AN ECO-FRIENDLY SYNTHESIS OF TELLURIUM NANO PARTICLES AND ITS APPLICATIONS USING *Conus betulinus*

Project in Chemistry

Submitted to St. Mary's College (Autonomous) in partial fulfillment for the award of the Degree of Bachelor of Science in

Chemistry

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DECLARATION

We hereby declare that the project entitled "**AN ECO-FRIENDLY SYNTHESIS OF TELLURIUM NANOPARTICLES AND ITS APPLICATIONS USING *Conus betulinus***" submitted to St. Mary's college (Autonomous) Thoothukudi affiliated to Manonmaniam Sundaranar University for the Degree of Bachelor of science is our original work and that it has not previously formed the basis for the award of any degree, Diploma or Similar title.

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CERTIFICATE

This is to certify that the report of the project in chemistry entitled **"AN ECO-FRIENDLY SYNTHESIS OF TELLURIUM NANOPARTICLES AND ITS APPLICATIONS USING *Conus betulinus* "** is submitted to St. Mary's College (Autonomous), in partial fulfillment for the award of the Degree of Bachelor of Science in Chemistry and is a record of the work done by the following students during the year 2021-2022.

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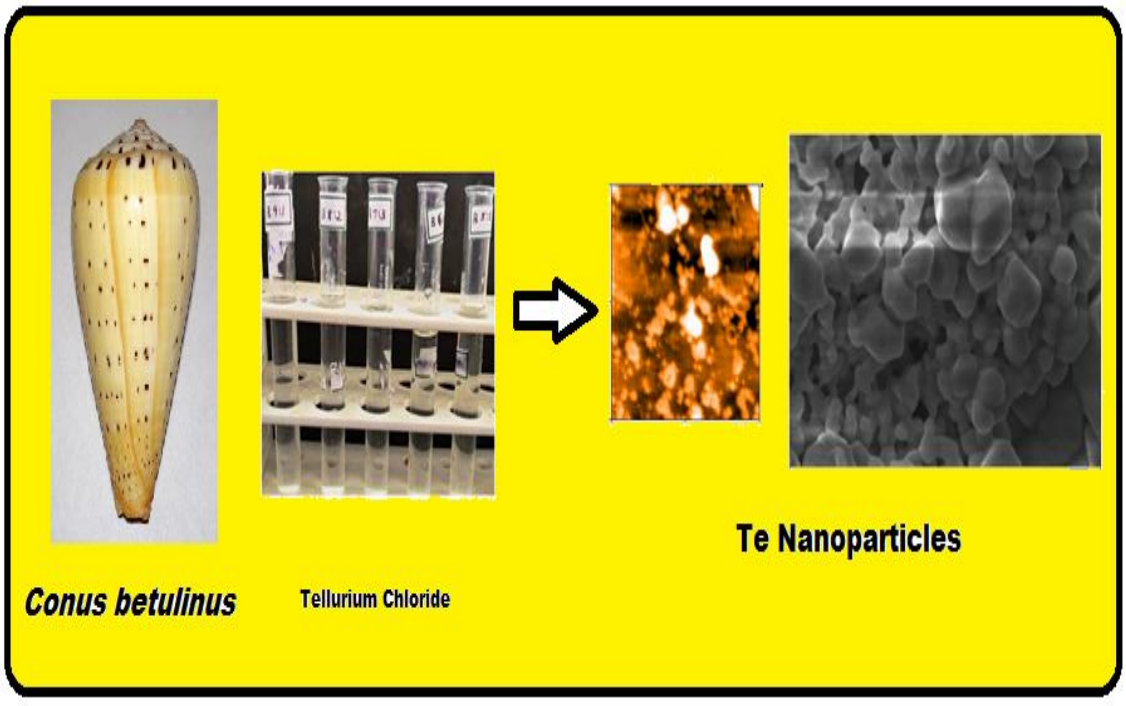
CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Eco-friendly synthesis of Tellurium Nanoparticles using *Conus betulinus* was reported. UV-Visible spectroscopy analysis confirmed the formation of Nanoparticles. Fourier Transform Infrared Spectroscopy indicates the presence of reducing agents. Atomic Force Microscopy analysis showed the nano particles formed are of agglomerated rocky shape. The Scanning electron microscopy images also confirmed the presence of the flaky nanostructures. The inhibitory activity of nanoparticles were studied and an excellent inhibition activity was observed against the fungus confirm its antibiotic property. DPPH scavenging and Hydrogen peroxide scavenging results shows excellent property towards anti-aging applications.



CHAPTER I

INTRODUCTION

INTRODUCTION

1.1 NANO

A nanoparticle is a small particle that ranges between 1 to 100 nanometers in size. Undetectable by the human eye, nanoparticles can exhibit significantly different physical and chemical properties to their larger material counterparts.

The definition given by the European Commission states that the particle size of at least half of the particles in the number size distribution must measure 100 nm or below. Most nanoparticles are made up of only a few hundred atoms.

The material properties change as their size approaches the atomic scale. This is due to the surface area to volume ratio increasing, resulting in the material's surface atoms dominating the material performance. Owing to their very small size, nanoparticles have a very large surface area to volume ratio when compared to bulk material, such as powders, plate and sheet. This feature enables nanoparticles to possess unexpected optical, physical and chemical properties, as they are small enough to confine their electrons and produce quantum effects.

1.2 APPLICATION OF NANOMATERIALS

Nanomaterials can occur naturally, be created as the by-products of combustion reactions, or be produced purposefully through engineering to perform a specialised function . Due to the ability to generate the materials in a particular way to play a specific role, the use of nanomaterials spans across awide variety of industries, from healthcare and cosmetics to environmental preservation and air purification.

The healthcare field, for example, utilises nanomaterials in a variety of ways, with one major use being drug delivery. One example of this process is whereby nanoparticles are being developed to assist the transportation of chemotherapy drugs directly to cancerous growths, as well as to deliver drugs to areas of arteries that are damaged in order to fight cardiovascular disease. Carbon nanotubes are also being developed in order to be used in processes such as the addition of antibodies to the nanotubes to create bacteria sensors.

