In-vitro and In-silico Studies for Various Biotechnological Aspects of *Vitex negundo*

A Thesis Submitted
In Partial Fulfilment of the Requirements
for the Degree of

Master of Science
in
Biotechnology

by

AYUSHI SINGH
2K22/MSCBIO/16

Under the Supervision of
DR. NAVNEETA BHARADVAJA

Department of Biotechnology

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahabad Daulatpur, Main Bawana Road, Delhi-110042. India

May,2024

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to everybody who made my master's thesis possible. Many people supported me in different ways; without them, such a dissertation would have been impossible.

First and foremost, I will thank my supervisor, Dr. Navneeta Bharadvaja, for her support, advice and understanding throughout the research. Her invaluable advice and enduring encouragement were indispensable. I owe her much gratitude for the opportunities and resources that brought me up as a researcher. I am equally grateful to the respected Head of Department, Prof. Yasha Hasija for her invaluable support.

A very special and heartfelt thanks to my lab senior, Mr. Sidharth Sharma for his continuous guidance and mentoring. His knowledge and willingness to assist have made my research experience much more enriched. I would also like to thank my other lab members Ms. Anuradha, Ms. Ishita and Ms. Taneem for their helpful advice and warm support which were essential in helping me get past a lot of obstacles along the road.

Additionally, I am grateful to the office staff, Mr. Chhail Bihari, Mr. Jitendra K Singh, and Mr. Lalit for their support in maintaining a conducive environment for my research.

I also want to mention my friends Ms. Akanksha Sahu and Ms. Aditi Singh for their incendiary and motivational talks they had with me throughout the thesis.

Lastly, I want to express my gratitude to my family for their constant encouragement and support during my academic career. Their confidence in me has always given me courage and motivation.

Thank you all for your support and contributions to this thesis.

AYUSHI SINGH

2K22/MSCBIO/16

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Shahabad Daulatpur, Main Bawana Road, Delhi-42

CANDIDATE'S DECLARATION

I AYUSHI SINGH, Roll Number: 2K22/MSCBIO/16 hereby certify that the work which is being presented the thesis entitled- **"In-vitro and In-silico Studies for Various Biotechnological Aspects of *Vitex negundo*"** in partial fulfilment of the requirements for the award of Master of Science, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from May 2023 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.

Candidate's Signature

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)
Shahabad Daulatpur, Main Bawana Road, Delhi-42

CERTIFICATE

Certified that **AYUSHI SINGH (2K22/MSCBIO/16)** has carried out their research work presented in this thesis entitled **"In-vitro and In-silico Studies for Various Biotechnological Aspects of *Vitex negundo*"** for the award of **Master of Science** from 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.

Place: Delhi
Date:

Dr.Navneeta Bharadvaja
Supervisor
Dept. of Biotechnology
Delhi Technological University

Prof. Yasha Hasija
H.O.D
Dept. of Biotechnology
Delhi Technological University

In-vitro and In-silico Studies for Various Biotechnological Aspects of *Vitex negundo*

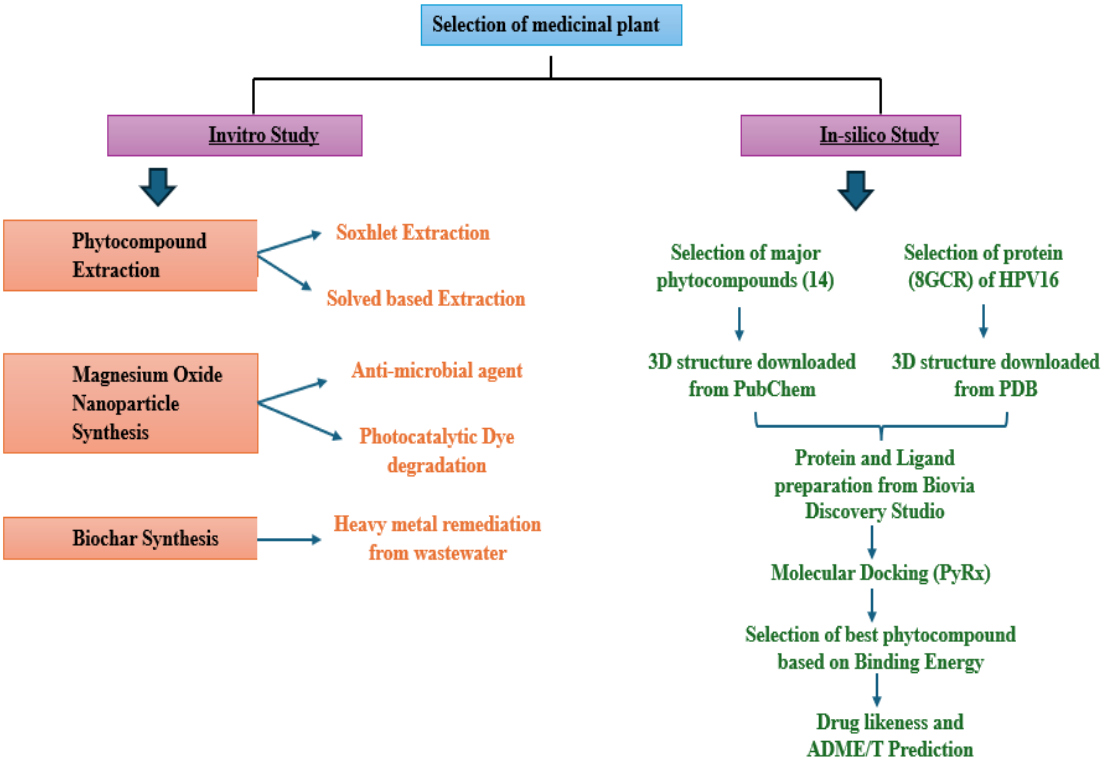
AYUSHI SINGH

ayushi.singhas26@gmail.com

ABSTRACT

Vitex negundo, also known as the Chinese chaste tree, is a medicinal plant famous for its healing qualities. This study aims at examining various biotechnological aspects of *V. negundo* using in vitro and in silico techniques so as to appreciate its probable utilities in nowadays medicine. Standard techniques were employed in analysing phytochemical content of the plant in order to identify pharmaceutical compounds in biological experiments. An investigation was done to explore different biotechnological attributes of *Vitex negundo*, such as its phytochemical composition, capability to produce nanoparticles, capacity to produce biochar, as well as multiple uses in different fields. 0.162gms of empty MgO-NPs and 0.094gms of plant extract based MgO-NPs was synthesized which showed maximum absorbance peak of at a wavelength of 238 nm, conforming the formation of particles at nanoscale. These nanoparticles showed anti-bacterial properties and also degraded dye showing its maximum efficiency on methyl red dye. The pyrolysis of *Vitex negundo* seeds yielded 13.8gms of biochar with an average yield of 27.6% (w/w) relative to the initial seed mass and when this biochar was used for heavy metal remediation maximum removal percentage was seen in case of nickel. By utilizing in-silico tools we predicted that Negundoside outperformed all other phytocompounds in terms of binding energy of -8.9 kcal/mol by using molecular docking methods. ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties and potential molecular targets of the bioactive compounds was also found in *Vitex* by using admetSAR. The computational analysis showed that multiple phytocompounds exhibited drug-like characteristics and had low toxicity in their ADMET profiles. Molecular docking research confirmed the results of the in vitro experiments in that it indicated strong protein affinities for these drugs associated with viral pathogenicity. The use of laboratory experiments and virtual simulations has led to a deep understanding of the biotechnological capacities of *Vitex negundo*, hence opening doors for more investigations and advancements in this area.

GRAPHICAL ABSTRACT



PUBLICATION:

Title of Paper - "Structure based Computational Investigation of Phytocompounds from *Vitex negundo* as Drug Candidate for Human Respiratory Syncytial Virus (hRSV)*"*.

Author Names – Ayushi Singh, Ishika, Navneeta Bharadvaja

Name of Conference - International Conference on Emerging Technologies in Science and Engineering (ICETSE)-2024 organised by the Akshaya Institute of Technology and technically co-sponsored by Hinweis Research.

Date of Conference – June 26-27, 2024

Indexing - Scopus Indexed

Status of Paper – Acceptance Received

Date of Acceptance – May 04, 2024

Date of Camera-Ready Submission and Registration – May 20, 2024

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

VN – *Vitex negundo*

MgO – Magnesium Oxide

MgO-NPs – Magnesium Oxide Nanoparticles

MIC – Minimum Inhibitory Concentration

ROS – Reactive Oxygen Species

LPS – Lipopolysaccharide

HPV – Human Papillomavirus

HR-HPV – High Risk- Human Papillomavirus

NaOH – Sodium Hydroxide

PDB – Protein Data Bank

RCSB PDB – Research Collaboratory for Structural Bioinformatics PDB

SDF – Simulation Description Format

PLIP – Protein-Ligand Interaction Profiler

ADMET – Absorption, Distribution, Metabolism, Excretion, and Toxicity

TPSA – Topological Polar Surface Area

CHAPTER 1

INTRODUCTION

1.1 Background

The World Health Organization states that plants provide an extremely important source for different types of medicines. About eight out of every ten individuals residing in the developed countries rely on modern drugs made from herbs in order to maintain their health standards. Nevertheless, there still need to be more studies aimed at giving a full account of how these herbs are like in terms of their quality, safety, and performance (Singh et al., 2022). Medicines have always incorporated uses of extracts from plants and their biologically active chemicals, but they could not be fully exploited due to ignorance of the biochemical and pharmacological systems in plants. The interest of the health sector in the *Vitex* genus which is based on the potential of their phytochemicals was revealed in a recent study (Thaçi et al., 2020). These phytochemicals have the potential to be used in numerous pills that are beneficial for the treatment and prevention of different ailments. They can also be combined with conventional medicine. Currently, there is ongoing research on new pharmacological agents taken from the *Vitex negundo* (Yao et al., 2016). *Vitex negundo* is known for being able to provide healthier reproductive systems for women and, also, reducing female libido (Arora et al., 2011). The primary phytoconstituents of VN are Isovitexin (Quercetin-3-O-rutinoside), Vitexin (Isorhamnetin-3-O-glucoside), Vitedoin ((7R,8S)-8-(4-hydroxy-3-methoxyphenyl)-6), and Negundin (7-bis(hydroxymethyl)-3-methoxy-7,8-dihydronaphthalen-2-ol). The use of the entire plant, including plant leaves has been for medicinal purposes since ancient times because it has a wide range of characteristics. Out of all these purposes, the best ones are treating and preventing diseases. Hence, rheumatoid arthritis bronchitis, gonorrhea, leukoderma, skin ulcers, toothache, are ocular problems are treated or avoided by it. Moreover, it can be used as an antibacterial, antipyretic, analgesic, tonic, emmenagogue, lactagogue, vermifuge and antihistaminic extract (Tiwari & Tripathi, 2007). It has antihistaminic effects; it has the capacity to protect the liver, as well as inhibit the central nervous system. The flowers are utilized as astringents, febrifuges, and anti-diarrheics, and are recommended for liver ailments. The oil extracted from the flowers has advantageous properties for treating sinus issues and scrofulous ulcers (Venkateswarlu, 2012). The fruits are used to stimulate the nerve system and promote menstrual flow, as well as to function as a vermifuge. The plant's stem and bark have demonstrated therapeutic effects in treating a range of illnesses including burns, cancer, irritable bladder,

dysentery, and rheumatism. The seeds possess antitoxic properties, while the roots have several applications such as serving as a tonic, reducing fever, aiding with respiratory disorders, and acting as a diuretic.

Nanotechnology, a captivating discipline within the realm of Materials Science, possesses the capability to fabricate a diverse range of materials at the nanoscale magnitude. Nanoparticles consist of a wide variety of materials that have diameters ranging from 1 to 100 nm. At the nanoscale, metal nanoparticles can exhibit substantially modified physiochemical properties. Nanoparticles find many uses in biomedical and pharmacological areas, including as replacements of conventional antibiotics due to high prevalence of infectious diseases and emergence of antibiotic-resistant strains (Essien et al., 2020).

Biochar is a solid substance created by subjecting biomass to high temperatures through a process known as pyrolysis. For many centuries this material has been used and is often called wood charcoal. The uses of this substance are many and encompass heating and power production, flue gas treatment, metallurgical processes, agricultural and animal farming methods, construction materials, and medical applications (Weber & Quicker, 2018). The primary objective of biochar production is to alter the chemical makeup of raw biomass, particularly by augmenting the carbon content. This is the outcome of the functional groups containing oxygen and hydrogen being separated. As a result, a greater carbon content and a lower hydrogen and oxygen content are obtained at a higher reaction temperature (Yang et al., 2007).

Both computer-aided drug design and plant-based medicines are regarded as useful resources for drug development and discovery. Molecular docking and molecular dynamic simulations are widely used in computational drug discovery to identify novel compounds through in silico approaches. The combination of both in-vitro and in-silico work on phytocompounds of medicinal importance will thus prove to be

1.2 Objectives of the Study

- To identify and quantify the phytochemicals present in the *Vitex negundo.*
- To optimize the extraction methods for obtaining maximum yield of phytocompounds.
- To synthesize metal oxide nanoparticle using phytochemicals extracted from the *Vitex*
- To evaluate the potential applications of the synthesized nanoparticles
- To produce biochar from *Vitex* seed biomass under controlled pyrolysis conditions.
- To investigate the efficacy of the synthesized biochar in adsorbing heavy metals from contaminated water
- To perform molecular docking to predict the interaction of phytocompounds against Human Papillomavirus.
- To evaluate the potential biological activities of phytocompounds based on ADMET studies

Integration Objective: To integrate findings from phytochemical analysis, nanoparticle synthesis, biochar synthesis, and in silico studies to propose a comprehensive approach for utilizing plant-based materials in environmental and biomedical applications.

CHAPTER 2

LITERATURE REVIEW

2.1 *Vitex negundo*

Vitex negundo Linn., The Verbenaceae family, commonly known as the "Five-leaved chaste tree," "Monk's Pepper," or "Nirgundi," has a special place in Ayurvedic medicine as a "Sarva Roga Nivarani.". This aromatic species has roughly 250 species spread across 5 genera, and its wood can reach heights of up to 27 feet. Its leaves are digitated, with three or five arranged in a palmate hand shape, each measuring between three and ten point two centimetres in length (Nirmalkumar, 2007). The plant has fragrant flowers ranging from violet to deep purple, and produces small, black, round fruit that is 3-4 mm in size. It is indigenous to Madagascar, Africa, Southeast Asia, Central Asia, and South Asia, and thrives only in dry regions and wooded areas with moisture, typically near water sources. In some parts of Asia, Europe, North America, and the West Indies, it is also grown as a crop for commercial purposes (K et al., 2022).

2.2 Phytocompounds of *Vitex negundo* and their Therapeutic Potential

The medicinal plant *Vitex negundo* is renowned for its various phytocompounds, such as flavonoids, terpenoids, polyphenols, saponins, steroids, and phenolics. A total number of 120-150 metabolites found in this plant have substantial pharmacologic effects and are thus desirable as therapeutic agent (Cokul Raj et al., 2023). These phytocompounds are utilized as bioactive substances in various industries including wastewater management, food, pharmaceuticals, textiles, and biotechnology. Folk medicine is widely used in healthcare systems in developing and underdeveloped countries where there are no major pharmaceutical companies (Mohammad et al. 2023).

