological synthesis were introduced into a solution of methylene blue colored dye, with a capacity of 100 mL.
- Prior to being exposed to the UV lamp, the suspension was kept on a magnetic stirrer at a speed of 600 revolutions per minute for a duration of 1 hour in a dark environment. The reaction was conducted with continuous magnetic agitation throughout the process.
- At regular interval the sample from the suspension was taken in the cuvette for absorbance. The degradation of eosin yellow was examined using a UV-Vis spectrophotometer at 660nm[53].
- Dye degradation percentage was obtained using the above formula.

3.9 Biochar Synthesis using *Euphorbia tithymaloides* leaves

- The leaves of *Euphorbia tithymaloides* were gathered from the plant and cleaned with the deionized water.
- The leaves were kept in the hot air oven at 60ºC to get dry.
- After drying the leaves, the leaves were crushed into the grinder in order to get the powder.
- The obtained powder of leaves was then impregnated with the concentrated phosphoric acid.

- Then carbonization of the combination was carried out in a combustion chamber lasting 200 ºC for 2-3 hr.
- Before taking out the carbonized material, it was left in the hot air oven to cool down.

3.9.1 pH of the biochar

The carbonized substances pH's must be maintained around 7. Thus, after the process of pyrolysis maintaining the material's pH must be done.

- Biochar was washed many times with distilled water while centrifuging the carbonized material.
- After each washing its pH was checked.
- Washing was continued until nearly the neutral pH of the biochar was achieved.
- After achieving the neutral pH of the biochar, the mixture was filtered out.
- After that, biochar was dried out at 80ºC in oven with hot air for 24 hr.

3.10 Utilizing Biochar for the elimination of Cadmium from artificial wastewater

The biochar fabricated utilizing Euphorbia *tithymaloides* leaves was then utilized as an adsorbent to treat artificial wastewater.

various amount of biochar was taken and checked for their ability as an adsorbent to remove the heavy metal from a 500-ppm solution of cadmium.

- A 10,000-ppm stock solution of Cadmium was prepared.
- A stock solution of 10000 ppm was made by dissolving 1g of Cd 100 ml distilled water.
- Out of the stock, a 500-ppm solution of cadmium was made by mixing 25 milliliters of the stock mixture in 25 ml sterile water and 4 duplicates of 500 ppm of Cd were made in 5 conical flasks.
- Weigh 50mg, 100mg, 150mg, 200mg, and 250mg biochar.
- Add 50mg biochar in the first flask and label it as 50, in the same manner, add other concentrations of biochar in different flasks and label them.
- The conical flasks were mounted on a rotary flask spinner and set at 100 rpm.

- UV-Vis reading at a regular interval was taken and the observation was recorded.
- The blank solution was made by adding the above-mentioned concentrations of biochar in distilled water, however, the heavy metal was absent in the blank solution.
- UV-Vis reading of blank was also recorded [49].

Fig. 3.14 Heavy metal (Cd) solution of 500 PPM containing different concentrations of biochar placed on a Rotary Flask Shaker at 100 rpm.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Biochemical assay to qualitatively determine the presence of bioactive compounds in *Euphorbia tithymaloides*.

Table 4.1 Qualitative test for the screening of different bioactive compounds in the leaves of *Euphorbia tithymaloides*.

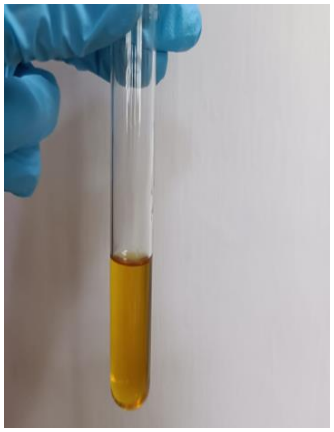
Qualitative test	Result (Water as a solvent)	Result (Methanol as a solvent)
Saponins	+	+
Flavonoid	+	+
Tannins	-	+
Glucoside	+	+
Glycoside	+	-
Cardiac glycoside	+	+
Terpenoids	-	+
Steroids	+	-

Fig. 4.1 Leaves extract obtained using (a) Water and (b) methanol.

Water as a solvent

Saponins Test

Flavonoid Test



Tannins Test

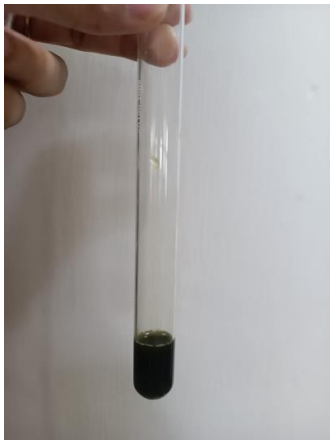
Methanol as a solvent

Saponins Test

Flavonoid Test

Tannins Test

Glucoside Test



Glucoside Test

Glycoside Test

Glycoside Test

Cardiac Glycoside

Cardiac Glycoside

Terpenoids and steroid Test

Terpenoids and steroid Test

Fig. 4.2 Results of qualitative test of different bioactive compounds.