In aerospace, carbon nanotubes can be used in the morphing of aircraft wings. The nanotubes are used in a composite form to bend in response to the application of an electric voltage.

Elsewhere, environmental preservation processes make use of nanomaterials too - in this case, nanowires. Applications are being developed to use the nanowires - zinc oxide nanowires - in flexible solar cells as well as to play a role in the treatment of polluted water.

In the cosmetics industry, mineral nanoparticles – such as titanium oxide – are used in sunscreen, due to the poor stability that conventional chemical UV protection offers in the long-term. Just as the bulk material would, titanium oxide nanoparticles are able to provide improved UV protection while also having the added advantage of removing the cosmetically unappealing whitening associated with sunscreen in their nano-form.

The sports industry has been producing baseball bats that have been made with carbon nanotubes, making the bats lighter and therefore improving their performance. Further use of nanomaterials in this industry can be identified in the use of antimicrobial nanotechnology in

items such as the towels and mats used by sportspeople, in order to prevent illnesses caused by bacteria.

Nanomaterials have also been developed for use in the military. One example is the use of mobile pigment nanoparticles being used to produce a better form of camouflage, through injection of the particles into the material of soldier'suniforms. Additionally, the military have developed sensor systems using nanomaterials, such as titanium dioxidethat can detect biological agents.

The use of nano-titanium dioxide also extends to use in coatings to form self-cleaning surfaces, such as those of plastic garden chairs. A sealed film of water is created on the coatingand any dirt dissolves in the film, after which the next shower will remove the dirt and essentially clean the chairs.

Controlling the size, shape and material of the nanoparticle enables engineers to design photovoltaics (PV) and solar thermal products with tailored solar absorption rates. Absorption of solar radiation is much higher in materials composed of nanoparticles than in thin films of continuous sheets of material.

The Sol-Gel process is a method for producing solid material from nanoparticles. Whilst it is generally viewed as a relatively new industrial technology, it is used extensively in a number of industries, such as abrasive powder manufacture, coatings production and optical fibres.

1.3 Betuline cone (*Conusbetulinus*)

Conusbetulinus, common name the **betuline cone**, is a species of sea snail, a marine gastropod mollusk in the family Conidae, the cone snails and their allies.

Figure1.1.*Conusbetulinus*

NAME	: Betuline cone
SCIENTIFIC NAME	: *Conusbetulinus*
KINGDOM	: Animalia
PHYLUM	: Mollusca
CLASS	: Gastropoda
SUBCLASS	: Caenogastropoda
ORDER	: Neogastropoda
SUPERFAMILY	: Conoidea
FAMILY	: Conidae
GENUS	: *Conus*
SPECIES	: *Conus betulinus*

1.4 TELLURIUM NANOPARTICLES

Tellurium (Te) nanoparticles, nanodots or nanopowder are black spherical high surface area particles. Nanoscale Tellurium Particles are typically 10 - 45 nanometers (nm) with specific surface area (SSA) in the 30 - 50 m^2/g range. Nano Tellurium Particles are also available in passivated and Ultra high purity and high purity and coated and dispersed forms. They are also available as a dispersion through the AE Nano fluid production group. Nano fluids are generally defined as suspended nanoparticles in solution either using surfactant or surface charge technology. Nanofluid dispersion and coating selection technical guidance is also available. Other nanostructures include nanorods, nanowhiskers, nanohorns, nanopyramids and other nano composites. Surface functionalized nanoparticles allow for the particles to be preferentially adsorbed at the surface interface using chemically bound polymers.

Tellurium (atomic symbol: Te, atomic number: 52) is a Block P, Group 16, Period 5 element with an atomic radius of 127.60. The number of electrons in each of tellurium's shells is 2, 8, 18, 18, 6 and its electron configuration is [Kr] $4d^{10}\ 5s^2\ 5p^4$. Tellurium was discovered by Franz Muller von Reichenstein in 1782 and first isolated by Martin Heinrich Klaproth in 1798. In its elemental form, tellurium has a silvery lustrous gray appearance. The tellurium atom has a radius of 140 pm and a Van der Waals radius of 206 pm. Tellurium is most commonly sourced from the anode sludges produced as a byproduct of copper refining. The name Tellurium originates from the Greek word *Tellus*, meaning Earth.

1.5 GREEN SYNTHESIS

Nanoparticles can be produced by physical , chemical and biological methods. Physical and chemical methods of synthesis pose a threat to environment due to the use of reducing and stabilizing agents that are known to be both toxic and non – biodegradable. Alternatively , biological synthesis or 'GREEN SYNTHESIS' of nanoparticles is considered a novel approach due to its numerous advantages such as eco-friendly nature, ease of production, feasible large scale synthesis and lack of requirement of harmful chemical agents.

1.6 ANTIBACTERIAL

Antibacterial usually refers to an antibiotic, aprincipal type of antimicrobial agent used mainly against bacteria; itmay kill or inhibit them.

Antibacterial may also refer to:

- Antiseptic, a principal type of antimicrobial agent used mainlyagainst bacteria; it may kill or inhibit them
- Disinfectant, an agent to impair microbes in cleaning/sanitation butnot taken internally as medicine; it may kill or inhibit them
- Bactericide, an agent that kills bacteria populations
- Bacteriostatic agent, an agent that does not kill bacteria populationsbut inhibits their growth

1.7 ANTI FUNGAL

An antifungal medication, also known as an antimyoticmedication, a pharmaceutical fungicide or fungistatic used to treat andprevent mycosis suchas ringworm, candidiasis (thrush), serious systemicinfections such

as cryptococcal meningitis, and others. Such drugs are usually obtained by a doctor's prescription, but a few are available overthe counter (OTC).