2.2.1 Iridoids are a type of cyclopenta[c]pyran monoterpenoids primarily found in plants, with most of them being of glycosidic origin. The first iridoids to be found in plants of the genus *Vitex* were called negundoside, also known as 2-p-hydroxybenzoyl mussaenosidic acid; they protect most well-differentiated hepatocellular carcinoma cell lines against carbon tetrachloride-induced necrosis and oxidative stress, (Tasduq et al., 2008) along with 6-p-hydroxybenzoyl mussaenosidic acid (Sehgal et al., 1982).

2.2.2 **Terpenoids** are one of the most prevalent classes of secondary metabolites present in *Vitex* plants. Research findings indicate that *V. negundo* might be identified chemosystematically by the presence of Vitexilactone B – a new labdane diterpenoid (Zheng et al., 2010). Negundol derived from *V. negundo* L, denotes two diterpene epimers of the labdane type. It has antifungal qualities. The compound has been s hown to stop the following fungi when concentration between 16 to 64 g/mL: *Trichophyton rubrum, Cryptococcus neoformans*, and *Candida albicans* (Zheng et al., 2012).

2.2.3 **Ecdysteroids,** also known as phytoecdysteroids, are made in enormous amounts in plants as a natural defence against insects that prey on them (Das et al., 2021). Consequently, for many years now, these phytocompounds are as chemical markers for classifying members of the genus *Vitex*. These are abundant in plants, used for keeping off plant eating insects (Filho et al., 2008).

2.2.4 **Flavonoids** are a group of coloured chemicals that are plentifully present in plants. These substances are key for the growth, development, and protection of plants (Steen & Kiel, 1983). Vitegnoside, a flavonoid discovered in *V. negundo* L., exhibited inhibitory effects against Trichophyton mentagrophytes, a dermatophyte fungus, and *Cryptococcus neoformans*, a yeast-like fungus. The minimum inhibitory concentration (MIC) value of vitegnoside was determined to be 6.25 g/mL (Sathiamoorthy et al., 2007).

Table I: List of phytochemicals of *Vitex negundo*

Plant part	IMPPAT Phytochemical Identifier	Phytochemical name
Leaf	IMPHY008689	Isovitexin
Leaf	IMPHY003738	Negundoside
Leaf	IMPHY004388	Kaempferol
Leaf	IMPHY004379	Chrysosplenol D
Seed	IMPHY005204	Vitexilactone
Seed	IMPHY005690	Vitedoin A
Leaf	IMPHY012058	Linalool
Bark	IMPHY004660	Luteolin
Leaf	IMPHY016053	Viridiflorol
Leaf	IMPHY004407	Artemetin
Leaf	IMPHY001801	Isoorientin
Fruit	IMPHY015022	Nerolidol
Flower	IMPHY012104	Citronellol
Seed	IMPHY014990	Linoleic acid

2.3 EXTRACTION METHODS OF PHYTOCOMPUNDS

Extraction is the process that separates a plants medicine producing constituent parts through the utilization of normal methods and special dissolving elements. Every extraction process is designed to wash-out the soluble plant metabolites but exclude the insoluble cellular residue. Pre-extraction and extraction techniques, which are crucial steps in the processing of the bioactive components from plant materials, are where the study of medicinal plants begins. The preservation of phytochemicals in the final extracts is influenced by the pre-preparation of plant materials, such as grinding and drying. To reduce the particle size of a sample, electric blenders, and mills as well as traditional mortars and pestles are frequently utilized (Azwanida, 2015). Continuous phytochemical extraction with a hot solvent is known as soxhlet extraction. Plant material that has been ground is put into a thimble, which is a porous bag composed of cellulose or firm filter paper. Within the compartment housing the Soxhlet paraphernalia is a thimble filled with ground plant material. You fill the bottom flask with extraction solvent (ethanol or methanol, for example), to extract the phytochemicals, the solvent is heated and vaporized in the sample thimble, condensed in the condenser atop the apparatus, and finally dripped back. Comparing extraction procedures based on maceration shows that they result in a higher yield. Phenolic compounds were extracted from leaves using 60 percent ethanol for 2 hours whereas fatty acids were extracted from hemp seeds at 70 °C for 8 hours (Alara et al., 2018). This extraction process is quite efficient, but the greater temperature of this approach poses a danger of thermolabile chemical degradation.

2.4 BIOGENIC MgO-NPs SYNTHESIS

In the past few years, “green synthesis” has been a very common idea and lots of scientists have been doing research on it as indicated by statistics that it was among those methods that ensure good health (Ahmed et al., 2016). For an eco-friendly approach, nanoparticles are produced and manipulated in healthcare in order to increase its effectiveness and efficiency. There are several ways of making nanoparticles referred to as "green synthesis" which have come up recently in this new frontier. Nanoparticles are created from a variety of natural sources other than just plant materials like in the case of bacteria algae yeast or fungus even though they are used also in making certain kinds of medicines. One example of this is the usage of plant sources, where different sections of plants such as leaves, roots, seeds, and fruits can be used (Karunakaran et al., 2017). The plant-mediated pathway, also known as the green method, has been getting serious interest because of its ease and eco-friendliness in producing metal and metal oxide nanoparticles than physical or chemical methods. It has been suggested that plant metabolites can act as metal capping and reducing agent for nanoparticle formation. The characteristics of the end product, such as its biocompatibility and antibacterial activity are influenced by the large number of phytochemicals found in the plant extracts that are used to prepare the metallic nanoparticles (Essien et al., 2020). Traditional chemical methods for nanoparticle production are expensive and strongly dangerous for human health and the surrounding environment because of the harm of the initial reagents and by-

products used in these approaches. MgO has recently become a focus for its eco-friendly preparation and broad application in medicine, pharmaceutical industry, industry, and technology areas. The biological field uses magnesium oxide in its green form for the purposes of disinfection, treatment of cancer and antimicrobial treatment. Furthermore, this material is widely used in electrical insulators due to its dielectric properties. However, apart from its wide usage in the insulation sector, MgO nanoparticles also have an immense capability for absorption and adsorption. Consequently, they find application in predominantly optical applications where environmental cleanliness plays a major role like the pollutant degradation process where dyes are extracted.

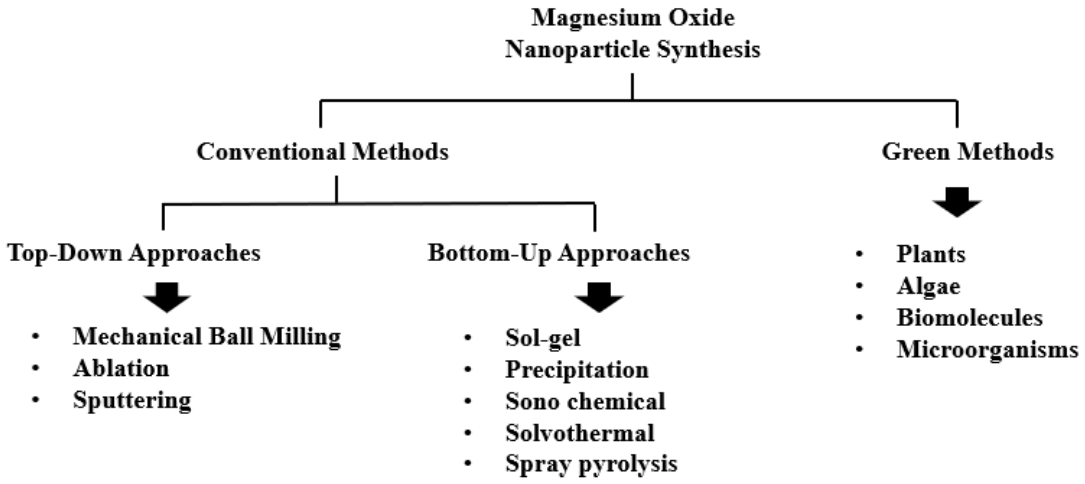


Fig.2.1 Methods of Nanoparticle synthesis (Silva et al., 2022)

In the ecologically friendly and sustainable production of magnesium oxide nanoparticles (MgO-NPs), the source of the metal ionic precursor incorporates resources from nature that are renewable and full of phytochemicals able to chelate, stabilize, reduce, or precipitate it. Green MgO-NPs may be formed using microorganisms, including bacteria, fungi, or algae. On the other hand, polymers can be synthesized from plant-based substances containing different polyphenols. The huge interest in exploiting various plant materials might be associated with the high demand for the production of nanosized MgO and the need to design ecological pathways (Pilarska et al., 2017). There is a growing interest in making green MgO-NPs using plant extracts as of recently. It is favoured because it is environmentally friendly and easy. As a result, hazardous waste from chemical reagents and solvents is being eliminated using this process. The green MgO-NPs have some unique characteristics including they are chelated by plant polyphenols which make them have excellent stability; their large surface area; and well-defined shape (Chandra et al., 2020).

2.5 APPLICATIONS OF MgO-NPs

Researchers are currently using magnesium oxide (MgO) nanoparticles because of their unique thermal, electrical, and biological properties.

Anti-microbial agents - Minimum inhibitory concentration is the lowest concentration of an active substance that effectively inhibit the development of microorganisms. MIC is important to establish the role of any bioactive compound against pathogenic microorganisms whenever such compounds are used in biological applications. The ability of biogenically synthesized MgO-NPs to suppress pathogenic Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis* growth), Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*), or unicellular fungi growth (*Candida albicans*) has largely been demonstrated by several scientific experiments in recent years in the field of emergent biomedicine (Saied et al., 2021).Several mechanisms account for why biogenic MgO-NPs act as inhibitors when they interact with microbial cell walls killing them, like production of reactive oxygen species (ROS) release of Mg^{2+} ions into the cell, or alkaline effectivity of MgO on the microbial cells (Al-Hazmi et al., 2012; Wang et al., 2017). Biosynthesized magnesium oxide nanoparticles (MgO-NPs) were found to be the most potent against Pseudomonas aeruginosa. This could be due to the dissimilarities in cell wall make up between Gram positive and Gram-negative microbes whereby Gram-negative bacteria have a thin lipopolysaccharides (LPS) coating with a thin peptidoglycan layer around them Gram positive organisms have both thick layers. The deposition of nanoparticles (NPs) on the bacterial cell membrane disrupts selective permeability due to the high electrostatic interaction between the positive charge of NPs and the negative charge of LPS. Moreover, MgO-NPs possess the capacity to impede quorum sensing, a process that enables microbial strains to communicate with each other. Consequently, the functioning of physiological systems and other microbial activities is impeded (Wong et al., 2020).

Dye degradation - The textile, pharmaceutical, food, cosmetic, plastics, paint, ink, photography, and paper industries all use dyes as colorants. Even though they are very helpful, dye structure and origin are to blame for the harmful effects that have accumulated in water systems. The dyes are classified into various structural forms, including metal-complex dyes, anthraquinone-based, disperse, azo, diazo, reactive, acidic, and basic dyes. These dyes primarily come from well-known carcinogens like naphthalene and benzidine. Therefore, when these dyes come into contact with biological systems like humans and animals, they can always be broken down by microorganisms and turn into carcinogens (Marimuthu et al., 2020). Dye's complex structure makes it challenging to break down. Thus, a permanent solution is required to remove the dyes. Traditional techniques are effective, but they are ineffective because the costly derivatives of the complex dyes stay in the water even after treatment. Furthermore, the bulk of the dyes are xenobiotics by nature, meaning they are resistant to standard removal techniques. Recently, it has been discovered that nanoparticles are completely effective (Banat et al., 1996). Without any restrictions, dyes could be treated and degraded effectively with the use of nanoparticles. The dye degradation efficiency was influenced by the size, shape, and effectiveness of the nanoparticles (Nandhini et al., 2019). They are also a better option for dye degradation

and removal due to their superior surface qualities and chemical reactivity. More effective nanoparticles are produced by the biological synthesis, also known as the "green synthesis," of nanoparticles than by any other technique. This process uses plant extracts and microorganisms like fungi, bacteria, and algae to reduce metal salts. It enhances and guards against environmental harm brought on by overuse of chemicals in the reduction of metal salts. Additionally, it gives better shape and size of nanoparticles for a variety of nanotechnological applications and gives control over the synthesis. It does not apply to the additional stabilizing agents that are needed for the physical and chemical processes during synthesis (Marimuthu et al., 2020).

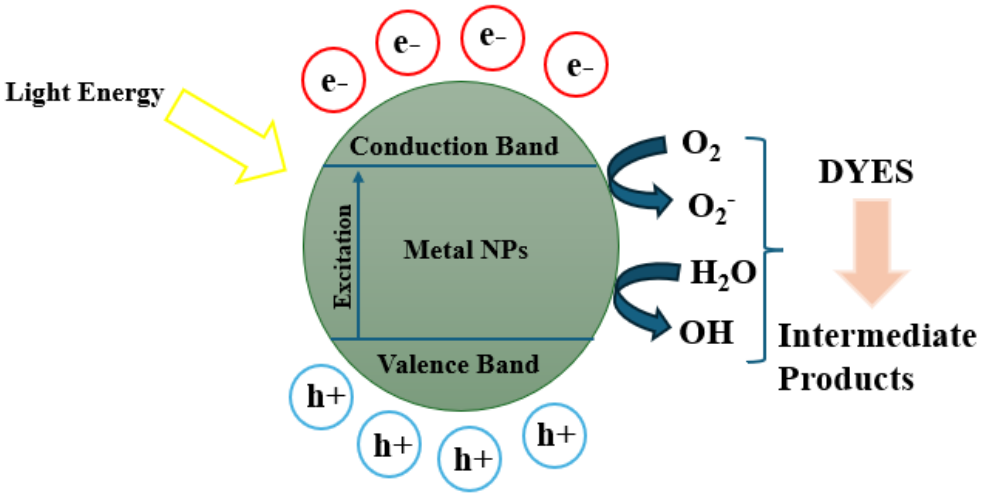


Fig 2.2. General mechanism of dye degradation by metal nanoparticles

Heavy metal remediation - Water resources are at risk from surface water pollution because pollutants have the ability to seep into groundwater and reach sources of drinking water. The need for increasingly advanced water remediation technology will increase as the world's population rises (Adeleye et al., 2016). It is essential to regularly monitor for non-biodegradable heavy metals like cadmium, nickel, arsenic, lead, and mercury in food, water, and the environment to prevent serious health issues from arising in the food chain. Various nanoparticles, including metals and their oxides, carbon materials, zeolites, and bimetallic NPs, have been identified as potential remedies for cleaning up heavy metal pollution in the environment (Latif et al., 2020). A number of illnesses, including lung adenocarcinoma, lung cancer, kidney failure, and bone fractures, are associated with cadmium exposure. Lead in the blood damages the nervous system and inhibits a number of enzymes. Excessive levels of nickel, copper, and chrome can also be harmful to people's health (Ekrami et al., 2022). Therefore, in order to lessen the health effects associated with these heavy metals, remediation is required. Nanomaterials can serve as a cost-effective substitute for chemical-based solutions in the process of in-situ water treatment.