4.2 Manufacturing Nanoparticles of zinc oxide originated from the leaves of *Euphorbia tithymaloides*

The moisture content of *Euphorbia tithymaloides leaves:*

The initial weight of the leaves before drying – 104.8g

The dry weight of the leaves – 9.8g

Moisture content (%) = $[(W_O – W_D) / W_O] \times 100$

W_O- initial weight of leaves

W_D- weight of leaves after drying

Moisture content (%) = [(104.8 – 9.8) / 104.8] x 100

Moisture content = 90.64%

4.3 Manufacturing of ZnO nanoparticles

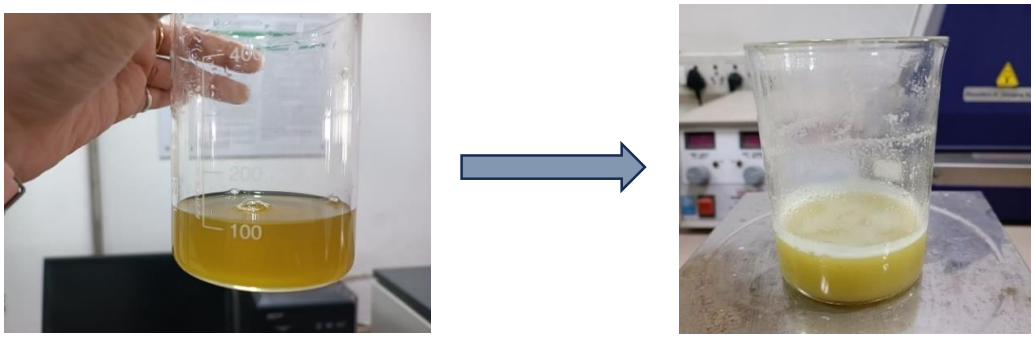
Yellow-white precipitates were formed following the addition of NaOH while maintaining the pH of the solution to 12. Following the washing with distilled water and ethanol, the pellet obtained was left in a Hot air oven at 80ºC for 5 hr for drying.

Fig. 4.3 ZnO nanoparticles synthesized utilizing the leaves of *Euphorbia tithymaloides* after drying.

4.4 Characterization of nanoparticles

4.4.1 Color change

When the extract of plants was incorporated into the salt of metal, zinc sulfate heptahydrate, the reaction mixture underwent a color change, signifying the formation of ZnO Onanoparticles.



Yellow-white precipitates

Fig. 4.4 The solution undergoes a noticeable color change to a yellow-white hue, indicating the successful creation of Zinc oxide nanoparticles.

4.4.2 UV-Vis spectrometry

The confirmation of the generated ZnONPs was conducted via UV-visible Spectrophotometer at ambient temperature. The aqueous leaf extract contains a significant amount of various biological substances, particularly flavonoids and

phenolics. These components exhibit various neutralizing and reducing characteristics when combined using Zn^{2+} ions, leading to the formation of ZnO[41]. The phenol components start the process of Zn^{2+} ions capping, while phenolic molecules form multiple chelating links and strengthen the ZnO NPs once they are formed, causing creation of nanoparticles of separate dimensions.

A peak of absorption was detected at a wavelength of 244nm, this lines up with ZnO peculiar band. This confirms the creation of ZnO nanoparticles. In another study, ZnO[54]*alsinoides* plant extract show a UV-Vis absorption peak at 264nm[54.] Fig. 4.5 dispaly the peak of absorption of ZnO NPs generated by *Euphorbia tithymaloides*.

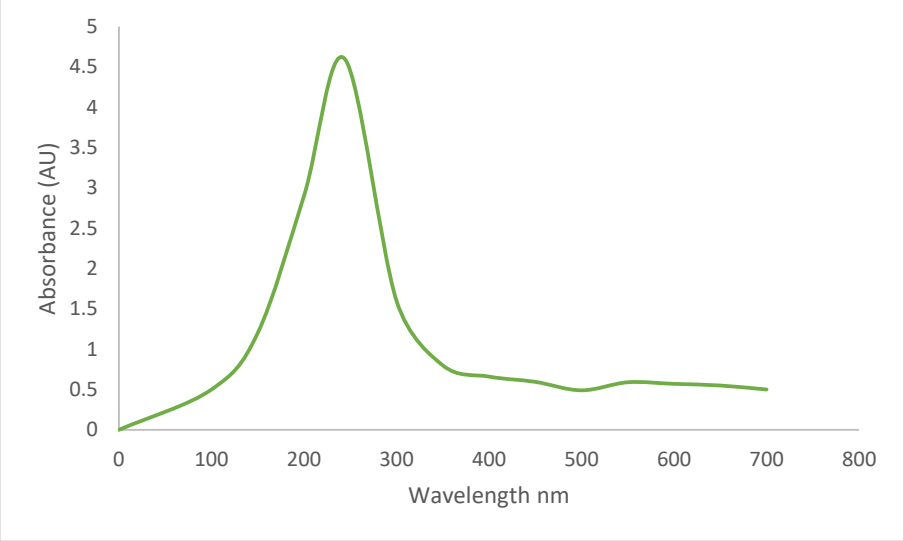


Fig. 4.5 UV-vis spectrum of ZnONPs.

4.5 Antibacterial performance of manufactured ZnONPs using *Euphorbia tithymaloides.*

Serial dilution of spore suspension of *Bacillus clausii* was done in order to get countable colonies between 30-300 colony numbers. Following the serial dilution 0.1 ml of dilution from each test tube from 10^{-3} to 10^{-10} dilutions were taken separately and transferred to the plates containing nutrient agar. It was observed that in nutrient agar plates with dilutions ranging from 10^{-1} – 10^{-3}, the colonies were too numerous to count (TNTC) while in the plates with dilutions ranging from 10^{-7} – 10^{-10} the colonies

were observed to be too less to count (TLTC). 10^{-4} nutrient agar plate consisting of 36 colonies and CFU was calculated.