1.8 DPPH

DPPH is a common abbreviation for the organic chemicalcompound 2,2-diphenyl-1-picrylhydrazyl. It is a dark-colored crystallinepowder composed of stable free radical molecules. DPPH has two majorapplications, both in laboratory research: one is a monitor of chemical reactions involving radicals, most notably it is a common antioxidant assay, and another is a standard of the position and intensity of electronparamagnetic resonance signals.

DPPH is a well-known radical and a trap ("scavenger") for otherradicals. Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction.Because of a strong absorption band centered at about 520 nm, theDPPH radical has a deep violet color in solution, and it becomescolorless or pale yellow when neutralized. This property allows visualmonitoring of the reaction, and the number of initial radicals can becounted from the change in the optical absorption at 520 nm or in theEPR signal of the DPPH.

1.9HYDROGEN PEROXIDE

Hydrogen peroxide is the simplest kind of peroxide available (oxygen-oxygen single bond). It is a colourless liquid and is used in aqueoussolution for safety reasons. It acts as a bleaching agent and is also usedas disinfectant. Concentrated hydrogen peroxide is a very reactiveoxygen species and is used as a propellant in rocketry. The chemicalformula for hydrogen peroxide is H 2 O 2 It is often referred to as waterwith one more oxygen atom. It is acidic in nature and PH is about 4.5. Itis 100 percent degradable compound.

CHAPTER II

SCOPE OF THE ART & SCOPE OF THE WORK

STATE OF THE ART & SCOPE OF THE WORK

2.1 LITERATURE SURVEY

Tellurium (TeNPs), using various microorganisms, as bacteria and fungi, and plants'extracts. It also discusses the methodologies followed by materials scientists andhighlights the impact of the experimental sets on the outcomes and shed light on theunderlying mechanisms. it features the unique properties displayed by these biogenicnanoparticles for a large range of emerging applications in medicine, agriculture,bioengineering, and bioremediation [1].

Tellurium nano particles (tnps) have pharmaceutical, medical, industrial andenvironmental importance. tellurium has also indications of traditional homeopathicusage. tellurium and selenium are two basic components of solar cells. as solar cellindustries expand over the next decades, increasing demand for tnps is inevitable.some organisms can incorporate tellurium in place of sulfur and selenium into aminoacids such as telluro-cysteine and telluro-methionine. some streptomycetes take uptellurium ions and reduce them to elemental tellurium [2].

The nanoparticles were synthesized using lactose and characterized with differentinstrumentation methods. The in vitro and in vivo cytotoxicity of telluriumnanoparticles and its effect on lipid profile were also evaluated. Hydroxyl-cappedtellurium nanoparticles were successfully fabricated by lactose. The results showedspherical tellurium nanoparticles with a mean size of 89 nm. The toxicological studyshowed that the tellurium nanoparticles did not exhibit any toxicity on the primarycells [3].

Synthesizing nanoparticles using tea extracts has gained considerable attention due to its simplicity, cost-effective and the usage of environmentally friendly reagents.The resulting nanoparticles were characterized in terms of morphology, structureand composition by Transmission Electron Microscopy (TEM), X-Ray PowderDiffraction (XRPD), Fourier Transform Infrared Attenuated Total Reflectance(FTIR-ATR) and UV–Vis spectroscopy [4].

Tellurium nanostructures: A simple solution-based process has been developedfor the synthesis of tellurium nanostructures with tunable shape and sizes, includingnanoparticles, nanorods, nanowires, and nanotubes. The size-dependent opticalproperties are explained based on modeling calculations using the joint density ofstates (JDOS) [5].

Tellurium nano particles (tnps) have pharmaceutical, medical, industrial andenvironmental importance. tellurium has also indications of traditional homeopathicusage. tellurium and selenium are two basic components of solar cells. as solar cellindustries expand over the next decades, increasing demand for tnps is inevitable.some organisms can incorporate tellurium in place of sulfur and selenium into aminoacids such as telluro-cysteine and telluro-methionine. some streptomycetes take uptellurium ions and reduce them to elemental tellurium, tnps [6].

Tellurium is a chalcogenide element, essential, in the development of renewableenergy solutions. However, substantial problems remain to synthesize zero-dimensional tellurium nanostructures in a facile and environmentally friendly way.This communication reports the first successful synthesis of pure telluriumnanoparticles by laser ablation in liquids.Finally, the most stable colloidal solutionas well as the smallest size distribution was obtained in acetone [7].

Tellurium nanoparticles (TeNPs) are extensively used in biomedicine, electronicsand some other industrial applications. Few microorganisms have been studied forthe production of TeNPs either under aerobic or anaerobic conditions [8].

The antioxidant, antimicrobial, and cytotoxicity of mycosynthesizedtellurium nanoparticles (TeNPs) were evaluated by using DPPH, microbrothdilution, and MTT tests, respectively. Overall, the biogenic nanostructured Teexhibited higher antioxidant and anticancer activity compared to potassiumtellurite. [9]

A clean and wet chemical method, employing sodium tellurite(Na2TeO3) as a source of tellurim and iron(II) as a reducing agent, has beendeveloped for the generation of tellurium nanoparticles in the size ranging from 25-80nm at room temperature [10].

Biogenic tellurium nanoparticles (TeNPs) weresuccessfully prepared using potassium tellurite (K_2TeO_3,$3H_2O$) via an eco-friendly and simple green approach by exploiting extracellular enzymes and biomolecules secreted from *Pencillium chrysogenum* PTCC 5031 at roomtemperature for the first time. It strongly suggested the P.chrysognum can be potential nano factory for the preparation of TeNPs due to several advantagesincluding non-pathogenic organism fast growth rate, and high capacity ofelements ions reduction, as well as facile and economical biomass handling [11].

Two highly tellurite-resistant bacteria were isolated from wastewater. Bothbacteria could perform tellurite reduction under an initial pH of 5–9, temperatureof 20–37 °C, and salinity conditions lower than 5%. Both bacteria produced tellurium nanorodsthat were accumulated intracellularly or extracellularly [12].