2.6 BIOCHAR

Biochar is produced by heating up organic material in a place with nothing but little oxygen. Because of its amazing ability to remove many kinds of pollutants, people are very curious about biochar. Biochar has the capability of binding heavy metals, immobilizing them, and making them less available or toxic when applied on the soil as a method of amendment or remediation for them. One can attain this through procedures like the exchange of ions, complex formation on the metal ion surface, co-precipitation, and physical adsorption (Mohamed et al., 2017). This can be used in energy production, soil management, CO_2 capture, wastewater treatment, supercapacitors production as well as other associated fields. Biochar has a structure that is disordered and at the same time pored too. The structure of this material at all levels from microscopic to macroscopic shows outstanding resistance to chemical, mechanical factors, and changes in surface properties. The capacity for substance adsorption is heavily influenced by its surface activity, which is dependent on the pores characteristics and surfaces containing functional groups such as -C=O, -COOH, -OH, lactone among others. Understanding these functional groups helps one understand why different dyes stick so well on these surfaces and at the same time what makes it ideal for filters targeting water. Hence, it remains one of the most outstandingly universal materials that would suffice when it comes to water filtration (Diaz-Uribe et al., 2022). Commercially available activated carbons are the most commonly used adsorbents. The most used adsorbent for removing ions and heavy metals from contaminated water is activated carbon (Barakat, 2011). Activated carbon remains high-priced adsorbent material for wastewater treatment although it is widely used in the industry for heavy metal elimination from industrial water because of its outstanding mechanical strength, significant porosity as well as large internal surface area that contributes to its ability to absorb great volume of metal ions from wastewater. Therefore, it is crucial to develop cost-effective carbon-based polymers for the purpose of adsorbing heavy metals from water (Abid et al., 2019). Biochar has pores and a large surface area because it has hydroxyl, carboxyl, carbonyl, and other functional groups. Next, increasing or decreasing the concentration of harmful metals (e.g., lead) in water using this technique can be effective for both eliminating organic chemicals such as pesticides from farms as well as industrial products like tannery dyestuffs and some antibiotic residues that contaminate our rivers due to discharge through hospitals or pharmaceutical industries (Inyang et al., 2016).

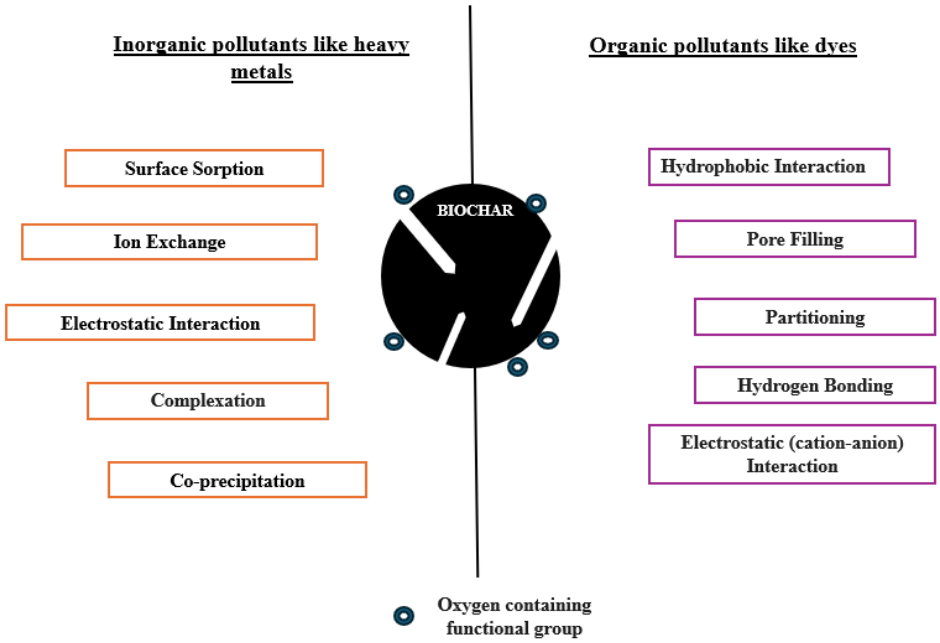


Figure 2.3. Mechanism of removal of organic and inorganic pollutants by biochar

2.7 IN-SILICO STUDY OF PHYTOCOMPUNDS AS INHIBITORS FOR HPV

In India, cervical cancer is the second most common cause of cancer in women. Every year, 96,922 new cases of cervical cancer are diagnosed, representing a crude incidence rate of 14.9 percent. About 70% of cervical cancer cases worldwide are caused by HR-HPVs, mainly types 16 and 18. These oncogenic HPV types are the cause of cervical cancer. The Catalan Institute of Oncology and Information Centre on HPV and Cancer (ICO/IARC) reports that 469 point one million Indian women who are 15 years of age or older are at risk of developing cervical cancer. The eight kilobyte (kb) circular, double-stranded, non-enveloped DNA viruses that make up HPVs are classified into three categories: early, late, and long control regions (LCR). The early region contains the protein coding regions E1, E2, E4, E5, E6, and E7, which are regularly active and have a significant impact on HPV oncogenicity (Rodríguez-Ruiz et al., 2019). As the three main indicators of HPV infection, E5, E6, and E7 are also critical for the emergence of HPV-positive carcinoma. E6 and E7 modulate the regulation of the cell cycle and aid in the upkeep of the viral genome. E6 overrides the cell cycle process by inhibiting p53 and causing a loss in cell cycle regulation. The best targets for immunotherapeutic agents are E6 and E7 (Pal & Kundu, 2020). Both computer-supported design of drugs and plant-derived medicines are considered as drug development and discovery useful resources. In the field of computational drug discovery, molecular docking, and molecular dynamic; in silico approaches are used widely to identify new compounds by computational means. One uses molecular docking, a simulation method, to establish and regulate the most preferred orientation

between two atoms, the ligand, and the receptor specifically. Its purpose is to recognize and examine desired binding conformers, as well as to check affinity using a good number of multifarious algorithms (Hussain Jaffar & Rasool, 2019). Virtually assessing the receptor-ligand combination through the intermolecular interactions, docking algorithms are also able to rate the ligand confirmations according to binding modalities and related energies. The process of molecular docking can generally be broken down into two main phases: preparation of the ligand molecule with assessment of its aromaticity, as well as receptor and rotamer protonation, binding site prediction; and post-docking analysis, being the accurate prediction of the protein-ligand complex and its conformational energy (Rallabandi et al., 2020). Molecular docking algorithms evaluate the binding energy of an expected ligand-receptor complex through scoring element. The variation in energy is attributed to the binding constant (Kd) and Gibbs Free Energy (GGL) during development of ligand-receptor structure (Foloppe & Hubbard, 2006). In order to predict the accompanying energy, we rate the most important physical and chemical happenings happening in ligand receptor binding, such as entropy effects, dehydration, and inter-molecular interaction (Huang et al., 2010). To ascertain whether a test compound could be a drug molecule, pharmacokinetic analysis checks if it has drug-like qualities. Keeping into account the RO5, which is the five Lipinski rules, the distinction of how well compound displays bioavailability is done.

CHAPTER 3

MATERIALS AND METHODOLOGY

IN-VITRO STUDY

3.1 SOXHLET EXTRACTION

The plant sample of *V. negundo* leaves (50 g) was added to the extraction apparatus after being weighed into a Soxhlet extractor thimble. A 250 mL conical flask was filled with 200 mL of distilled water. The mixture was refluxed for a range of extraction times, from one to three hours, using a heating mantle. The solvent is heated, vaporized in the sample thimble, condensed in the condenser on top of the apparatus, and then dripped back in order to extract the phytochemicals. Following the extraction period, the extract solution was allowed to cool to room temperature and refrigerated.

Figure 3.1. Soxhlet apparatus

3.2 SOLVENT BASED EXTRACTION

The *V. negundo* seed plant sample (20 g) was measured and pulverized using a mortar and pestle. Subsequently, the substance was moved into a glass beaker and positioned on a heating mantle, which was adjusted to match the boiling temperatures of the various solvents employed, namely 56ºC for acetone, 61ºC for chloroform, and 65ºC for methanol. The seed and solvent mixture is heated until it reaches its boiling point and then left to cool. The solvents are maintained covered to inhibit their evaporation. After undergoing a brief period of cooling, the mixture is passed through a Whatman filter paper to separate and collect the final filtrate, which is thereafter kept for subsequent tests.

3.3 QUALITATIVE ESTIMATION OF PHYTOCHEMICALS

Terpenoids - Transfer 2 millilitres of extracts into four separate test tubes. Add 2 millilitres of chloroform and 2 millilitres of concentrated sulfuric acid to each test tube. Observe any resulting changes.

Flavonoids - Transfer 2 millilitres of extracts into four separate test tubes and add 1 millilitre of a 10% solution of lead acetate. Observe any changes in colour.

Tannins - Transfer 2 millilitres of extracts into four separate test tubes and add a little amount of 5% ferric chloride solution to each tube in order to notice any colour changes.

Steroids: Fill each of four test tubes with two millilitres of extracts. To each test tube, then add two millilitres of pure sulfuric acid and chloroform. Go on and closely check any changes that take place.

Glucoside - Add two millilitres of extracts equally among four test tubes. Slightly add concentrated sulfuric acid to each test tube. See what adjustments come about.

Saponin - Add two millilitres of extracts equally among four test tubes. Pour a small amount of distilled water into each test tube, then closely watch for any changes that come about.

3.4 PLANT MEDIATED SYNTHESIS OF MgO-NPs

The synthesis employed an aqueous seed extract of *Vitex negundo* obtained from Soxhlet. Because VN has a rich phytochemical composition—especially a high concentration of phenolic compounds and reducing agents, which are known to help with the creation of nanoparticles—it was chosen. Plant-mediated synthesis demonstrated the potential of VN extract as a reducing and stabilizing agent by enabling the synthesis of clearly characterized MgO-NPs under mild reaction conditions. A volume of 25mL of a solution containing 0.1M concentration of Magnesium nitrate ($Mg(NO_3)_2$) was carefully measured and mixed with 100mL of VN seed extract. The mixture was then subjected to rotation on a magnetic stirrer for approximately 45 minutes. 15mL of a 0.2M solution of NaOH was gradually added dropwise to this combination. Once the nanoparticles are formed by this procedure, the entire mixture is moved to a falcon tube and subjected to three rounds of washing with distilled water. This is achieved by centrifuging the mixture at a speed of 6000rpm for a duration of 5 minutes each time. To obtain nanoparticles, remove the liquid supernatant after washing, and then use a hot air oven at 60ºC for 12 hours to dry the concentrated solution and form solid pellets. Place in refrigerator for further characterisation and analysis. The same procedure was repeated without the use of plant extract again to synthesize empty MgO-NPs. For comparative studies, these empty nanoparticles will be later used.

Figure 3.2. Plant extract nanoparticles

3.5 CHARACTERIZATION OF MgO-NPs USING UV-Vis

Magnesium oxide nanoparticles are utilized for various purposes, such as environmental cleanup, medicine, and catalysis. Nonetheless, for these purposes, it is important to have a full understanding of the optical properties of these materials. UV-Vis spectroscopy is widely used to investigate the optical properties of nanoparticles due to its ability to provide information about the energy band gap and absorption behaviour of materials. In order to ensure a uniform suspension, a predetermined concentration of MgO-NPs was dispersed in distilled water for a duration of five minutes using a vortex. To calibrate the UV-Vis spectrophotometer and alter the baseline, distilled water was used as a blank solvent. After being moved to a quartz cuvette, the MgO-NPs suspension was put inside the UV-Vis spectrophotometer. The range of 200–700 nm was used to record the absorbance spectrum. To ascertain the distinctive absorption peak of the MgO-NPs, the absorbance data were analysed.

3.6 ANTI-BACTERIAL EFFECT OF MGO-NPS

Under aseptic conditions, the bacterial strain of *Bacillus clausii* which was isolated in the laboratory itself was inoculated into Nutrient agar media (Beef extract 3.0g, Peptone 5.0g, Agar 20.0g, NaCl 0.5g, Distilled water 1000mL). With the disc diffusion method, MgO-NPs' antibacterial activity was assessed. To find the minimum inhibitory concentrations (MIC), four filter papers (0.7 cm in diameter) were prepared in the inoculation plates and soaked with 100 µL of biosynthesized MgO-NPs. The plates were incubated at 35 ± 2 °C for 24 hours after being refrigerated for 1 hour. The diameters of the inhibition zones (ZOI) that developed around each well were measured at the conclusion of the incubation period. The MIC value was determined as the lowest concentration of MgO-NPs that effectively stopped bacterial growth from being seen.

3.7 PHOTOCATALYTIC DYE DEGRADATION USING MgO-NPs

The process of using photocatalysis to break down organic dyes is highly effective and has no impact on the environment. This method shows great potential for detoxifying wastewater. We investigated the efficacy of magnesium oxide nanoparticles (MgO-NPs) in degrading methylene blue, methyl red, lactophenol cotton blue and eosin yellow using photocatalysis. After preparing the dye solution at a particular concentration, MgO-NPs were introduced. Upon introducing the MgO-NPs suspension into the prepared dye solution, the mixture was subjected to a specific duration of blue light irradiation. In order to monitor the degradation of the dye, samples were collected at regular intervals and the absorbance spectra were recorded using a UV-Vis spectrophotometer.

Figure 3.3. Photocatalytic dye degradation

3.8 BIOCHAR SYNTHESIS

Dehydrate 50gms of *V. negundo* seeds in a hot air oven at a temperature of 80ºC for a duration of 48 hours, and thereafter grind them into a fine powder. Coat the powdered seeds with a 50% concentration of phosphoric acid (H_3PO_4), ensuring that the ratio of seed powder to acid is 1:1.5. Abstain from agitating the mixture for a minimum of 24 hours. Transfer the mixture into falcon tubes and perform two washes by centrifuging at 5000 revolutions per minute. Set the pH to 7.0 and then proceed to dehydrate the biochar in a hot air oven at a temperature of 80ºC for a duration of 24 hours. Measure the weight of the sample and document the measurement.

3.9 BIOCHAR BASED HEAVY METAL REMEDIATION

Using analytical grade metal salts, a stock solution of three distinct heavy metals (Cd, Ni, and Pb) was prepared in order to examine the effectiveness of biochar as a remediation technique for heavy metal-contaminated soils. For nickel and lead a stock of 1g/L and for cadmium 10g/L were made. For the purpose of facilitating adsorption, biochar was added to each metal solution at varying dosages (0.5%, 1%, 2%, 3%, and 4% mg/mL) and stirred for 24 hours. The reference treatments were control treatments devoid of biochar. Samples were taken on a regular basis (1 hr, 2 hrs and 24 hrs), and the remaining metal concentrations were measured using UV-Vis analysis. Following biochar treatment, the results demonstrated a significant drop in heavy metal concentrations, with higher removal efficiencies seen at higher biochar dosages. A positive correlation between the dosage of biochar and the effectiveness of metal removal was found through statistical analysis. Nevertheless, the efficiency of removal differed among the various metals, with nickel demonstrating the greatest removal efficiency.