CFU/mL = No. of colonies / (dilution X Volume of dilution plated)

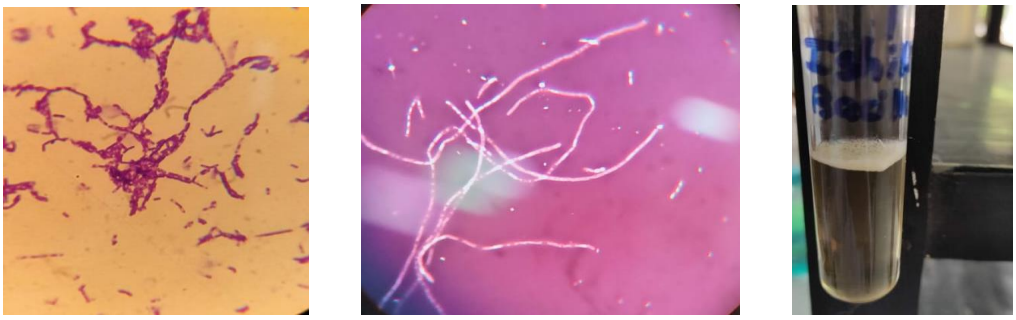
CFU/mL = 36 / (10^{-4} X 0.1**)**

CFU/mL = 3.6 X 10^{6}

Fig. 4.6 *Bacillus clausii* growth in Nutrient agar and Nutrient broth.

4.5.1 Characterization of the bacteria

Negative staining was done by picking up one of the colonies. Rod-shaped bacillus was observed under a bright field microscope. Further confirmation was done by performing gram staining where rod-shaped bacillus in chains was observed stained in purple color thus confirming that the colonies formed on the nutrient agar were of a gram-positive bacterium, *Bacillus clausii*. Further growth pattern of the bacteria was observed by inoculating them in a nutrient broth and it was observed that the bacteria form a pellicle-like growth pattern and are obligate aerobes.



(a) (b) (c)

Fig. 4.7 Characterization of Bacteria by (a) Gram staining, (b) Negative Staining, (c) growth in nutrient broth.

4.5.2 Microbicidal performance of ZnO NPs with drug-resistant bacteria

The microbicidal performance of biologically synthesized zinc oxide NPs was tested against a poly antibiotic-resistant *Bacillus clausii* strain. The greatest zone of inhibition was demonstrated via ZnONPs with a 22-millimeter diameter of zone of inhibition followed by the methanolic extract with a 12 mm diameter of inhibitory zone and then by the plant extract with a 7-millimeter diameter of inhibitory area obtained using water. The antibiotic Rifampicin exhibited no inhibition zone which is due to the reason that the bacteria show resistance to multiple drugs. Blank ZnO nanoparticles exhibited no inhibition zone which ultimately proves that green synthesized nanoparticles exhibit a potent bactericidal effect compared to those synthesized by chemical means.

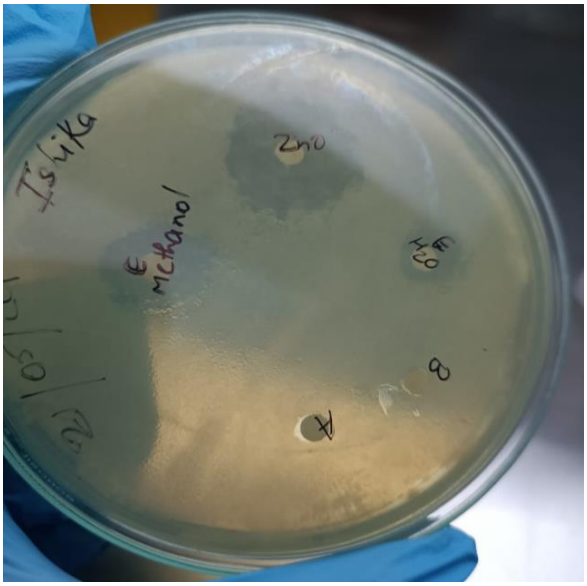


Fig. 4.8 Investigation of antibacterial efficiency of ZnO NPs against poly antibiotic-resistant *Bacillus clausii* employing the diffusion technique of agar wells.

4.5.3 *In Silico* analysis of the antibacterial activity of phytocompounds of *Euphorbia tithymaloides* against *Staphylococcus aureus*

Among four phyto-compounds, Ursolic acid exhibited the highest binding affinity with the LtaS enzyme with -8.9 Kcal/mol. The binding energies of the bioactive compounds are mentioned in Table. 4.1 Fig.4.9 represents the 2D interaction among phytocompounds and LtaS enzymes.

Table 4.2 Binding affinity of Bioactive compounds of *Euphorbia tithymaloides*.