Extracellular synthesis of telluriumnanoparticles was investigated under optimized reaction conditions. Our resultsshow that the extracellular spherical-shaped tellurium nanoparticles with averagesize of 31 nm were formed in an optimal tellurite concentration of 1. 5 mM,optimal initial biomass concentration 40 g/l at the optimal pH 7. 5 and optimaltemperature of 30o C after 120 h of incubation under resting cell conditions. Thisis the first report on biosynthesis of tellurium nanoparticles by *brevibacillussp.strain* TR2211 [13].

TeO nanoparticles possessantimicrobial and biofilm eradication activity against Escherichiacoli JM109, Pseudomonas aeruginosa PAO1, and Staphylococcus aureus ATCC25923. In particular, SeO nanoparticles exhibited antimicrobial activity at quite low concentrations, below that of selenite [14].

TeNPs showed an important antibacterial activity against both Gram-negative and -positive bacteria in arange concentrations from 5 to 50 μg mL−1 over a 24-hour time period. Besides,nanoparticles showed an anticancer effect towards human melanoma cells over48 hours at concentrations up to 50 μg mL−1[15].

Nucleation ofTeNPs takes place over the entire cell growth period although the addition ofnew tellurium Te0 to pre-formed TeNPs is the main strategy used by R.capsulatus to generate TeNPs outside the cells [16].

Tellurium nanotubes have been synthesized via a hydrothermal redox route starting from Na2TeO3 andNa2SO3. X-ray diffraction reveals that the product is a pure trigonal phase andtransmission electron microscope indicates the widths of the nanotubes are in therange of 100–400 nm [17].

An efficient and practical synthesis of tellurim tetrachloride from elemental tellurium and sulfuryl chloride is described [18].Te nanotubes show p-typesemiconducting property with the field effect carrier mobility of approx.0.01 cm2/V s which is relatively lower than other 1D nanostructure. Lowmobility might be attributed to porous morphology with small grain size(<10 nm) [19].

A safe-by-design plug-and-play approach for continuous gas flowproduction of silver (or copper)-doped tellurium (Ag- or Cu–Te) nanoparticleswith safer antimicrobial activity. the use of ratios between biocompatibility andantimicrobial activity as safety indices (SIs) for evaluations of nanoparticleapplications. Approximately 6% atomic Ag in Ag–Te particles exhibited anoptimal SI and significantly reduced the minimum inhibitory concentration ofindividual Te nanoparticles [20].

Te(0) crystals occur internally within butmainly externally from the cells, and each microorganism forms a distinctlydifferent structure. Those formed by Bacillus selenitireducens initially arenanorods (~10-nm diameter by 200-nm length) [21]. TeNPs similarly affect violacein production and the P. aeruginosa biofilm structurebut at lower concentration levels. The results obtained suggest an importantdisruption of the QS signalling system by SeNPs and TeNPs, supporting nanotechnology as a promising tool to fight against the emerging problem ofbacterial resistance related to bacterial biofilm formation [22].

Mechanisms of bacterial killing include the production of reactive oxygen species, cation release,biomolecule damages, ATP depletion, and membrane interaction. Finally, acomprehensive analysis of the effects of NPs on the regulation of genes and proteins(transcriptomic and proteomic) profiles is reported [23].

Se- or Te-nanomaterials will explore the most used chemical and/orphysical methods exploited to generate different morphologies of metalloid-nanostructures, focusing also the attention on the major advantages, drawbacks aswell as the safety related to these synthetic procedures[24].

Controlled synthesis of crystalline tellurium nanorods, nanowires, nanobelts and related structures by a self-seeding solution process[25]. The local structure of Tellurium nanoparticles synthesized by arc plasma were studied by X-ray absorption spectroscopy.Nanoparticles were characterized by using different techniques to reveal particles size, morphology and composition. Te K-edge EXAFS spectra reveal a structure similar to trigonal Te, where both the inter-chain and intra-chain bonds, typical ofTe crystal structure, are reported [26].

A facile route to colloidal Te nanocrystals with binary uniform size distributions at room temperature. The binary-sized Te nanocrystals were well separated into two size regimes andassembled into films by electrophoretic deposition. The research provides a newplatform for nanomaterials to be efficiently synthesized and manipulated [27]. Tellurium (Te) is an appealing material for thermoelectric andmany other applications due to its layered structures and highly anisotropic nature [28].

Atomic Force Microscope (AFM) and Transmission Electron Microscope (TEM) analysis were used to characterize the size and size distributions of the metals NPs on the levels of tri-iodothyronine.[29, 30].

Metal nanoparticles have been known to be synthesized in glycerol within limited experimental conditions including high temperatures, alkaline pH conditions and the irradianceof ultraviolet light. Herein, we report that silver nanoparticles have been formed inglycerol under completely

green conditions (e.g., room temperature, neutral pHconditions and without irradiance of ultraviolet light) [31].

The synthesis of silver nanoparticles is very common due to their numerous applications in variousfields. Silver nanoparticles have unique properties such as: optical and catalytic properties, which, depend on the size and shape of the produced nanoparticles. So,today the production of silver nanoparticles with different shapes which havevarious uses in different fields such as medicine are noted by many researchers. Thisarticle, is an attempt to present an overview of the shape-controlled synthesis of silver nano particles using various methods [32].

Silver nanoparticles were synthesised using *clitoriaternatea*and *solanumnigrum*. Further investigation of the shape and size ofnanoparticle was done by X-ray diffraction and scanning electron microscopicstudies. A silver nanoparticle at different concentration was assessed for itsantibacterial effect against various nosocomial pathogens[33].

Green synthesis of silver nanoparticles (AgNPs) has gained much interest from chemists and researchers. In this concern, Indian flora has yet to divulge innumerable sources ofcost-effective non-hazardous reducing and stabilizing compounds utilized in preparing AgNPs. An efficient and sustainable route of AgNP preparation from 1 mM aqueous $AgNO_3$ using leaf extracts of three plants, *musa balbisiana* (banana), *azadira chtaindica* (neem) and *ocimum tenuiflorum* (blacktulsi), well adorned for their wide availability and medicinal property was reported[34].