IN-SILICO STUDY

3.10 MOLECULAR DOCKING AND ANALYSIS

Using the structure of the HPV16 E6 oncoprotein (PDB: 8GCR) as a template, an in-silico analysis was carried out to characterize the molecular effects. A resolution of 3.38 Å was downloaded for the 8GCR 3D crystal structure. Utilizing the RCSB PDB (https://www. http://rcsb.org). In its original state, the protein was not suitable for molecular docking. In order to remove heteroatoms, ligands, and water molecules, it was imported into Biovia Discovery Studio. The objective of this procedure was to stabilize the interactions between proteins and ligands and increase the accuracy of projected electrostatic interactions. These elements can prevent particular ligands from attaching themselves to the target, but they are not necessary for the protein to function. To improve the accuracy and efficiency of the docking computations, they are usually removed. The PubChem database provided the SDF format for all ligands' three-dimensional structures, including Negundoside, Isoorientin, Luteolin, Vitexilactone, Isovitexin, Kaempferol, Chrysosplenol D, Artemetin, Vitedoin A, Viridiflorol, Nerolidol, Linoleic acid, Linalool, and Citronellol. The PyRx program, which can be found at https://pyrx.sourceforge.io/, was used to perform molecular docking with an exhaustiveness of 100. The application accepts proteins in PDB format and ligands in SDF format. The docking process calculated the binding energy of the target protein with each of the ligands. A two-dimensional representation of protein-ligand interactions was made after this procedure. Using PyMol (https://pymol.org/2/), we could import the receptor protein and the result file to create a complex that would allow us to observe the protein-ligand interaction from three dimensions. The abovementioned complex was saved using the PDB format and further analysed via PLIP to find the amino acids involved in the ligand interactions as well as to investigate the major protein-ligand interactions.

3.11 ADMET PROFILES

The admetSAR tool (http://lmmd.ecust.edu.cn/admetsar2/) is used to assess the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of chemical compounds. AdmetSAR forecasts different molecular properties which are important in the Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) of compounds and pharmaceuticals. It employs several quantitative structure–activity relationship (QSAR) models to predict parameters such as human intestinal absorption, blood-brain barrier penetration and potential toxicity. This study obtained and utilized canonical smiles for each molecule as input to the tool. The resulting predicted values for every property were recorded. With this approach, it becomes easier to identify early on possible candidates possessing good pharmacokinetics or less toxicity thus making drug development easier. Combining computational predictions with experimental confirmation strengthens the reliability of our results. To achieve this, the canonical smiles of the molecule were fetched; thus,

serving as inputs into such tool that would help in understanding the predicted values associated with every property. Consequently, when applied to drugs discovery, this greatly assists in discovering potential leads that are biologically active early enough in the pharmacokinetic important assays, which is helpful in assessing toxicity at the same time.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 SOXHLET EXTRACTION

Water was used as the solvent in the Soxhlet extraction process. The potential of Soxhlet extraction for the extraction of bioactive compounds with pharmaceutical or nutraceutical applications is demonstrated by the extraction yield that was achieved from *Vitex* seeds. Even though Soxhlet extraction worked well to extract bioactive compounds from *Vitex* seeds, more research is necessary to fully explore the range of compounds present in the seeds and optimize extraction conditions.

Figure 4.1. *Vitex* seed extract from Soxhlet

4.2 SOLVENT BASED EXTRACTION

Depending on the solvent employed, the solvent-based extraction method produced extracts from *Vitex* seeds with variable yields. Our results support earlier studies showing that certain classes of compounds are selectively soluble in methanol, acetone, and chloroform. While the presence of flavonoids in the methanol extract indicates their polar nature, the predominance of terpenoids in the acetone and chloroform extracts is consistent with the literature on the chemical composition of VN seeds. With its non-polar nature, chloroform effectively extracts lipophilic compounds, while methanol and acetone, which have polar and intermediate properties, respectively, are better suited for extracting distinct classes of phytochemicals. The particular compounds of interest and their intended applications should serve as a guide when selecting a solvent. The yield and quality of the extracts for use in pharmaceutical, nutraceutical, and cosmetic applications may be further

improved by optimizing extraction parameters like solvent concentration and extraction time.

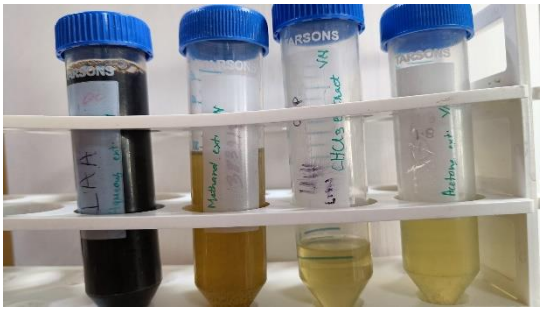
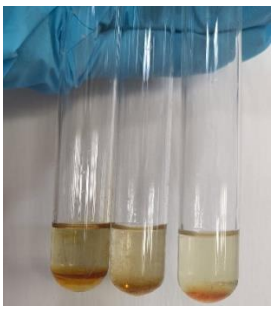


Figure 4.2. Different solvent extracts of *Vitex*

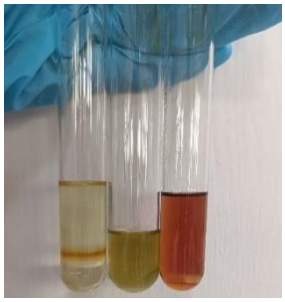
4.3 QUALITATIVE ESTIMATION OF PHYTOCHEMICALS

Table II: Phytochemical analysis

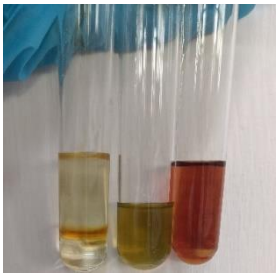
Extracts	Terpenoids	Flavonoids	Steroid	Glucoside	Saponin	Tannins
Aqueous	✓	✓	✓	✓	×	✓
Chloroform	×	✓	✓	✓	×	×
Methanol	✓	✓	×	✓	×	✓
Acetone	✓	✓	×	✓	×	×



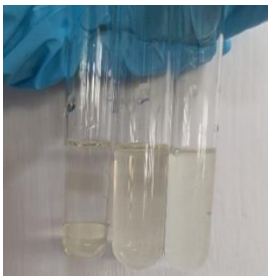
Glucoside



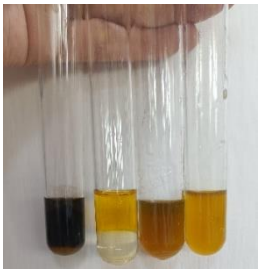
Steroid



Terpenoids



Saponin



Tannins

Figure 4.3. Phytochemical analysis

4.4 PLANT MEDIATED SYNTHESIS OF MAGNESIUM OXIDE NANOPARTICLES

0.162gms of empty MgO-NPs and 0.094gms of plant extract based MgO-NPs was synthesized. Out of the 0.094gms of plant extract nanoparticle, 0.032 was dissolved and washed in ethanol and 0.062gms was washed in distilled water and then further characterization was performed.

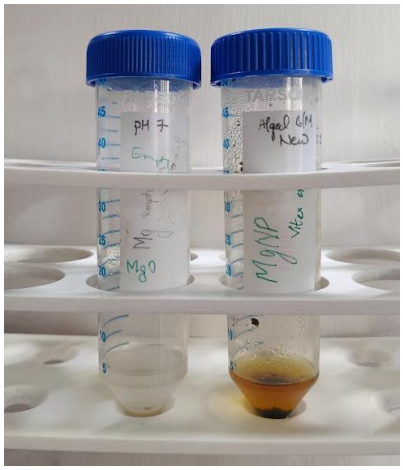


Figure 4.4. 1. Empty MgO-NPs 2. Plant based MgO-NPs

4.5 CHARACTERIZATION OF MgO-NPs USING UV-VIS

The maximum absorbance peak of biogenic MgO-NPs was detected at a wavelength of 238 nm, which confirms the formation of particles at nanoscale.

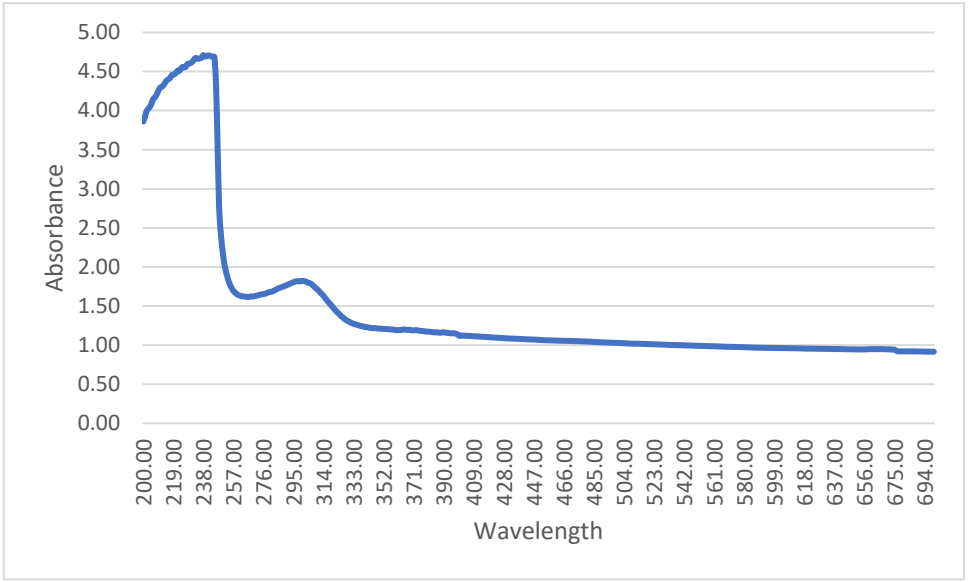


Figure 4.5. MgO-NPs dissolved in water

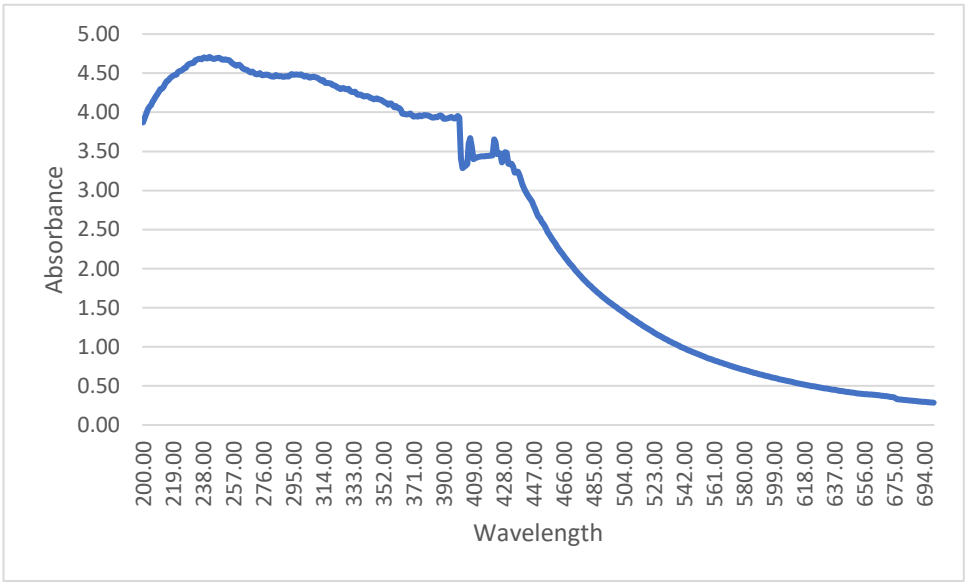


Figure 4.6. MgO-NPs dissolved in ethanol

4.6 ANTI-BACTERIAL EFFECT OF MGONPS

The significant antimicrobial activity of magnesium oxide nanoparticles (MgO-NPs) noted in this investigation is consistent with earlier studies showcasing their effectiveness against an array of bacterial pathogens. The generation of ROS and the release of Mg^{2+} ions by MgO-NPs have been shown to cause damage to bacterial cell membranes, proteins, and DNA, ultimately resulting in cell death. These results demonstrate MgO-NPs' potential as potent antibacterial agents. To completely comprehend the underlying mechanisms and assess the long-term safety and environmental impact of MgO-NPs, more research is necessary.

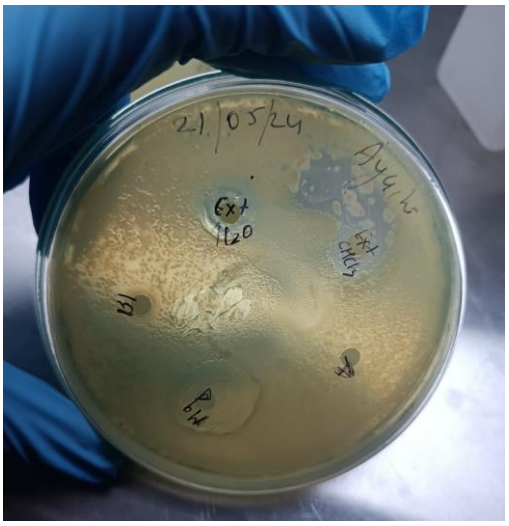
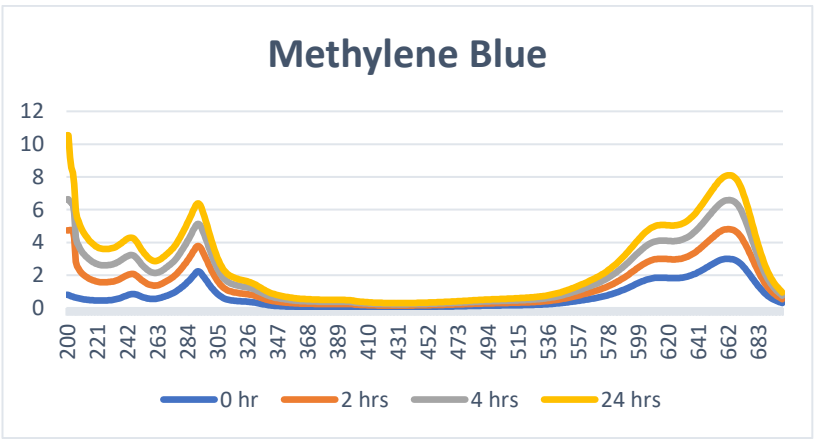


Figure 4.7. Zone of inhibition of MgO-NPs

4.7 PHOTOCATALYTIC DYE DEGRADATION USING MGO-NPS

With increasing concentration of MgO-NPs and irradiation time, it was discovered that the degradation efficiency of dyes increased. MgO-NPs capacity to produce ROS in response to light irradiation is responsible for the photocatalytic activity that has been observed. ROS aids in the oxidative processes that break down organic dyes.