Bioactive Compound	Binding affinity (Kcal/mol)
Ursolic acid	-8.9
Epifriedelanyl acetate	-8.7
Friedelanol	-8.6
β-sitosterol	-7.6

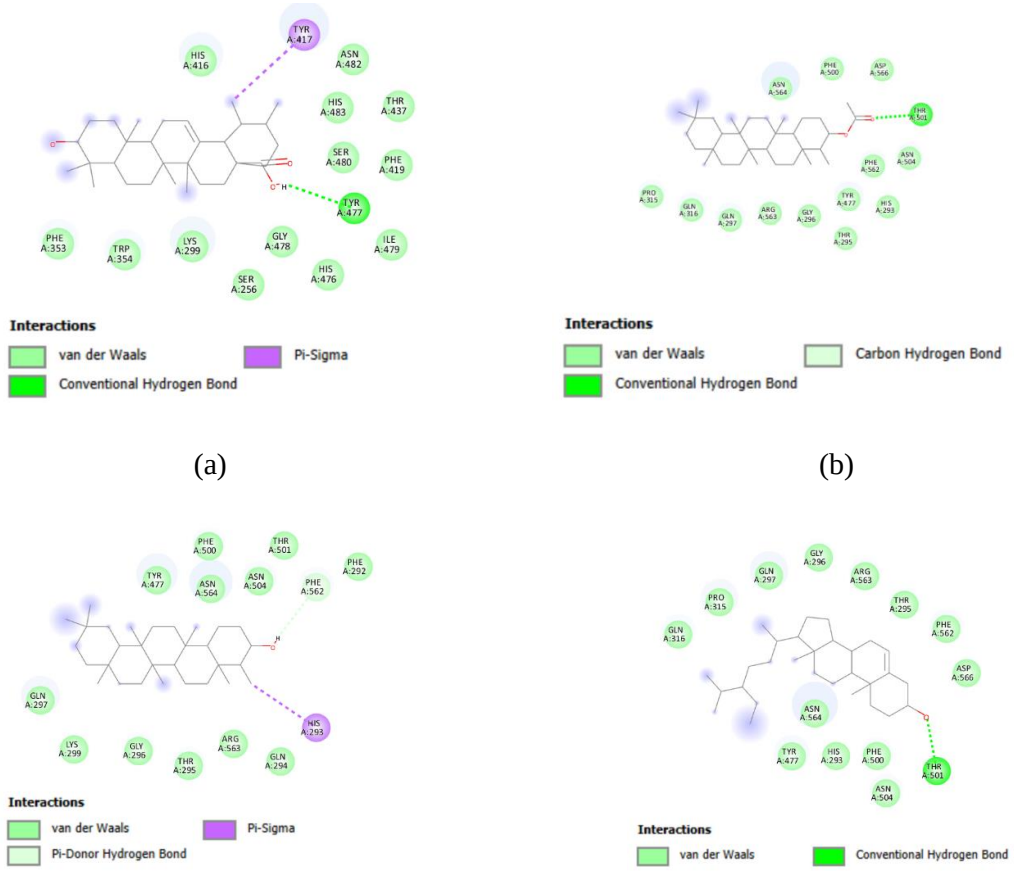


Fig. 4.9 2D interactions of (a) Ursolic acid, (b) Epifriedelanyl acetate, (c) Friedelanol, and (d) β-sitosterol with Lipoteichoic acid synthase enzyme of *Staphylococcus aureus*.

4.6 Photocatalytic efficiency of zinc oxide NPs

4.6.1 Decolorisation of Eosin Yellow using Zinc Oxide NPs

Fig. 4.11 shows the absorption spectra of the solution of Eosin Yellow. It illustrates the time-dependent absorption spectrum of UV-Vis while the photocatalysis takes place. fig. 4.10 demonstrates a consistent decrease in the absorption spectrum maximum of Eosin Yellow as the duration of UV light exposure ranges from 0 to 360

minutes. This indicates the occurrence of photodecomposition of the dye. The percentage of dye removed by the ZnO nanoparticle was observed to be:

$$\text{Dye removal (\%)} = (C_0 - C) / C_0 \times 100$$

$$\text{Dye removal (\%)} = (2.5634\text{-}0.2962) / 2.5634 \times 100$$

Dye removal = 88.44%

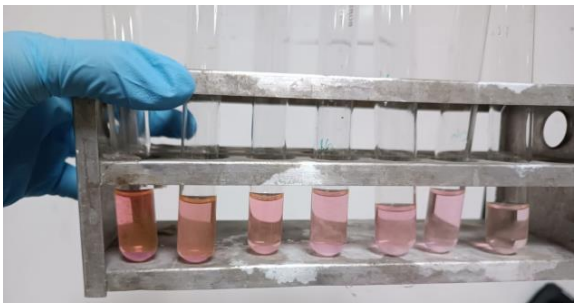


Fig. 4.10 Decolorization of Eosin Y using manufactured ZnO nanoparticles.

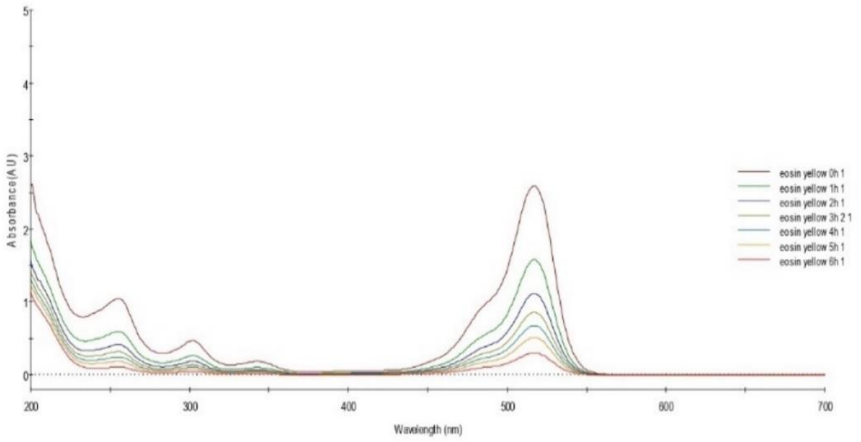


Fig. 4.11 Time-dependent absorption spectrum of UV-Vis while the photocatalytic reaction of eosin Y takes place by ZnO nanoparticles.