A novel approach for the green synthesis of silver nanoparticles using aqueous leaves extracts of *catharanthus roseus* (C. roseus) Linn. G. Don which has been proven active against malaria parasite *plasmodium falciparum*(P.falciparum) [35].

Magnetic nanoparticles constitute an important class of inorganic nanoparticles, which find applications in different areas by virtue of their several unique properties. Nevertheless, in comparison with biological synthesis protocolsfor noble metal nanoparticles, limited study has been carried out with respect tobiological synthesis of magnetic nanoparticles[36].

Iron oxide nanoparticles (NPs) have attracted much consideration due to their unique properties, such as super paramagnetism, surface-to-volume ratio, greater surface area and easy separation methodology. Various physical, chemical and biological methods havebeen adopted to synthesize magnetic NPs with suitable surface chemistry [37].

Synthesis of nano particles by utilizing some of the green chemistry principles offersa viable and sustainable approach for nanotechnology. Iron nano particles (Fe NPs) were synthesised using Artocarpus heterophyllus (Jackfruit) peel extract. The peel with its high antioxidant content serves as a potential source of valuable biomolecules which act as the bio-reductants, capping and stabilizing agents for green synthesis of nanoparticles [38].

Iron nanoparticles with strong redox and adsorption abilities have been applied in a range of different fields including medicine, sensing, catalysis, optics,electronics and environmental remediation. Iron nanoparticles have been experimentally proven to effectively decrease the potential leachability of heavy metals via in situ immobilization and prevent transport of these heavy metals into deeper soil layers, rivers and ground water. Nanotechnology is currently a relatively mature remediation method for water pollution. However, soil complexity has limited using iron nanoparticles for soil remediation [39].

Gold nanoparticles (AuNPs) are important components for biomedical applications. AuNPs have been widely employed for diagnostics and have seen increasing use in the area of therapeutics. AuNPs highlight a selection of recent applications of these materials inbionanotechnology [40].

Gold nanoparticles demonstrate special advantages in this field due to their unique properties, small size and high surface area-to-volumeratio. These particles have been widely used in various biomedical applications and drug delivery systems due to their inert nature, stability, high dispersity, non-cytotoxicity and biocompatibility[41].

Glyco-gold nano particles combine in a single entity the peculiar properties of gold nanoparticles with the biological activity of carbohydrates. The result is an exciting nanosystem, able to mimic the natural multivalent presentation of saccharide moieties and to exploit the peculiaroptical properties of the metallic core. Recent advances on glyco-gold nanoparticle applications in different biological fields, highlightingthe key parameters which inspire the glyco nanoparticle design[42].

The AuNPs have been successfully used in bioelectrochemistry and found to efficiently enhance interfacial electrochemical electron transfer of the metalloprotein yeast cytochrome c in homogeneous solution. The synthesis has been extended successfully to direct use of starch-rich foods such as potato, carrotand onion to synthesize AuNPs [43].

Platinum nanoparticles (PtNPs) are widely used for biomedical applications, including imaging, implants, photothermal therapy and drug delivery. Indeed, PtNPs possesses intrinsic antimicrobial, antioxidant, and anticancer properties. Also, due to their remarkable catalytic

activity, they are able to reduce the intracellular reactive oxygen species (ROS) levels and impair the downstream pathways leading to inflammation. Various approaches including both physical and chemical methods are currently employed for synthesis of PtNPs [44].

Platinum (Pt) nanoparticles have been synthesized froma precursor solution of potassium tetrachloroplatinate (K_2PtCl_4) using a matrix of bacterial cellulose (BC). The formation of Pt nanoparticles occurs at the surface and the inside of the BC membrane by reducing the precursor solution with a hydrogen gas reductant [45]. Platinum (Pt) nanoparticles were synthesized by pulsed laser ablation in liquid (PLAL) technique in different liquids (acetone,ethanol, and methanol). Ablation was performed using a Q-switched Nd:YAG laser with output energy of 230 mJ/pulse for 532 nm wavelength[46].

Se nanoparticles exhibited dose-dependent antibacterial activity against all the four bacterial strains tested. Noticeably, PVA-SeNPs exhibited significant effect against S. epidermidis (MIC 125 ppm) and S. aureus (MIC 125ppm). Se nanoparticles can be potentially used as antimicrobial and antioxidant agents [47]. The cytotoxic studies clearly determined that PF-SeNPs was much less toxic and safer related to sodiumselenite. PF-SeNPs could find suitable application as antioxidant and antimicrobial agent in food, biomedical and pharmaceutical industry[48].

Selenium nanoparticles have the potential to be used for various applications. Green synthesis using plant extracts has gained popularity because it requires non-toxic solvents and moderate temperatures [49]. Selenium (Se) is an essential element to human health that can be obtained in nature through several sources. In the human body, it is incorporated into seleno cysteine, an amino acid used to synthesize several seleno proteins which have an active center usually dependent on the presence of Se [50].

2.2 SCOPE OF THE WORK

Chemical method of synthesizing nanoparticles and green method of synthesizing nanoparticles were widely studied by the researchers. Among this , Green method of synthesis is very important due to its advantage in controlling particle size, morphology very effectively. This method also involve less time consuming process for getting the desired nanoparticle size. Hence for this investigation ,Green method is chosen to synthesize nanoparticles.

Tellurium is mainly used for various applications. This work innovation gives importance for reducing the hazardous nature. In this investigation, we have synthesized Te nanoparticles from Tellurium chloride. It was then characterized by UV-visible spectroscopy , IR spectroscopy ,AFM ,Scanning Electron spectroscopy to confirm the nature of the particles. The application of synthesized nanoparticles were studied . Anti-Bacterial, Anti-Fungal activities, DPPH scavenging effect and Hydrogen Peroxide scavenging effect shows the excellent application of Te nanoparticles.