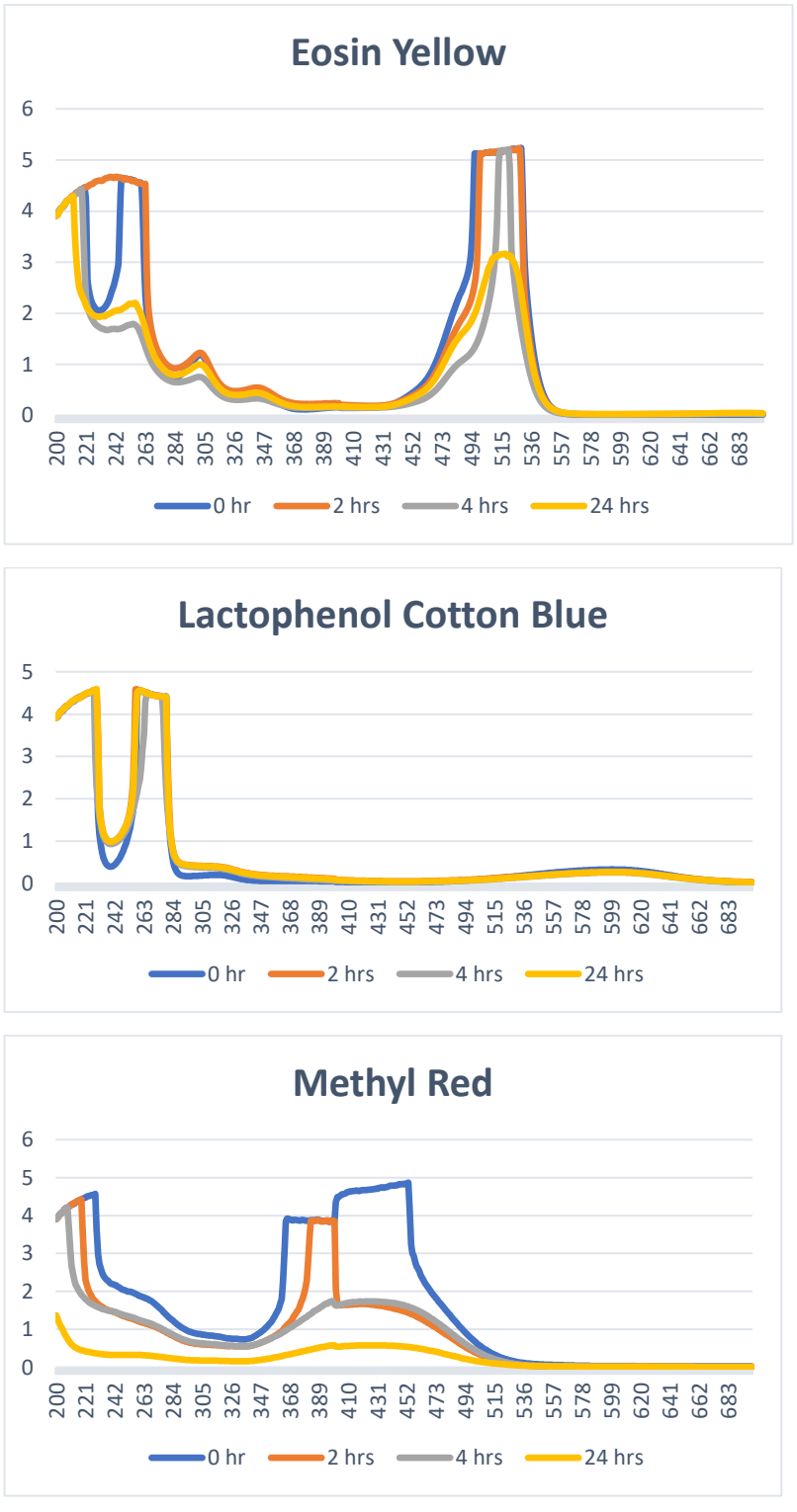


Figure 4.8. Degradation of Dyes using MgO-NPs

4.8 BIOCHAR SYNTHESIS

The pyrolysis of *Vitex negundo* seeds yielded 13.8gms of biochar with an average yield of 27.6% (w/w) relative to the initial seed mass of 50gms. The produced biochar had a granular morphology and was dark black in colour. VN seeds have been successfully used to synthesize biochar, demonstrating the potential of using plant seeds as a sustainable precursor for biochar production.

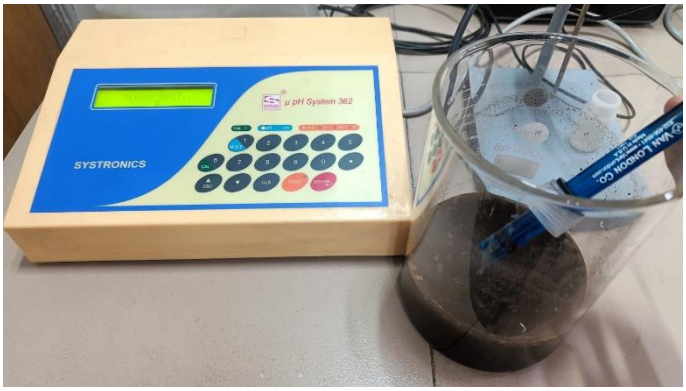


Figure 4.9. pH balance of Biochar

Figure 4.10. 1. Biochar before washing and drying 2. Biochar after washing and drying

4.9 BIOCHAR BASED HEAVY METAL REMEDIATION

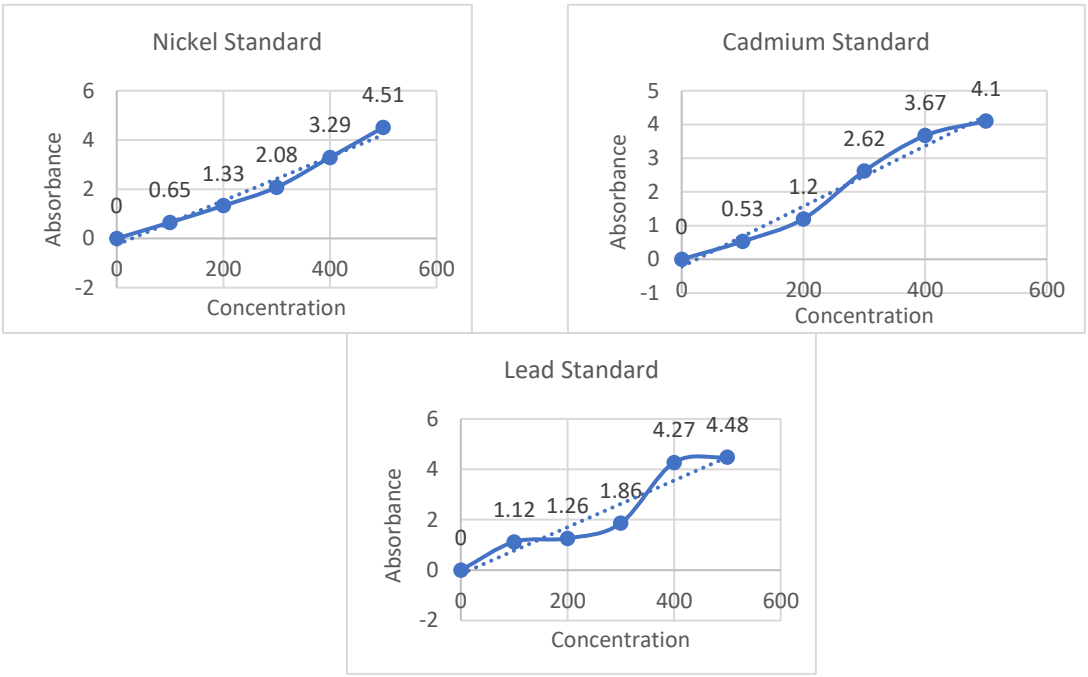
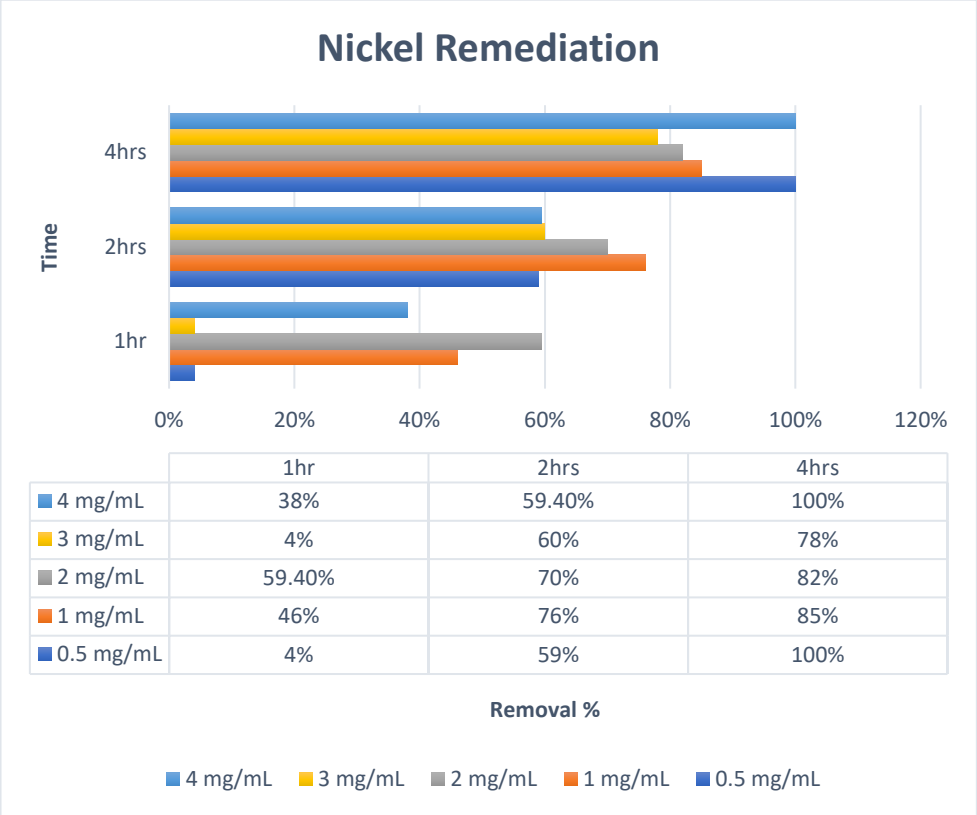


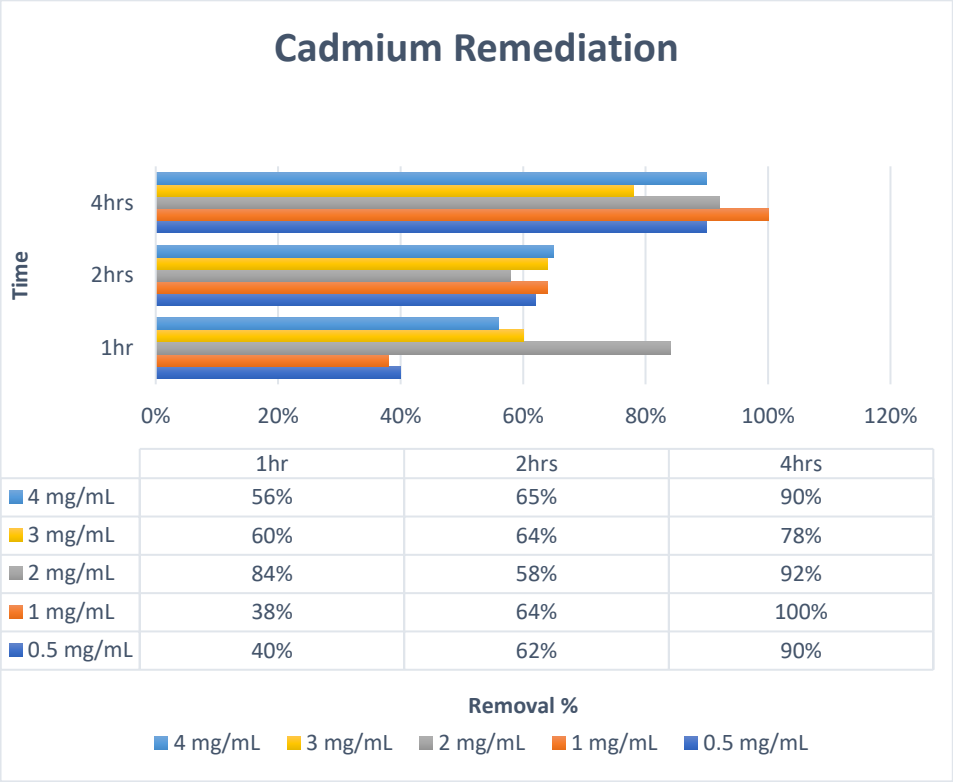
Figure 4.11. Standard curves for Ni, Cd and Pb

The absorbance of lead (Pb), cadmium (Cd), and nickel (Ni) in the untreated wastewater was measured to be 4.48, 4.51, and 4.1, respectively, prior to the application of biochar. There were notable drops in the concentrations of heavy metals after adding biochar to the wastewater. To document the observations, a cell scan was conducted one, two, and four hours into the treatment. The figure and table illustrate the additional decrease in concentrations that occurred after a 24-hour treatment period. These findings show that applying biochar to wastewater efficiently and in a time-dependent manner removes heavy metals. In order to evaluate the precise role that biochar plays in heavy metal remediation; the study also included an untreated wastewater control group. The control group's metal concentrations did not significantly decrease over the course of the experiment; instead, they stayed mostly unchanged. This emphasizes how valuable biochar is as a heavy metal remediation agent for wastewater.



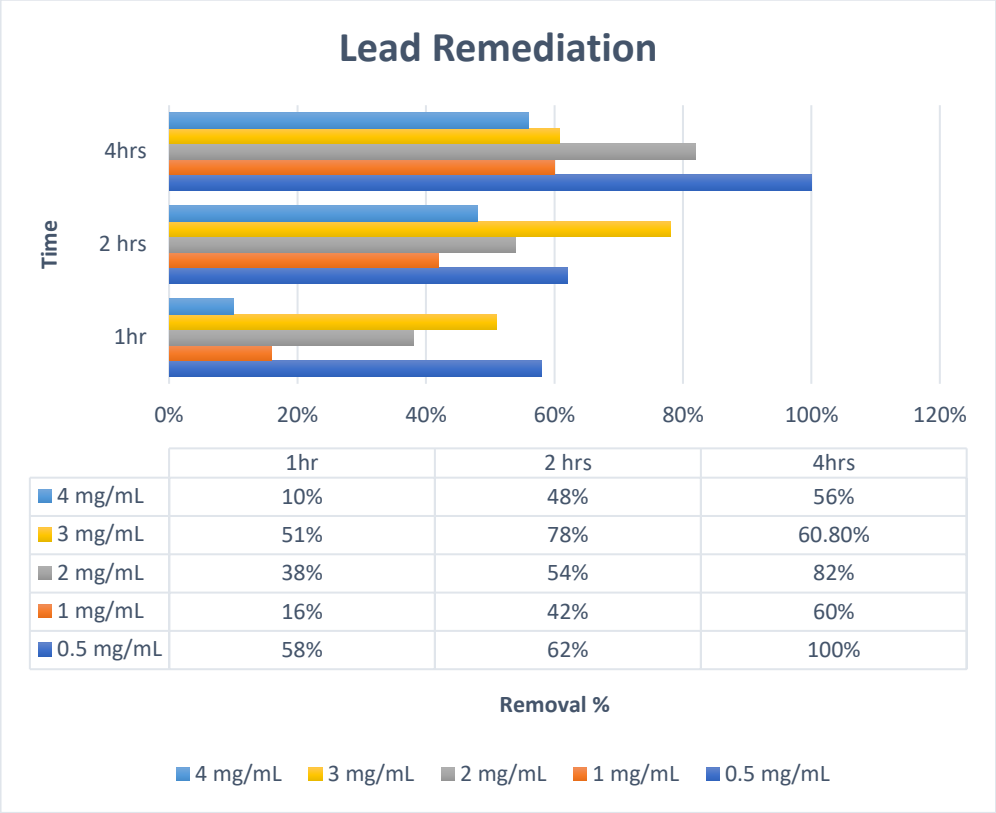
	1hr	2hrs	4hrs
4 mg/mL	38%	59.40%	100%
3 mg/mL	4%	60%	78%
2 mg/mL	59.40%	70%	82%
1 mg/mL	46%	76%	85%
0.5 mg/mL	4%	59%	100%

Figure 4.12. Nickel Remediation w.r.t time



	1hr	2hrs	4hrs
4 mg/mL	56%	65%	90%
3 mg/mL	60%	64%	78%
2 mg/mL	84%	58%	92%
1 mg/mL	38%	64%	100%
0.5 mg/mL	40%	62%	90%

Figure 4.13. Cadmium Remediation w.r.t time



	1hr	2 hrs	4hrs
4 mg/mL	10%	48%	56%
3 mg/mL	51%	78%	60.80%
2 mg/mL	38%	54%	82%
1 mg/mL	16%	42%	60%
0.5 mg/mL	58%	62%	100%

Figure 4.14 Lead Remediation w.r.t time

The significant reduction in metal concentrations after biochar treatment reveals the effectiveness of biochar as a heavy metal remediation agent in wastewater. Biochar's high surface area and porous structure probably make it easier for metal ions to adsorb and be removed from the aqueous phase. Because removal is time-dependent, equilibrium processes like adsorption kinetics appear to be important in biochar-mediated remediation. These results lend credence to the use of biochar as a viable and affordable heavy metal pollution mitigation strategy for wastewater streams.