4.6.2 Decolorisation of Methylene Blue (MB) dye utilizing ZnO NPs

Fig. 4.13 displays the spectral absorption features of the Methylene Blue (MB) coloring solution. It illustrates the time-dependent absorption spectrum of UV-Vis while the photocatalytic reaction of Methylene Blue takes place. Fig. 4.12

demonstrates a consistent decrease in the absorption spectrum maximum of Eosin Yellow duration of UV light exposure ranges from 0 to 360 minutes. This indicates the occurrence of photodecomposition of the dye. The percentage of dye removed by the ZnO nanoparticle was observed to be:

$$\text{Dye removal (\%)} = (C_0 - C) / C_0 \times 100$$

$$\text{Dye removal (\%)} = (0.7971\text{-}0.1780) / 0.7971 \times 100$$

Dye removal = 77.66%

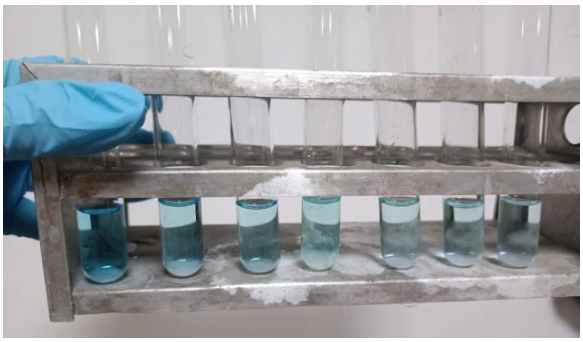


Fig. 4.12 Decolorization of MB dye employing manufactured ZnO NPs.

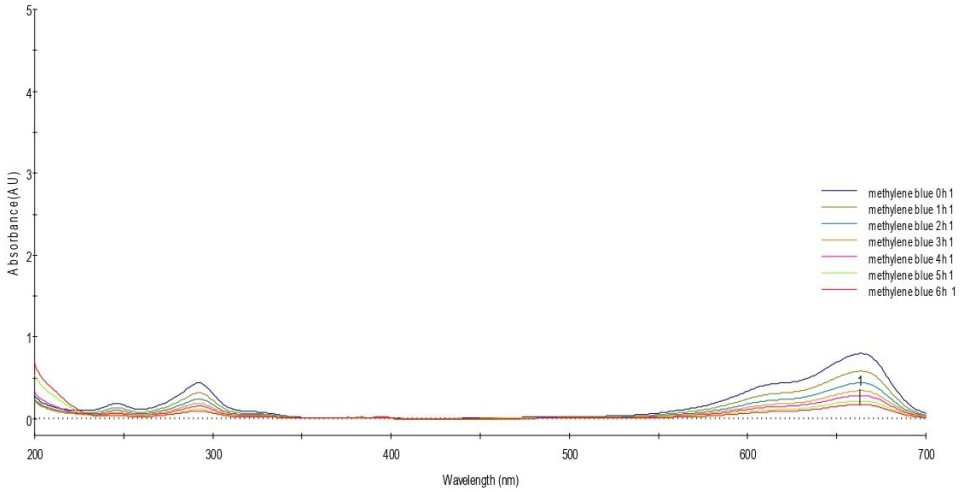


Fig. 4.13 Time-dependent absorption spectrum of UV-Vis while the photocatalytic reaction of eosin Y takes place by ZnO nanoparticles.

4.7 Biochar Synthesis using the leaves of *Euphorbia tithymaloides*

Biochar was synthesized using the leaves of *Euphorbia tithymaloides* by the process of pyrolysis. the pH of the biochar needs to be maintained around 7 thus after the carbonization of the material several washing of biochar was done until the pH of biochar came to neutral. Then the mixture was filtered out and the biochar was left to dry at 80ºC.

Fig. shows the dried biochar which was neutralized after several washing and dried at 80ºC.

Fig. 4.14 Biochar synthesized using the leaves of *Euphorbia tithymaloides*.

4.8 Utilizing Biochar for the elimination of Cadmium from artificial wastewater

The biochar created using the biomass of *Euphorbia tithymaloides* was employed to cleanse Cd from the artificially made wastewater. Different concentrations of biochar were examined for their ability to adsorb cadmium from the wastewater.

The study focused on the influence of the period of contact and quantity of adsorbent while keeping constant starting heavy metal concentration. The findings suggest that prolonging the contact duration has a beneficial impact on the adsorption of Cadmium. This means that a longer contact time leads to a significant removal of heavy metals.

It was noted that as the amount of Biochar increased from 50mg to 250mg the adsorption of Cadmium by biochar increased by time. OD of the blank solution containing different concentrations of biochar was taken and OD at regular intervals of time was taken of a solution containing 500 PPM of Cd and a biochar concentration from 50 mg to 250 mg. The final OD was calculated after subtracting the OD of the

solution containing heavy metal and biochar from the blank to get the final concentration of Cd present in the solution, the values have been mentioned in Table. 4.2. Fig 4.15 represents the graph between absorbance vs biochar concentrations at different intervals.

Table 4.3 Absorbance at 206nm of blank biochar synthesized from the leaves of *Euphorbia tithymaloides* and test solution containing Cadmium and biochar of different concentrations.