2.3 OBJECTIVES OF THE WORK

The main objectives of this investigation are

1. To synthesize nanoparticles through Eco-Friendly Method.

2. To utilize sea shells for synthesizing Nanoparticles.

3. To evaluate the presence of Metal Nanoparticles through various techniques such as UV-visible spectroscopy, FTIR, AFM and SEM.

4. To study the surface morphology of nanoparticle.

5. To study the Applications of Nanoparticles.

6. To study Anti-Bacterial, Anti-Fungal and Anti-Aging Applications.

CHAPTER III

MATERIALS & METHODS

MATERIALS ANDMETHODS

A brief outline of the materials and methods used in this project work is present here.

3.1 MATERIALS REQUIRED FOR SYNTHESIS

1. *Conus betulinus*

2. Double distilled water

3.Tellurium chloride and centrifuge tube.

3.2 PROCEDURE

Synthesis of Tellurium nanoparticles is carried out using 0.01M Tellurium chloride in double-distilled water using *Conus betulinus*. Tellurium chloride and *Conus betulinus* were mixed together in a ratio of (9:1, 8:2, 7:3, 6:4, and 5:5). In this different ratio concentration ,a 5:5 ratio concentration was selected for the bulk preparation because it shows a higher production than other ratios stirred at 800 rpm using a magnetic stirrer. The mixture turned into milk-white color within 1 hr. The whole reaction was carried out in the dark. The obtained suspension was centrifuged at 15,000 rpm for 15 min. The pellet containing tellurium nanoparticles was washed 3–4 times with deionized water to remove impurities. The precipitated nanoparticles were lyophilized. Lyophilized nanoparticles were stored in a cool, dry and dark place and further characterization was carried out.

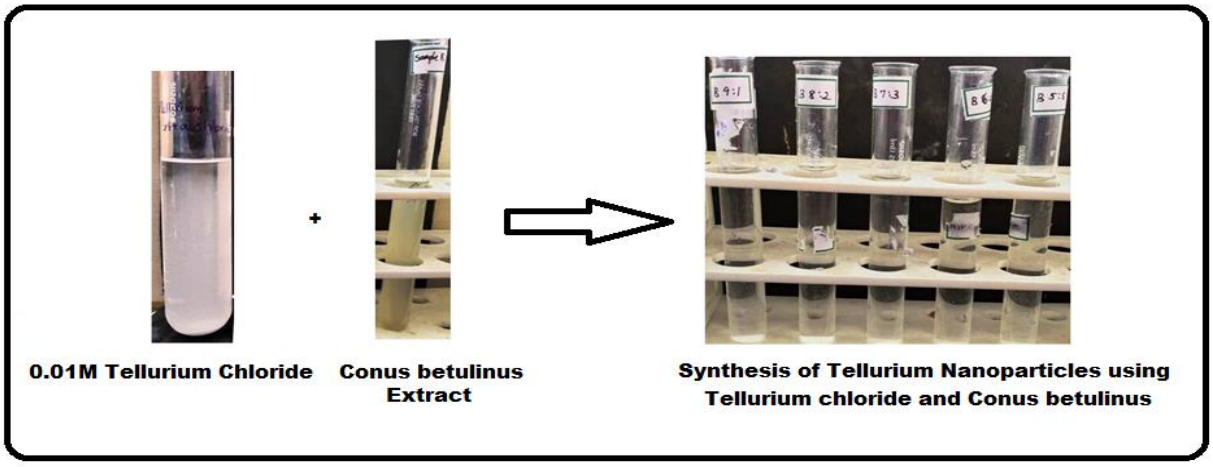


Figure 3.1 Synthesis of Tellurium nanoparticles

Figure 3.2 Synthesized Tellurium nanoparticles

3.3 METHODS

3.3.1 UV – SPECTROSCOPY

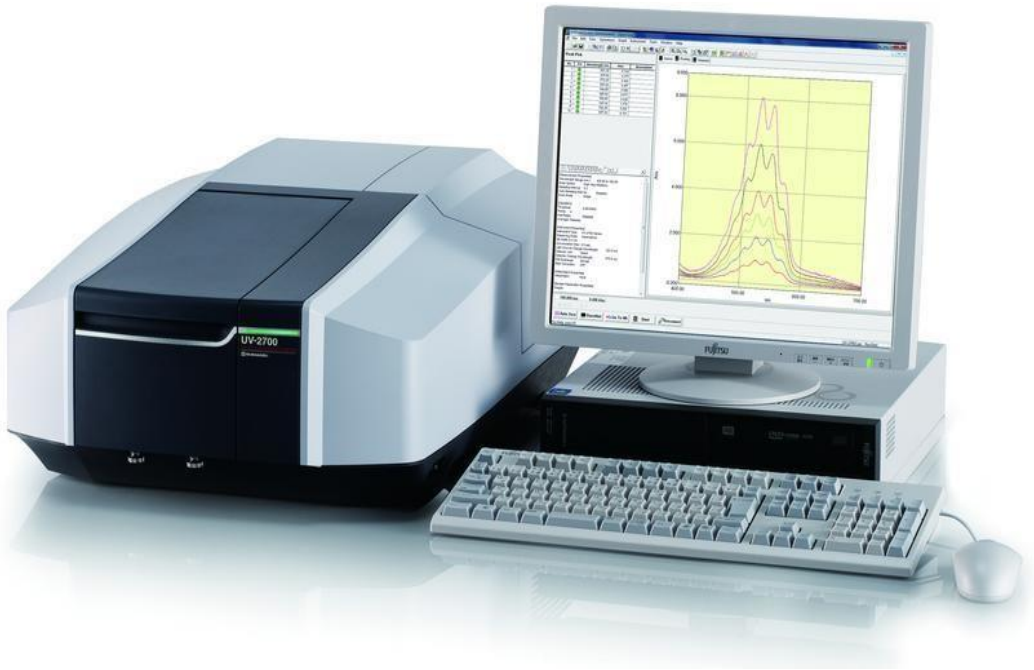


Figure 3.3 UV-VIS SPECTROMETER (UV-VIS)

Ultraviolet–visible spectroscopy (UV) refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full, adjacent visible spectral regions. Molecules containing bonding and non-bonding electrons (n-electrons) can absorb energy in the form of ultraviolet or visible light to excite these electrons to higher antibonding molecular orbitals.