4.10 MOLECULAR DOCKING AND ANALYSIS

Figure 4.15. 1. 8GCR structure from RCSB PDB 2. 8GCR structure after preparation

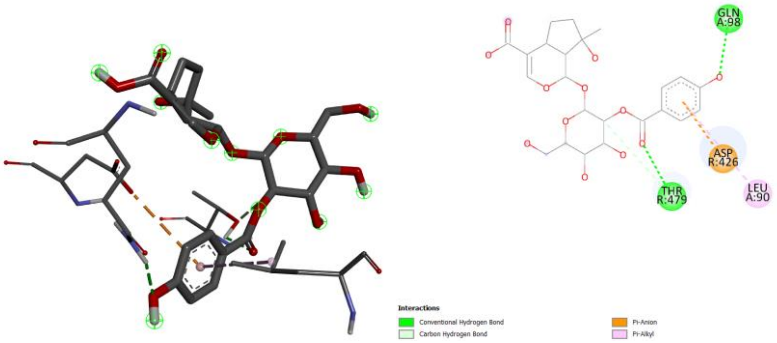
Table III: Binding Affinity of phytocompounds

Phytocompounds	Binding Affinities	Interacting Amino Acids
Negundoside (PubChem ID: 9935561)	-8.9	88Tyr, 373Lys, 470Glu, 475Phe, 479Thr, 98Gln, 376Lys, 426Asp, 474Lys, 476Ser
Isoorientin (114776)	-8.6	86Tyr, 499Ile, 500Arg, 79Lys, 133His, 142Arg, 464Tyr, 496Asp, 500Arg
Luteolin (5280445)	-8.6	86Tyr, 423Pro, 467Phe, 499Ile, 745Pro, 421Lys, 464Tyr, 500Arg, 743Glu
Vitexilactone (21636178)	-8.1	21Gln, 24Thr, 29Thr, 131Asn, 102Thr, 269Ser, 164Lys
Isovitexin (162350)	-8.4	147Val, 419Asn, 136Arg, 147Val, 150Thr, 220Tyr, 230Thr
Kaempferol (5280863)	-8	446Phe, 481Pro, 751Arg, 752Pro, 380Tyr, 424Arg, 439Cys, 489Lys
Chrysosplenol D (5280699)	-7.9	24Thr, 130Leu, 289Leu, 30Ile, 100Gln, 132Lys, 164Lys

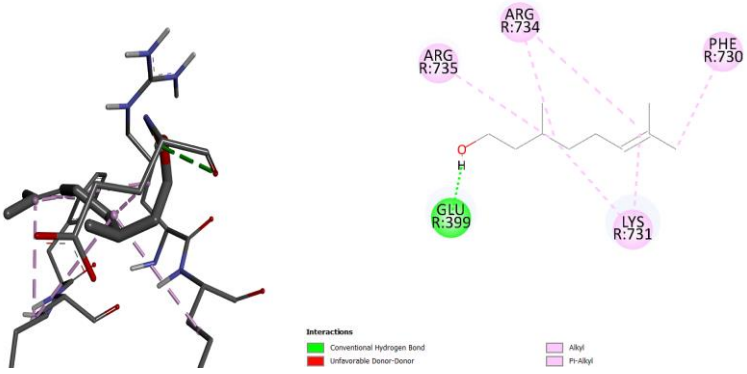
continued on page no. 30

Table III (continued)

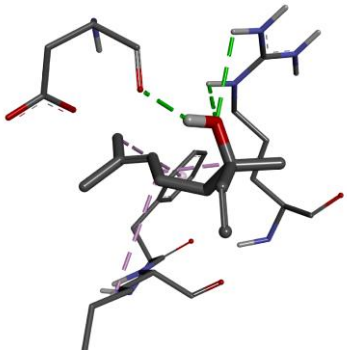
Phytocompounds	Binding Affinities	Interacting Amino Acids
Artemetin (5320351)	-7.9	468Lys, 499Ile, 464Tyr, 500Arg, 743Glu
Vitedoin A (21574226)	-7.4	470Glu, 475Phe, 97Gln, 471Thr
Viridiflorol (11996452)	-6.8	54Phe, 115Lys, 116Pro
Nerolidol (5284507)	-6.5	86Tyr, 467Phe, 468Lys, 745Pro
Linoleic acid (5280450)	-5.8	82Glu, 83Tyr, 499Ile, 86Tyr
Linalool (6549)	-5.7	399Glu, 730Phe, 731Lys, 734Arg, 397Asp, 399Glu, 334Arg
Citronellol (8842)	-5.5	730Phe, 734Arg, 399Glu

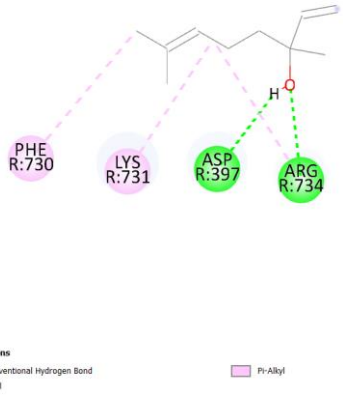


Negundoside

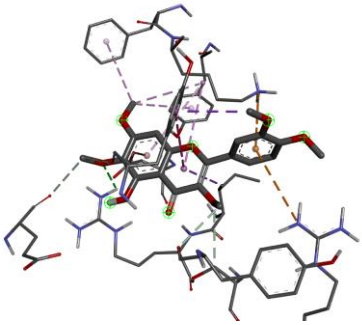


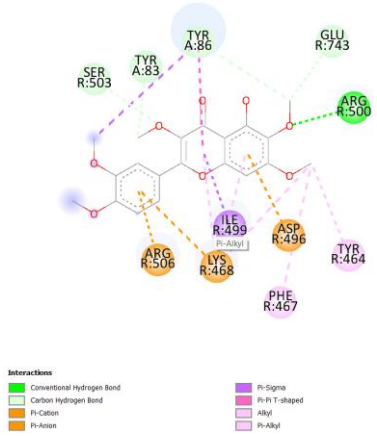
Citronellol



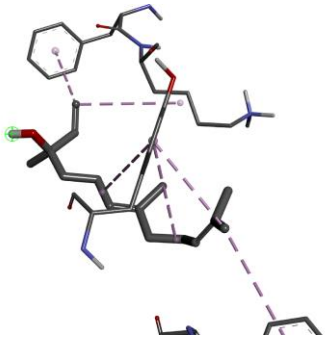


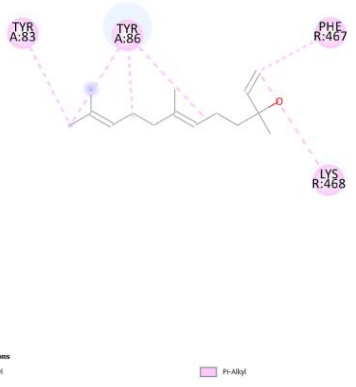
Linalool



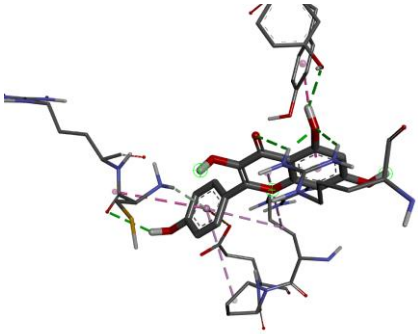


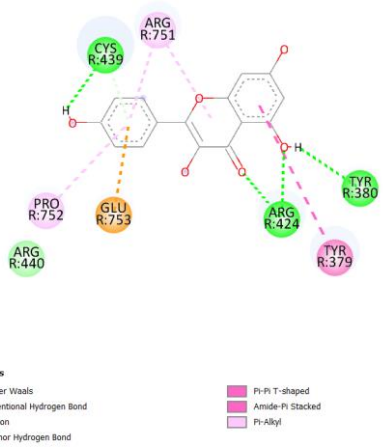
Armtemetin



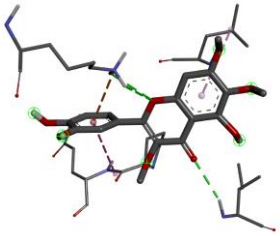


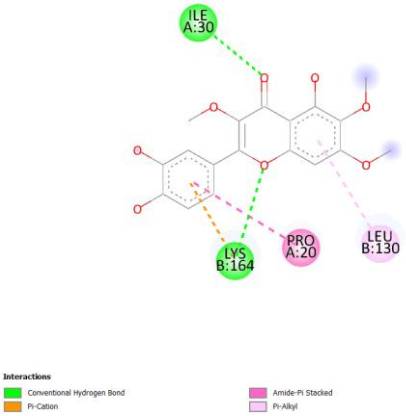
Nerolidol



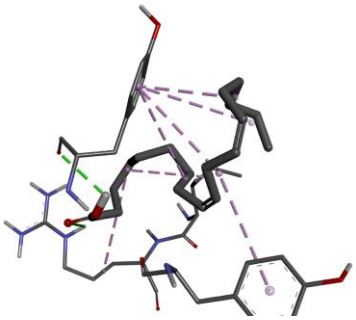


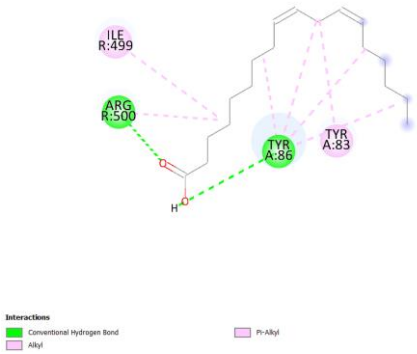
Kaempferol



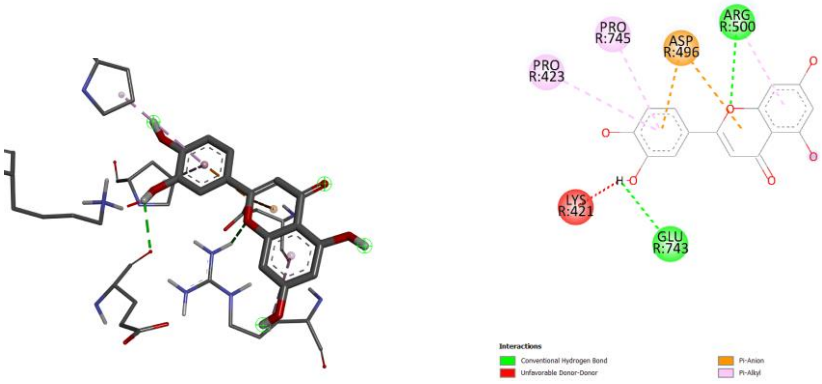


Chrysosplenol D

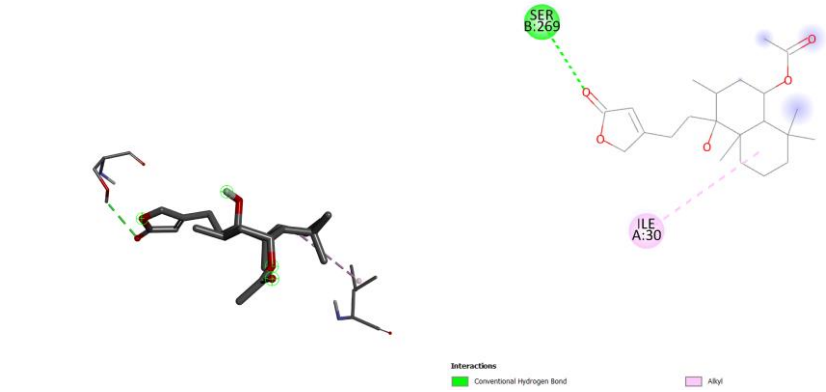




Linoleic Acid

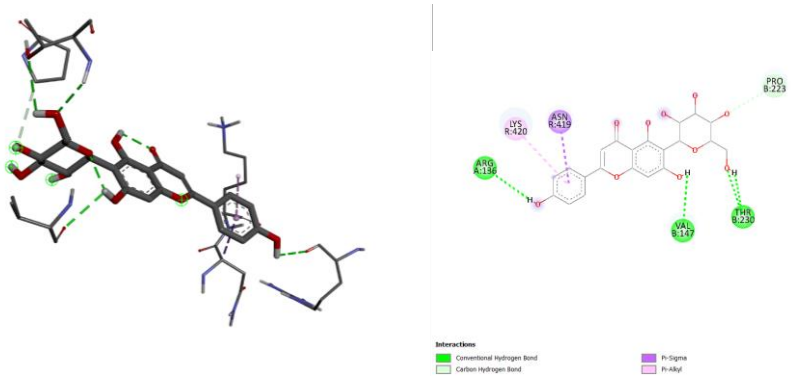


Luteolin

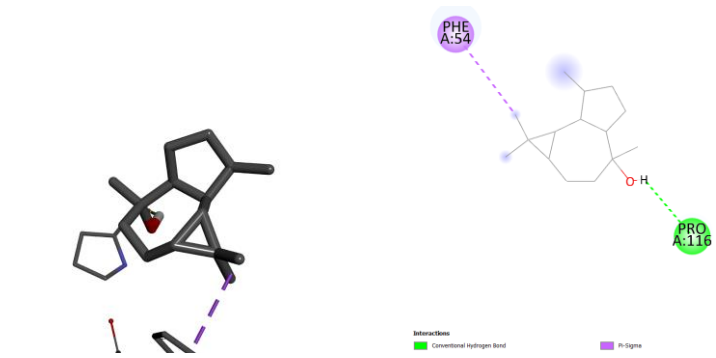


Vitexilactone

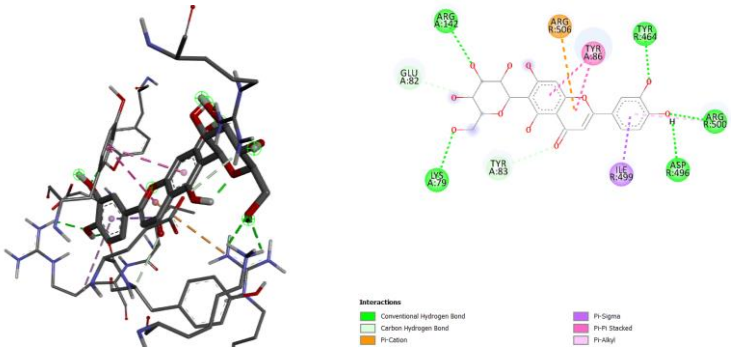
Vitedoin A



Isovitexin



Viridiflorol



Isoorientin

Figure 4.16. 2-D interactions of docked phytocompounds with 8GCR

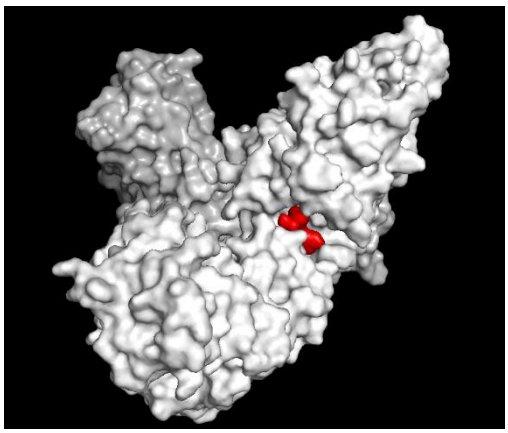


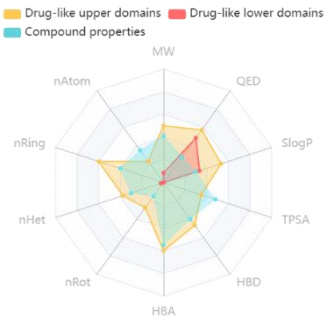
Figure 4.17. Binding site of the protein 8GCR