Concentration of biochar (mg)	Absorbance (206nm) (Blank)	Absorbance (206nm) (Test Solution after 1h)	Absorbance (206nm) (Test Solution after 2h)	Absorbance (206nm) (Test Solution after 4h)	Absorbance (206nm) (Test Solution after 24h)
50	0.135	2.7788	2.3049	1.8839	1.2132
100	0.176	2.3014	1.7945	1.4679	0.5197
150	0.268	1.4054	1.3014	1.0529	0.3594
200	0.301	0.6836	0.484	0.383	0.128
250	0.477	0.207	0.1519	0.1162	0.0164

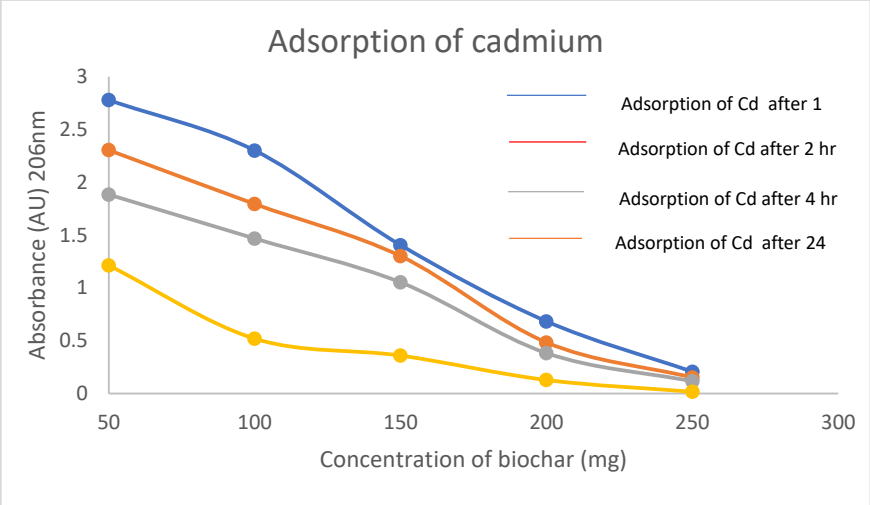


Fig. 4.15 Removal of cadmium by different concentrations of biochar by adsorption.

The removal percentage was calculated by the following formula.

$$\textbf{Heavy metal removal (\%)} = (C_0 - C)/C_0 \times 100$$

C_O – starting heavy metal concentration

C- ultimate heavy metal concentration

The removal percentage of cadmium by different concentrations of biochar was calculated and plotted in a graph.

Table 4.4 Removal percentage of Cadmium by different concentrations of biochar.

Biochar concentration (50mg)	Removal percentage of heavy metal
50	56.34%
100	77.41%
150	74.42%
200	81.27%
250	92.07%

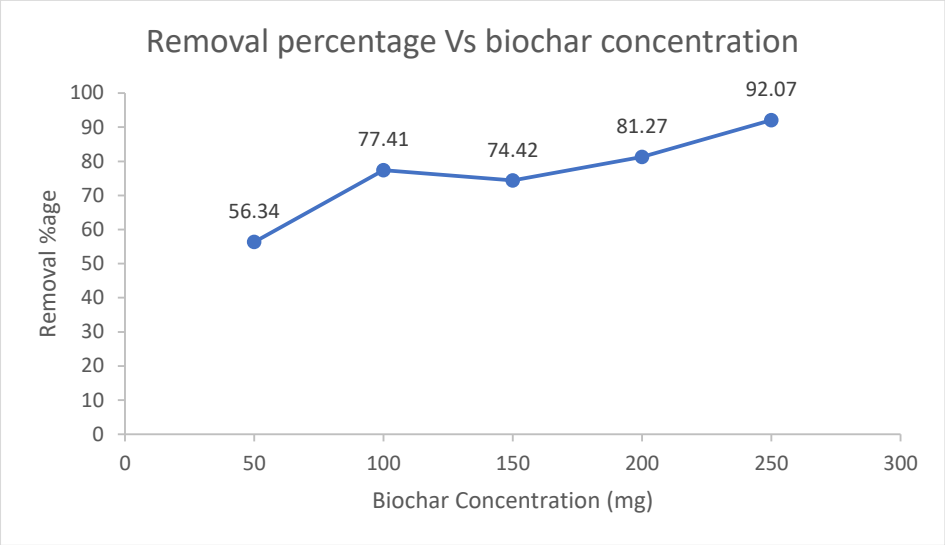


Fig. 4.16 A plot between Removal percentage and different concentrations of biochar.