3.3.2 INFRARED SPECTROSCOPY

An infrared spectrophotometer is an instrument that passes infrared light through an organic molecule and produces a spectrum that contains a plot of the amount of light transmitted on the vertical axis against the wavelength of infrared radiation on the horizontal axis.

Figure 3.4 INFRARED SPECTROSCOPY

3.3.3 ATOMIC FORCE MICROSCOPY (AFM)

Figure 3.5ATOMIC FORCE MICROSCOPY (AFM)

The AFM works much the same way a profilometer works only on a much, smaller scale: a very sharp tip is dragged across a sample surface and the change in the vertical position (denoted the "z" axis) reflects the topography of the surface. By collecting the height data for a succession of lines it is possible to form a three dimensional map of the surface features.

The AFM has three major abilities force measurement, imaging, and manipulation. The Atomic force microscopy analysis using the Nanosurf easy2 scanBT02218 is profilometer.

3.3.4 SCANNING ELECTRON MICROSCOPY

Figure 3.6 SCANNING ELECTRON MICROSCOPY

A typical SEM instrument, showing the electron column, sample chamber, EDS detector, electronics console, and visual display monitors. The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens.

CHAPTER IV

RESULTS & DISSCUSSON

4.1Characterisation of Tellurium nanoparticles

4.1.1 Ultraviolet Spectroscopy

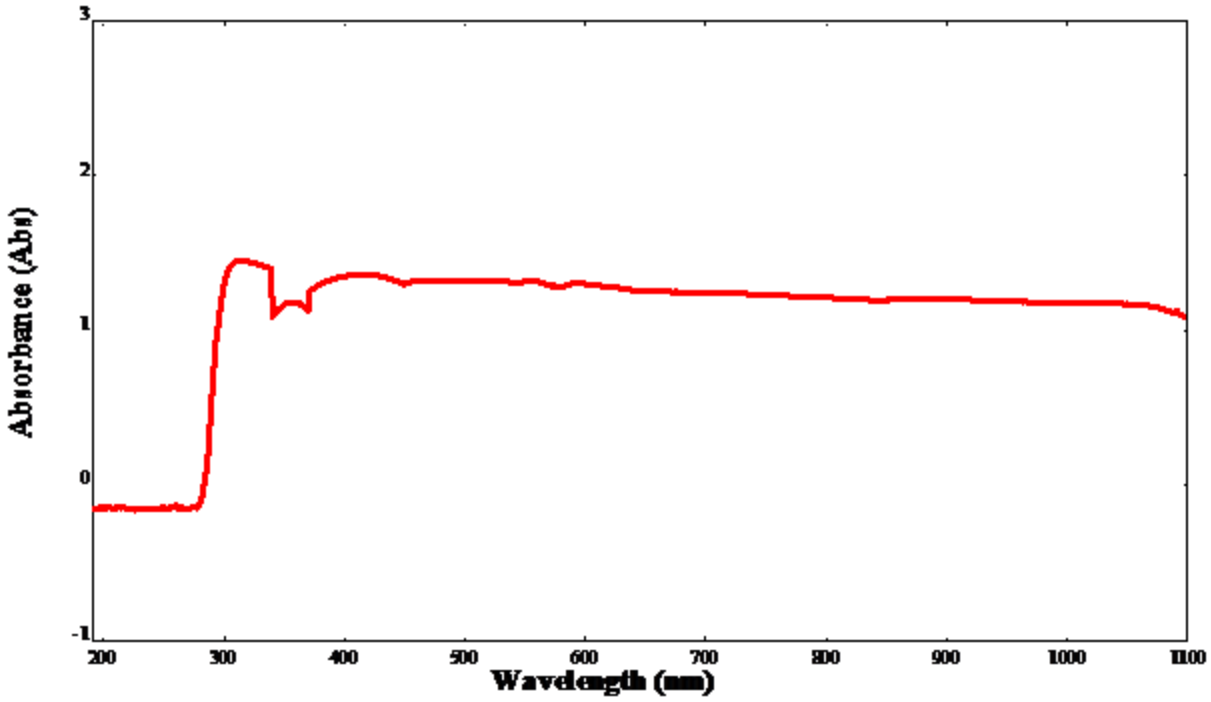


Figure 4.1 UV-VISBLE SPECTRA OF TELLURIUM NANOPARTICLES USING *Conus betulinus*

The optical absorbance properties of the synthesized Tellurium Nanoparticles were investigated using extract of *Conus betulinus* in the wavelength range 200-1100 nm at ambient temperature. Fig.4.1 indicates the UV –Visible diffuse reflectance spectra of Tellurium Nanoparticles using extract of *conus betulinus* . There is a strong absorption peak around 300nm which confirms the presence of Te nanoparticles.