4.11 ADMET PROFILES

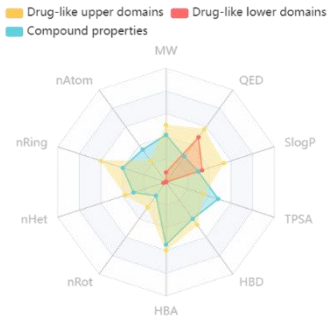
The ADMET analysis focused on the five phytocompounds with the lowest binding affinity values. The analysis recorded their molecular weight, number of hydrogen bond acceptors, number of hydrogen bond donors, Topological Polar Surface Area (TPSA) value, and logarithm of octanol-water partition coefficient (logP). The results were presented in the table below, and radar plots of these compounds were included in the study.

Table IV: ADMET profiling results

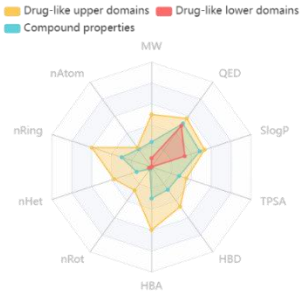
Phytocompounds	Mol. Wt.	Hydrogen acceptors	Hydrogen donors	TPSA	logP
Negundoside	496.47	11	6	192.44	-0.53
Isoorientin	448.38	11	8	201.28	-0.2
Luteolin	286.24	6	4	111.13	2.28
Vitexilactone	378.91	5	1	72.48	3.79
Isovitexin	432.38	10	7	181.05	0.09



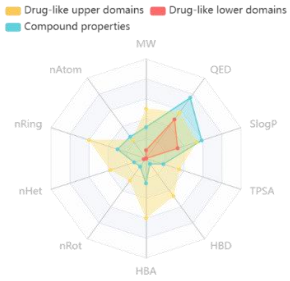
Negundoside



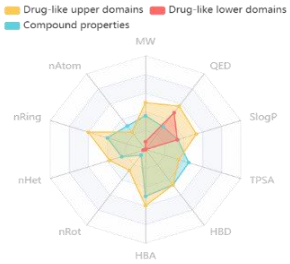
Isorientin



Luteolin



Vitexilactone



Isovitexin

Figure 4.18. Radar plots from ADMET analysis

CHAPTER 5

CONCLUSION AND FUTURE PROSPECTS

Vitex negundo is a class of herbal medicinal plant, which have a solid theoretical base to support their use. This plant species holds great promise for the pharmaceutical industry as a potential treatment. For years, the talk of research and development in medicine has revolved around plant-based medicine. However, without taking any further steps towards modern medical standards, we cannot say with certainty whether these phytochemicals could serve as one possibility for introducing immunomodulation, nutrient supplementation, or treatment option. Using a combination of laboratory experiments carried out in a test tube (in-vitro) and computer modelling attempts (in-silico), we now understand many aspects concerning the compound nature at play from different angles. This revelation then led to more trials leading to its full exploitation both within the field of biotechnology as well as its related subjects. Our phytochemical investigation has shown that *Vitex negundo* possesses a wide range of secondary metabolites, indicating a significant chemical variety. These compounds may have potential medical use. The basis for research on its pharmacological properties and the development of more sophisticated therapeutic options can be attributed to a thorough understanding of its molecular composition. The use of VN extract in the production of magnesium nanoparticles is a novel method that holds great promise for use in the fields of biology, catalysis, and nanotechnology. Plant extracts are able to be the basis of nanomaterials synthesis owing to an environmentally friendly technique for its production using them. It is also important to consider the biochar received from *Vitex negundo* biomass which testifies to its capability to enhance soil quality and assist in managing wastes. In different fields like nanotechnology, medicine, or agriculture, among others, the VN research shows different ways it can be applied hence emphasizing how important it is as a versatile commodity. As such, with the biotechnological capability of this plant there are several social issues that can be solved by saving money as well as improving quality of life because environmental degradation will not exist anymore. Further investigation is needed to develop a better understanding of what really happens to bioactive compounds of VN. The focus of such an investigation would be looking into the mechanisms that are involved in it, while improving methodologies of producing *Vitex negundo* compound nanoparticles should also be taken on board at the same time. In addition, the biochar produced from its biomass should be explored for alternative applications as well. To fully delve into the potential of VN as a vital resource for biotechnological innovation, interdisciplinary collaboration will be critical. Through encouraging biologists, chemists, engineers, and agricultural scientists to collaborate, we can efficiently combine everyone's skills and knowledge to deal with some of the major global challenges there are and promote sustainable development. The paper

adds to the ever-growing pool of information on *Vitex negundo*, underscoring its importance as a hub for nanomaterials, bioactive molecules, and sustainable bio-resources. By combining in vitro testing and in-silico modelling, we have achieved substantial advancements in understanding this remarkable plant and opened up novel possibilities for biotechnology research and development.

REFERENCES

Abid, M. K., Ibrahim, H. Bin, & Zulkifli, S. Z. (2019). Synthesis and characterization of biochar from peel and seed of jackfruit plant waste for the adsorption of copper metal ion from water. *Research Journal of Pharmacy and Technology*, *12*(9), 4182–4188. https://doi.org/10.5958/0974-360X.2019.00720.0

Adeleye, A. S., Conway, J. R., Garner, K., Huang, Y., Su, Y., & Keller, A. A. (2016). Engineered nanomaterials for water treatment and remediation: Costs, benefits, and applicability. Chemical Engineering Journal, 286, 640–662. https://doi.org/10.1016/j.cej.2015.10.105

Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. *Journal of Advanced Research*, *7*(1), 17–28. https://doi.org/10.1016/j.jare.2015.02.007

Alara, O. R., Abdurahman, N. H., & Ukaegbu, C. I. (2018). Soxhlet extraction of phenolic compounds from Vernonia cinerea leaves and its antioxidant activity. *Journal of Applied Research on Medicinal and Aromatic Plants*, *11*, 12–17. https://doi.org/10.1016/j.jarmap.2018.07.003

Al-Hazmi, F., Alnowaiser, F., Al-Ghamdi, A. A., Al-Ghamdi, A. A., Aly, M. M., Al-Tuwirqi, R. M., & El-Tantawy, F. (2012). A new large – Scale synthesis of magnesium oxide nanowires: Structural and antibacterial properties. *Superlattices and Microstructures*, *52*(2), 200–209. https://doi.org/10.1016/j.spmi.2012.04.013

Arora, V., Anil, B., Vimal, A., Vikram, L., & Singhal, S. (2011). *Vitex negundo: A Chinese Chaste Tree Development of targeted drug delivery system for treatment of pancreatic cancer View project Method development and validation of Etoricoxib and Thiocolchicoside in combined pharmaceutical solid dosage form by RP-HPLC method View project Vitex negundo:-A Chinese Chaste Tree*. *1*(5). http://www.ijpi.org

Azwanida. (2015). A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. *Medicinal & Aromatic Plants*, *04*(03). https://doi.org/10.4172/2167-0412.1000196

Banat, I. M., Nigam, P., Singh, D., & Marchant, R. (1996). Microbial decolorization of textile-dyecontaining effluents: A review. *Bioresource Technology*, *58*(3), 217–227. https://doi.org/10.1016/S0960-8524(96)00113-7

Barakat, M. A. (2011). New trends in removing heavy metals from industrial wastewater. *Arabian Journal of Chemistry*, *4*(4), 361–377. https://doi.org/10.1016/j.arabjc.2010.07.019

Chandra, H., Kumari, P., Bontempi, E., & Yadav, S. (2020). Medicinal plants: Treasure trove for green synthesis of metallic nanoparticles and their biomedical applications.

Biocatalysis and Agricultural Biotechnology, *24*, 101518. https://doi.org/10.1016/j.bcab.2020.101518

Das, N., Mishra, S. K., Bishayee, A., Ali, E. S., & Bishayee, A. (2021). The phytochemical, biological, and medicinal attributes of phytoecdysteroids: An updated review. Acta Pharmaceutica Sinica B, 11(7), 1740–1766. https://doi.org/10.1016/j.apsb.2020.10.012

Diaz-Uribe, C., Walteros, L., Duran, F., Vallejo, W., & Romero Bohórquez, A. R. (2022). Prosopis juliflora Seed Waste as Biochar for the Removal of Blue Methylene: A Thermodynamic and Kinetic Study. *ACS Omega*, *7*(47), 42916–42925. https://doi.org/10.1021/acsomega.2c05007

Ekrami, E., Pouresmaieli, M., Hashemiyoon, E. sadat, Noorbakhsh, N., & Mahmoudifard, M. (2022). Nanotechnology: A sustainable solution for heavy metals remediation. *Environmental Nanotechnology, Monitoring & Management*, *18*, 100718. https://doi.org/10.1016/j.enmm.2022.100718

Essien, E. R., Atasie, V. N., Okeafor, A. O., & Nwude, D. O. (2020). Biogenic synthesis of magnesium oxide nanoparticles using Manihot esculenta (Crantz) leaf extract. *International Nano Letters*, *10*(1), 43–48. https://doi.org/10.1007/s40089-019-00290-w

Filho, J. G. S., Duringer, J., Maia, G. L. A., Tavares, J. F., Xavier, H. S., Da Silva, M. S., da-Cunha, E. V. L., & Barbosa-Filho, J. M. (2008). Ecdysteroids fromVitex Species: Distribution and Compilation of Their13C-NMR Spectral Data. Chemistry & Biodiversity, 5(5), 707–713. https://doi.org/10.1002/cbdv.200890067

Foloppe, N., & Hubbard, R. (2006). Towards Predictive Ligand Design With Free-Energy Based Computational Methods? *Current Medicinal Chemistry*, *13*(29), 3583–3608. https://doi.org/10.2174/092986706779026165

Havsteen, B. (1983). Flavonoids, a class of natural products of high pharmacological potency. Biochemical Pharmacology, 32(7), 1141–1148. https://doi.org/10.1016/0006-2952(83)90262-9

Huang, S. Y., Grinter, S. Z., & Zou, X. (2010). Scoring functions and their evaluation methods for protein–ligand docking: recent advances and future directions. Physical Chemistry Chemical Physics/PCCP. Physical Chemistry Chemical Physics, 12(40), 12899. https://doi.org/10.1039/c0cp00151a

Hussain Jaffar, T., & Rasool, R. (2019). Computational drug design and exploration of potent phytochemicals against cancer through in silico approaches. In *Article in Biomedical Letters*. https://www.researchgate.net/publication/350976038

Inyang, M. I., Gao, B., Yao, Y., Xue, Y., Zimmerman, A., Mosa, A., Pullammanappallil, P., Ok, Y. S., & Cao, X. (2016). A review of biochar as a low-cost adsorbent for aqueous heavy metal removal. *Critical Reviews in Environmental Science and Technology*, *46*(4), 406–433. https://doi.org/10.1080/10643389.2015.1096880

K, S. C., Kumar, N. S., & Kumar, S. M. (2022). A REVIEW ON: THERAPEUTIC USES OF Vitex negundo LINN. *Certified Journal* | *Swaliha et al. World Journal of Pharmaceutical Research*, *11*. https://doi.org/10.20959/wjpr20224-23615

Karunakaran, G., Jagathambal, M., Venkatesh, M., Suresh Kumar, G., Kolesnikov, E., Dmitry, A., Gusev, A., & Kuznetsov, D. (2017). Hydrangea paniculata flower extract-mediated green synthesis of MgNPs and AgNPs for health care applications. *Powder Technology*, *305*, 488–494. https://doi.org/10.1016/j.powtec.2016.10.034

Latif, A., Sheng, D., Sun, K., Si, Y., Azeem, M., Abbas, A., & Bilal, M. (2020). Remediation of heavy metals polluted environment using Fe-based nanoparticles: Mechanisms, influencing factors, and environmental implications. *Environmental Pollution*, *264*, 114728. https://doi.org/10.1016/j.envpol.2020.114728

Marimuthu, S., Antonisamy, A. J., Malayandi, S., Rajendran, K., Tsai, P.-C., Pugazhendhi, A., & Ponnusamy, V. K. (2020). Silver nanoparticles in dye effluent treatment: A review on synthesis, treatment methods, mechanisms, photocatalytic degradation, toxic effects and mitigation of toxicity. *Journal of Photochemistry and Photobiology B: Biology*, *205*, 111823. https://doi.org/10.1016/j.jphotobiol.2020.111823

Mohamed, B. A., Ellis, N., Kim, C. S., & Bi, X. (2017). The role of tailored biochar in increasing plant growth, and reducing bioavailability, phytotoxicity, and uptake of heavy metals in contaminated soil. *Environmental Pollution*, *230*, 329–338. https://doi.org/10.1016/j.envpol.2017.06.075

Mohammad, M. S., & Shyam, P. (2023). Bioactivity studies and Insilico studies of the isolated compound from ancient plant Vitex negundo against breast cancer. Journal of Molecular Structure, 1288, 135762. https://doi.org/10.1016/j.molstruc.2023.135762

Nandhini, N. T., Rajeshkumar, S., & Mythili, S. (2019). The possible mechanism of eco-friendly synthesized nanoparticles on hazardous dyes degradation. *Biocatalysis and Agricultural Biotechnology*, *19*, 101138. https://doi.org/10.1016/j.bcab.2019.101138

Nirmalkumar, N. (2007). Pharmacognostic and Phytochemical Analysis of Vitex Negundo.L. In *International Journal of Innovative Research in Science, Engineering and Technology (An ISO* (Vol. 3297). www.ijirset.com

Pal, A., & Kundu, R. (2020). Human Papillomavirus E6 and E7: The Cervical Cancer Hallmarks and Targets for Therapy. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.03116