CHAPTER 5

CONCLUSION

Green synthesis is advantageous over traditional chemical synthesis due to its cost-effectiveness, enhancement of environmental and human health safety, and pollution reduction. Green materials consist of proteins and polyphenols which can substitute chemical compounds in lowering ionized metal to a lower valence state. Metal nanoparticles can be synthesized when green materials are present and the appropriate conditions, such as concentration, ambient air, and temperature are met. Under specific circumstances, a better standard of metal NPs might be generated by green approaches that can exceed that of nanoparticles synthesized through chemical methods. *Euphorbia tithymaloides* is rich in various phytochemicals such as saponins, tannins, steroids, coumarins, triterpenes, etc. Thus, because of the presence of a diverse spectrum of biologically active substances in a plant, it was employed in the manufacturing of ZnO nanoparticles. The rise of antimicrobial resistance among pathogenic bacteria has grown into a significant issue for health. The rationale behind choosing ZnO nanoparticles for this study is that ZnO, being a metal oxide, exhibits greater stability and longevity compared to disinfectants and antimicrobial agents based on organic compounds. Moreover, zinc oxide demonstrates a notable efficiency in inhibiting the progress of microorganisms. Zinc is a vital component for cell growth and participates in preventing the action of bacterial catalysts like dehydrogenase. It also supports the function of protective enzymes such as thiol peroxidase and glutathione reductase. More specifically, ZnO nanoparticles are of particular concern due to their flexibility in challenging process conditions and their safety for individual use. The antibacterial capability of ZnO NPs manufactured using the leaves of Euphorbia tithymaloides was assessed against a poly antibiotic-resistant *Bacillus clausii* strain. The nanoparticles displayed a potent microbicidal activity on Bacillus clausii. The discharge of untreated wastewater containing Methylene blue from various industries can result in significant health hazards. For instance, in humans, the administration of this dye can cause a range of health problems The ZnO nanoparticles were further utilized as a photocatalyst in the process of decolorization of synthetic

dyes, Eosin Yellow and Methylene Blue. Biochar is abundant in durable carbon and contains a substantial quantity of essential nutrients. Its numerous surface micropore nanostructures resulted in huge surface areas, along with its diverse functional groups, making it highly valuable for applications in carbon sequestration and soil improvement. Biochar can serve as adsorbents and passivators to eliminate heavy metals from water. Leaves of *Euphorbia tithymaloides* were successfully employed in the synthesis of Biochar. Further, the synthesized Biochar was adapted as an adsorber for the elimination of cadmium from the artificial wastewater. The Biochar exhibited a potent adsorbent property in the time-based removal of cadmium. It turned out that as time progressed, the biochar's capability to capture Cd from the solution. Thus, leaves of *Euphorbia tithymaloides* were utilized here for dual purposes, for both fabrication of zinc oxide nanoparticles and Biochar.

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LIST OF PUBLICATIONS AND THEIR PROOF

1. A review paper entitled “Biogenic Nanoparticles from Phytochemicals: A New Frontier in Fighting Antimicrobial Resistance and Biofilm Formation” has been accepted in the Research Journal of Biotechnology.

---------- Forwarded message ---------
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Date: Wed, May 15, 2024 at 4:49 PM
Subject: Re: request for fast track review of manuscript
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It is a pleasure to accept your manuscript entitled "Biogenic Nanoparticles from Phytochemicals: A New Frontier in Fighting Antimicrobial Resistance and Biofilm Formation" in its current form for publication in the Research Journal of Biotechnology.

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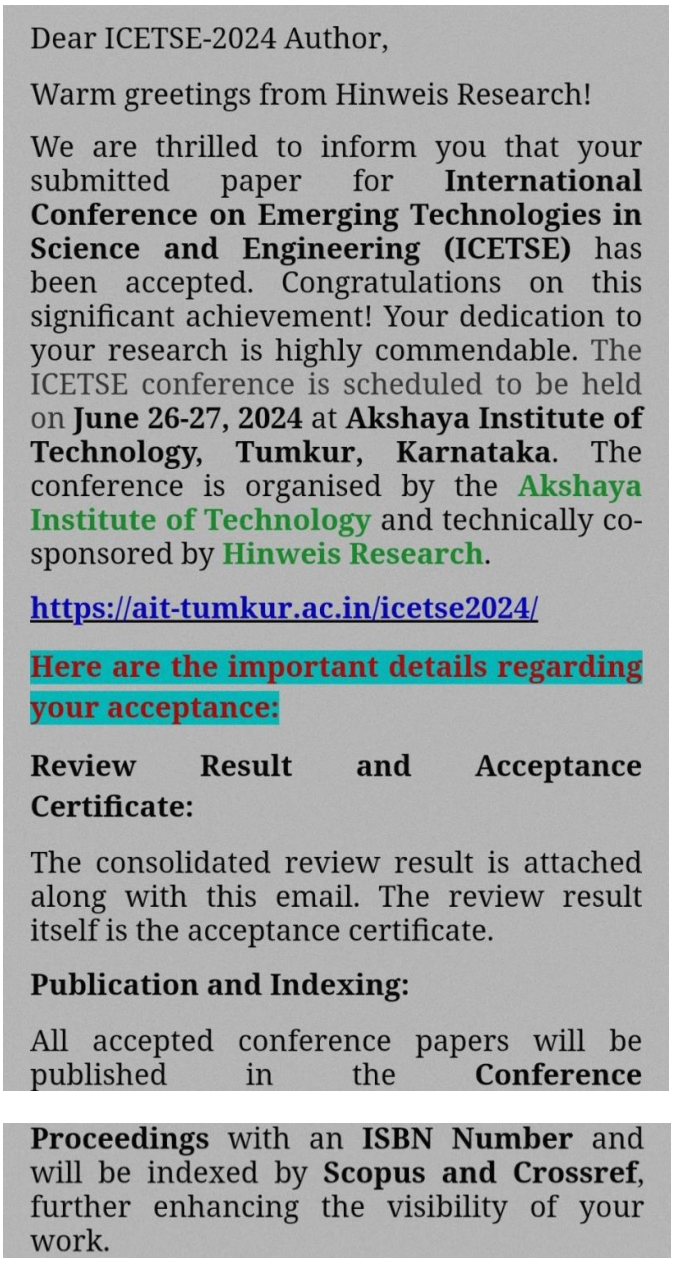
2. A Paper entitled “Structure based Computational Investigation of Phytocompounds from *Vitex negundo* as Drug Candidate for Human Respiratory Syncytial Virus (hRSV)” has been accepted at International Conference on Emerging Technologies in Science and Engineering (ICETSE) – 2024.