Pilarska, A. A., Klapiszewski, Ł., & Jesionowski, T. (2017). Recent development in the synthesis, modification and application of Mg(OH)2 and MgO: A review. *Powder Technology*, *319*, 373–407. https://doi.org/10.1016/j.powtec.2017.07.009

Rallabandi, H. R., Mekapogu, M., Natesan, K., Saindane, M., Dhupal, M., Swamy, M. K., & Vasamsetti, B. M. K. (2020). Computational methods used in phytocompound-based drug discovery. In *Plant-derived Bioactives: Chemistry and Mode of Action* (pp. 549–573). Springer Singapore. https://doi.org/10.1007/978-981-15-2361-8_25

Rodríguez-Ruiz, H. A., Garibay-Cerdenares, O. L., Illades-Aguiar, B., Montaño, S., Jiang, X., & Leyva-Vázquez, M. A. (2019). In silico prediction of structural changes in human papillomavirus type 16 (HPV16) E6 oncoprotein and its variants. *BMC Molecular and Cell Biology*, *20*(1). https://doi.org/10.1186/s12860-019-0217-0

Sathiamoorthy, B., Gupta, P., Kumar, M., Chaturvedi, A. K., Shukla, P., & Maurya, R. (2007). New antifungal flavonoid glycoside from Vitex negundo. Bioorganic & Medicinal Chemistry Letters, 17(1), 239–242. https://doi.org/10.1016/j.bmcl.2006.09.051

Sehgal, C., Taneja, S., Dhar, K., & Atal, C. (1982). 2′-p-Hydroxybenzoyl mussaenosidic acid, a new iridoid glucoside from Vitex negundo. Phytochemistry, 21(2), 363–366. https://doi.org/10.1016/s0031-9422(00)95267-1

Saied, E., Eid, A. M., Hassan, S. E. D., Salem, S. S., Radwan, A. A., Halawa, M., Saleh, F. M., Saad, H. A., Saied, E. M., & Fouda, A. (2021). The Catalytic Activity of Biosynthesized

Magnesium Oxide Nanoparticles (MgO-NPs) for Inhibiting the Growth of Pathogenic Microbes, Tanning Effluent Treatment, and Chromium Ion Removal. Catalysts, 11(7), 821. https://doi.org/10.3390/catal11070821

Silva, A. A., Sousa, A. M. F., Furtado, C. R. G., & Carvalho, N. M. F. (2022). Green magnesium oxide prepared by plant extracts: synthesis, properties and applications. *Materials Today Sustainability*, *20*, 100203. https://doi.org/10.1016/j.mtsust.2022.100203

Singh, Y., Mishra, P., Kannojia, P., Kumar Singh, P., & Kumar Gangwar, A. (2022). Phytochemical Screening Of Different Extracts Of Vitex Negundo Leaves. In *European Journal of Molecular & Clinical Medicine* (Vol. 09).

Thaçi, S., Krasniqi, B., Cela, D., Dërmaku-Sopjani, M., Bahtiri, E., Abazi, S., & Sopjani, M. (2020). Mechanisms Underlying the Tracheorelaxant Effect of Vitex agnus-castus Extract. *Revista Brasileira de Farmacognosia*, *30*(1), 103–110. https://doi.org/10.1007/s43450-020-00009-5

Tiwari, O. P., & Tripathi, Y. B. (2007). Antioxidant properties of different fractions of Vitex negundo Linn. *Food Chemistry*, *100*(3), 1170–1176. https://doi.org/10.1016/j.foodchem.2005.10.069

Venkateswarlu, K. (n.d.). International Journal of Pharmaceutical and Phytopharmacological Research Vitex negundo: Medicinal Values, Biological Activities,Toxicity Studies and Phytopharmacological Actions. In *Int.J.Pharm.Phytopharmacol.Res* (Vol. 2012, Issue 2). www.eijppr.com

Wang, L., Hu, C., & Shao, L. (2017). The antimicrobial activity of nanoparticles: present situation and prospects for the future. *International Journal of Nanomedicine*, *Volume 12*, 1227–1249. https://doi.org/10.2147/IJN.S121956

Weber, K., & Quicker, P. (2018). Properties of biochar. *Fuel*, *217*, 240–261. https://doi.org/10.1016/j.fuel.2017.12.054

Wong, C. W., Chan, Y. S., Jeevanandam, J., Pal, K., Bechelany, M., Abd Elkodous, M., & El-Sayyad, G. S. (2020). Response Surface Methodology Optimization of Mono-dispersed MgO Nanoparticles Fabricated by Ultrasonic-Assisted Sol–Gel Method for Outstanding Antimicrobial and Antibiofilm Activities. *Journal of Cluster Science*, *31*(2), 367–389. https://doi.org/10.1007/s10876-019-01651-3

Yang, H., Yan, R., Chen, H., Lee, D. H., & Zheng, C. (2007). Characteristics of hemicellulose, cellulose and lignin pyrolysis. *Fuel*, *86*(12–13), 1781–1788. https://doi.org/10.1016/j.fuel.2006.12.013

Yao, J. L., Fang, S. M., Liu, R., Oppong, M. B., Liu, E. W., Fan, G. W., & Zhang, H. (2016). A review on the terpenes from genus vitex. In *Molecules* (Vol. 21, Issue 9). MDPI AG. https://doi.org/10.3390/molecules21091179

Zheng, C. J., Huang, B. K., Wu, Y. B., Han, T., Zhang, Q. Y., Zhang, H., & Qin, L. P. (2010). Terpenoids from Vitex negundo seeds. Biochemical Systematics and Ecology, 38(2), 247–249. https://doi.org/10.1016/j.bse.2010.01.002

Zheng, C. J., Lan, X. P., Wang, Y., Huang, B. K., Han, T., Zhang, Q. Y., & Qin, L. P. (2012). A new labdane diterpene fromVitex negundo. Pharmaceutical Biology, 50(6), 687–690. https://doi.org/10.3109/13880209.2011.597410

LIST OF PUBLICATIONS AND THEIR PROOF

"Structure based Computational Investigation of Phytocompounds from *Vitex negundo* as Drug Candidate for Human Respiratory Syncytial Virus (hRSV)"

Gmail Ayushi Singh <ayushi.singhas26@gmail.com>

ICETSE2024 :: Acceptance Confirmation and Registration Details
2 messages

Akshaya Institute Of Technology icetse2024 <icetse2024@gmail.com> 4 May 2024 at 11:55
To: Ayushi Singh <ayushi.singhas26@gmail.com>, ishikasuman03@gmail.com, navneetab@dce.ac.in

Dear ICETSE-2024 Author,

Warm greetings from Hinweis Research!

We are thrilled to inform you that your submitted paper for **International Conference on Emerging Technologies in Science and Engineering (ICETSE)** has been accepted. Congratulations on this significant achievement! Your dedication to your research is highly commendable. The ICETSE conference is scheduled to be held on **June 26-27, 2024** at **Akshaya Institute of Technology, Tumkur, Karnataka**. The conference is organised by the Akshaya Institute of Technology and technically co-sponsored by Hinweis Research.

https://ait-tumkur.ac.in/icetse2024/

Here are the important details regarding your acceptance:

Review Result and Acceptance Certificate:

The consolidated review result is attached along with this email. The review result itself is the acceptance certificate.

Publication and Indexing:

All accepted conference papers will be published in the **Conference Proceedings** with an **ISBN Number** and will be indexed by **Scopus and Crossref**, further enhancing the visibility of your work.

Camera Ready Submission:

To proceed with the publication process, please ensure that your paper adheres to the standard format. Your camera-ready submission should be in Microsoft Word DOC or DOCX format, and it should not exceed the stipulated page limit, including text, figures, tables, and references. Kindly send your camera-ready submission to icetse2024@gmail.com

Payment Mode:

You can conveniently make the registration payments using the provided bank account details of the Akshaya Education Trust, or by scanning the QR code using your UPI app given in the website.

Bank Details for Payment:

Account Name: Akshaya Education Trust

Account No.: 50200024166335

Bank: HDFC Bank

IFSC Code: HDFC0002090

Branch: Tumkur, Karnataka, India

For more details, kindly go through the website

https://ait-tumkur.ac.in/icetse2024/payment.html

PAPER NAME

THESIS -Vitex negundo.docx

WORD COUNT

10824 Words

CHARACTER COUNT

64534 Characters

PAGE COUNT

58 Pages

FILE SIZE

6.7MB

SUBMISSION DATE

May 30, 2024 7:24 PM GMT+5:30

REPORT DATE

May 30, 2024 7:25 PM GMT+5:30

8% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

- 6% Internet database
- 4% Publications database
- Crossref database
- Crossref Posted Content database
- 6% Submitted Works database

Excluded from Similarity Report

- Bibliographic material
- Quoted material
- Cited material

8% Overall Similarity

Top sources found in the following databases:

- 6% Internet database
- Crossref database
- 6% Submitted Works database
- 4% Publications database
- Crossref Posted Content database

TOP SOURCES

The sources with the highest number of matches within the submission. Overlapping sources will not be displayed.

1	dspace.dtu.ac.in:8080 Internet	1%
2	Delhi Technological University on 2024-05-28 Submitted works	1%
3	123dok.com Internet	<1%
4	dtusimilarity on 2024-05-29 Submitted works	<1%
5	Chibuye Bitwell, Indra Singh Sen, Chimuka Luke, Maseka Kenneth Kako... Crossref	<1%
6	core.ac.uk Internet	<1%
7	dokumen.pub Internet	<1%
8	Fatema Yasmin Nisa, Md. Atiar Rahman, Md. Khalid Juhani Rafi, Md. A... Crossref	<1%

9	Sivasankari Marimuthu, Arul Jayanthi Antonisamy, Sankar Malayandi, ... Crossref	<1%
10	edoc.hu-berlin.de Internet	<1%
11	University of Hull on 2021-10-04 Submitted works	<1%
12	rsdjournal.org Internet	<1%
13	K.M. Rajeshwari, M.R. Suhasini, S. Bindya, A.B. Hemavathi et al. "Photo... Crossref	<1%
14	N.T. Nandhini, S. Rajeshkumar, S. Mythili. "The possible mechanism of ... Crossref	<1%
15	labees.civil.fau.edu Internet	<1%
16	opac.wbut.ac.in Internet	<1%
17	frontiersin.org Internet	<1%
18	sro.sussex.ac.uk Internet	<1%
19	researchgate.net Internet	<1%
20	Amr Fouda, Khalid S. Alshallash, Mohammed I. Alghonaim, Ahmed M. ... Crossref	<1%

21	Hesam Kamyab, Shreeshivadasan Chelliapan, Gasim Hayder, Mohamm... Crossref	<1%
22	Swinburne University of Technology on 2015-09-03 Submitted works	<1%
23	thieme-connect.de Internet	<1%
24	Aremu, Ali Agaka. "Computational Studies of Anti-Erectile Dysfunction ... Publication	<1%
25	virologyj.biomedcentral.com Internet	<1%
26	grin.com Internet	<1%
27	Hong Kong Baptist University on 2022-05-11 Submitted works	<1%
28	College of Natural Resources, RUB on 2020-04-29 Submitted works	<1%
29	Ibri College of Technology on 2024-04-03 Submitted works	<1%
30	SASTRA University on 2014-05-28 Submitted works	<1%
31	University of Strathclyde on 2012-02-29 Submitted works	<1%
32	digitalcommons.library.uab.edu Internet	<1%

33	ninercommons.charlotte.edu Internet	<1%
34	Ain Farina Farizan, Hanis Mohd Yusoff, Nurhanna Badar, Irshad Ul Haq ... Crossref	<1%
35	Curtin University of Technology on 2020-05-03 Submitted works	<1%
36	De La Salle University on 2022-01-18 Submitted works	<1%
37	International Medical University on 2019-05-06 Submitted works	<1%
38	Trinity College Dublin on 2010-09-29 Submitted works	<1%
39	Uttar Pradesh Technical University on 2019-05-13 Submitted works	<1%
40	Vunain, E., AK Mishra, and BB Mamba. "Dendrimers, mesoporous silica... Crossref	<1%
41	coek.info Internet	<1%
42	gyan.iitg.ac.in Internet	<1%
43	link.springer.com Internet	<1%
44	pubag.nal.usda.gov Internet	<1%

45	dspace.dtu.ac.in:8080 Internet	<1%
46	Vels University on 2017-11-24 Submitted works	<1%
47	qspace.library.queensu.ca Internet	<1%
48	Elena Ekrami, Mahdi Pouresmaieli, Elham sadat Hashemiyoon, Negin N... Crossref	<1%
49	Nilai University College on 2020-01-05 Submitted works	<1%
50	Oluwaseun R. Alara, Nour H. Abdurahman, Chinonso I. Ukaegbu. "Soxhl... Crossref	<1%
51	Priyanka Rawat, Asmita Das. "Differential expression of disparate trans... Crossref	<1%
52	archive-ouverte.unige.ch Internet	<1%

AYUSHI SINGH

CONTACT

8404885777

ayushi.singhas26@gmail.com

https://www.linkedin.com/in/ayushi-singh26/

ANALYTICAL SKILLS

Research and Data mining

Data Interpretation and Organization

Collaborative skills

Creating and keeping deadlines

Verbal and written communication skills

EDUCATION

Post-Graduation

Delhi Technological University

2022-2024 (Pursuing)

GPA 8.08

Graduation

Shoolini University

2019-2022

OGPA 8.83

Intermediate

KN Memo Academy

Percentage - 79%

High School

Mount Assisi School

Percentage - 89%

COMPUTER

Excel

C++

WORK EXPERIENCE

- As a **Subject Matter Expert** in two top E-learning platforms (Doubtnut and Brainly) - Freelancer

INTERNSHIPS

- TMS Foundation (India Youth) - **Bioinformatics** Intern
- As a **Content Development Intern** at Medguru Digital Learning

TECHNICAL KNOWLEDGE

Instrumentation:

- Laminar Airflow
- Laboratory Incubator
- Autoclave
- Centrifugation Techniques
- Spectrophotometric Techniques

Microbiology skills:

- Streaking
- Serial Dilution
- Algal media formulations

Molecular biology skills:

- DNA isolation
- Electrophoresis

WORKSHOPS & ONLINE CERTIFICATIONS

- Cancer Biology Specialization
- Algae Biotechnology
- The Science of Stem Cells
- AI for Everyone
- Critical Thinking and Problem-Solving
- Watson AI
- Online webinar "Gel extraction and PCR clean up - Tips to maximize your yield and tricks for DNA quantification"
- Virtual Event Focusing on Exosomes- Stem Cells Derived Nanoparticles
- Online Workshop on Computational Applications in Secondary Metabolite Discovery (CAiSMD) 2022

PROJECTS

- Optimizing macronutrients for *Chlorella pyrenoidosa* culture for biomass production
- Isolation of ligninolytic bacteria and their use in agricultural wastewater purification

EXTRACURRICULAR ACHIEVEMENTS AND ACTIVITIES

- Content and Research and Event and Collaboration Member in BioSoc-DTU
- Student President of the Literature Club of the University during Graduation
- Student Body President of NSS Unit at University during Graduation
- Member of Poetry Wing in Sahitya, the Literary & Debating Society of DTU
- National Integration Camp (NIC) Certificate Holder
- NCC Certificate Holder
- NSS Certificate Holder