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CURRICULUM VITAE

Name: ISHIKA
Date of Birth: 03/01/2002
Contact No.:8684890609
Email: ishikasuman03@gmail.com
Academic Qualification:

Class	University / Board	Institution and department	Percentage/ CGPA	Year of Passing	Remark
MSc	**Delhi Technological University**	**Department of biotechnology**	**Sem I: 8.36 Sem II: 9.27 Sem III: 9.36 Sem IV: currently in Sem IV**	**2024**	
B.Sc	**University of Delhi**	**Bhaskaracharya College of Applied Sciences Department of microbiology**	**Sem I: 7.82 Sem II: 8.27 Sem III: 8.57 Sem IV: 9.21 SEM V: 9.0 SEM VI: 7.25 OVERALL: 8.3**	**2022**	**Passed**
12th	**CBSE**	**Navyug Convent School, Sainik Enclave- jharoda, Delhi**	**89.4%**	**2019**	**Passed**
10th	**CBSE**	**Rao Pahlad Singh Public School Baliar kalan, Rewari, Haryana.**	**9.8 CGPA**	**2017**	**Passed**

Awards/Scholarship:

- 1st prize in a poster-making competition organized by ECO Club and Gandhian Study Centre

Internship/ Training

- Completed hands-on training in Instrumentation and Biotechnique from Ganesh Scientific Research Foundation, Delhi in December 2022.
- Completed practical training for 1 month (June 2023- July 2023) in HPLC.
- Undergone practical training in the microbiology department from Auriga Research Private Limited.

Accepted Review Paper

"Biogenic Nanoparticles from Phytochemicals: A New Frontier in Fighting Antimicrobial Resistance and Biofilm Formation" has been accepted in the Research Journal of Biotechnology.

Conference

"Structure-based Computational Investigation of Phytocompounds from *Vitex negundo* as Drug Candidate for Human Respiratory Syncytial Virus (hRSV)" has been accepted at the International Conference on Emerging Technologies in Science and Engineering (ICETSE) – 2024.

Poster Presentation

Presented a poster entitled "Role of Quorum Sensing Inhibitors in Inhibiting the biofilm formation" at Innovation in Biotechnology, a workshop organized by Delhi Technological University

Workshops and seminars Attended:

- Online Short-Term Certificate Course on "Immunological Techniques and their Applications" organized by the Department of Zoology of Bhaskaracharya College of Applied Sciences (under the aegis of IQAC) in collaboration with the Department of Zoology, University of Delhi from October 22 - November 09, 2021.
- National Webinar on Injurious Invaders: Our Immune System and the Vaccines for Defense organized by the NAAC Committee, BCAS held on 23 July 2021.
- Participated workshop titled 'Analysing Spectrophotometric Data through MS Excel for Quantitative Estimation of Biomolecules' organized by Sukshmjeev Society, Department of Microbiology in association with the Department of Biochemistry on July 5, 2021.
- Participated talk titled 'Gene Editing: Present Scenario and Future Perspectives" by Dr. Bhupendra Kumar Verma, Assistant Professor, Department of Biotechnology, AIIMS during Webinar Series 2021 on Advances in Microbiology: An Interdisciplinary Approach as part of Microquest 2021 organized by Sukshmjeev Society, Department of Microbiology, BCAS on March 13, 2021.
- Participated talk titled 'Microbial Biofilms: Significance, Impact and Combatives" by Dr. Neetu Kumra Taneja, Assistant Professor, Department of Basic and Applied Sciences, National Institute of Food Technology Entrepreneurship and Management (NIFTEM), Sonepat, Haryana during Webinar Series 2021 on Advances in Microbiology: An Interdisciplinary

Approach as part of Microquest 2021 organized by Sukshmjeev Society, Department of Microbiology, BCAS on March 23, 2021.

- A Talk titled ‘‘Automation in Clinical Microbiology" during Webinar Series 2021 on Advances in Microbiology: An Interdisciplinary Approach as part of Microquest 2021 organized by Sukshmjeev Society, Department of Microbiology, BCAS on March 9, 2021.
- Participated talk titled ‘‘Molecular strategies to combat COVID-19 virus" held on July 15, 2020, during Webinar Series 2020 on COVID-19 Pandemic: The Road Map to Recovery, organized by Sukshmjeev Society, Department of Microbiology, BCAS.
- Participated talk titled ‘‘Natural Molecules Against SARS-CoV-2: A Molecular Modelling Study" held on July 7, 2020, during Webinar Series 2020 on COVID-19 Pandemic: The Road Map to Recovery, organized by Sukshmjeev Society, Department of Microbiology, BCAS.

Curricular/Extra-Curricular Activity:

- Participated in HPV Kavach – a social awareness campaign against cancers caused by human papillomavirus**.**
- Participated Sir alexander flemming birthday quiz organized by PG Department of microbiology, sacred heart college, Tirupati, Tamil Nadu held on 6 august 2021
- Food Safety Awareness Quiz, 2020 on the Occasion of World Food Safety Day 6/7/2020, with a passing score of 70%