4.2INFRARED SPECTROSCOPY

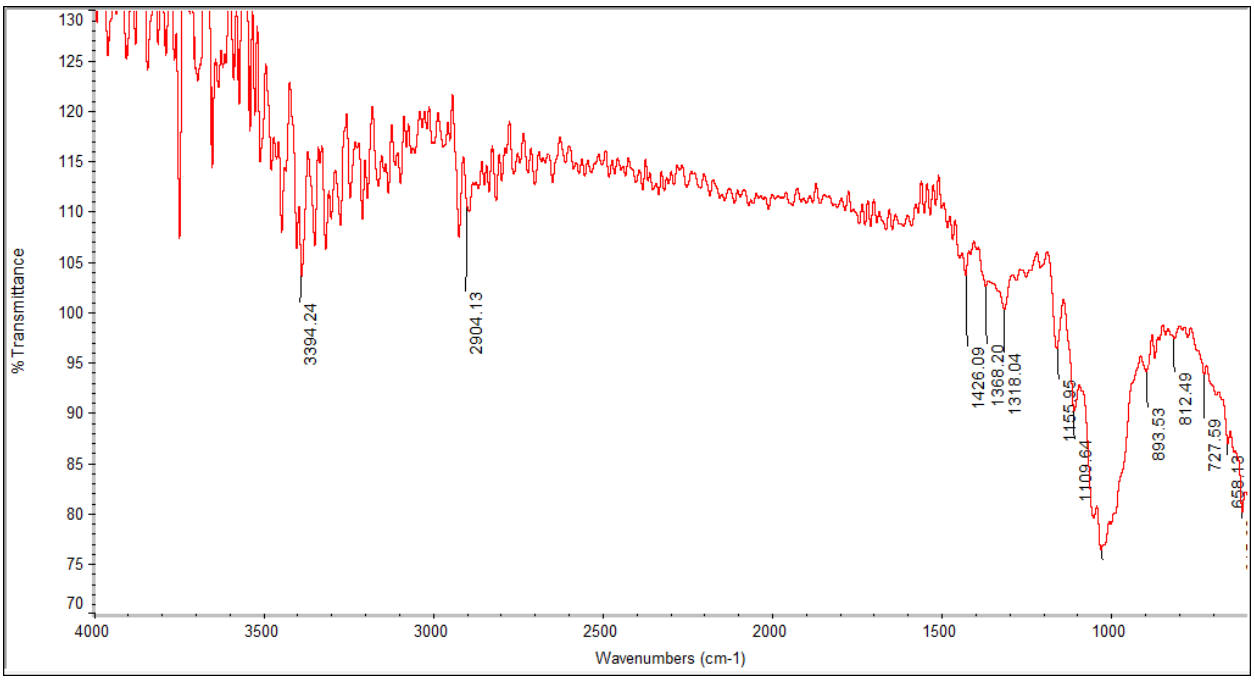


Figure 4.2 INFRAREDSPECTRA OF TELLURIUM NANOPARTICLES USING *Conu sbetulinus*

An infrared Spectroscopy image of *conus betulinus* is recorded. The image shows a strong absorption peak around 3324cm^{-1} to 2904cm^{-1} which shows the presence of C-H stretching vibration. A peak around 800cm^{-1} to 1100 cm^{-1} shows the presence of C-O stretching frequency. A peak around finger print region confirms the presence of Te Nanoparticles.

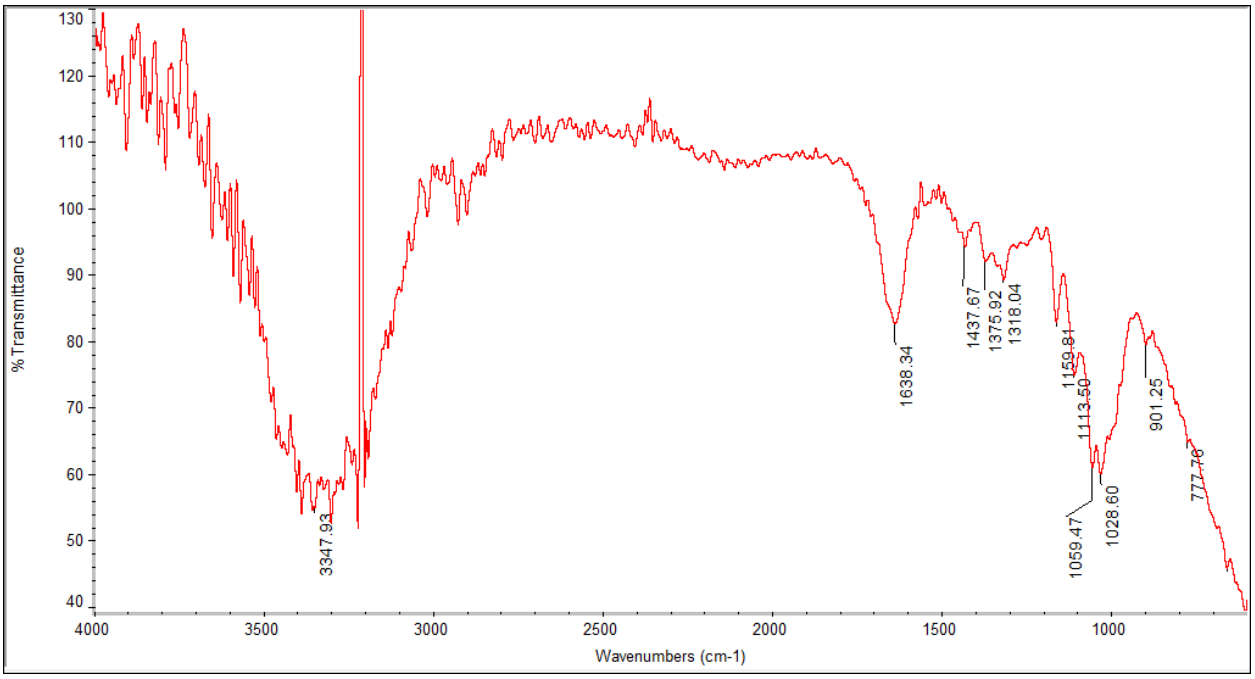


FIGURE 4.3 INFRARED SPECTRA IMAGES OF *Conus betulinus*

An infrared Spectroscopy image of *Conus betulinus* is recorded. The image shows a strong absorption peak around 3324cm^{-1} which shows the presence of N-H stretching frequencies, 1638 cm^{-1} due to CO stretching frequency and peak around 700cm^{-1} to 1100 cm^{1}due to C-O stretching frequency. IR Spectra shows the presence of biogenic reducing agents.

4.3 ATOMIC FORCE MICROSCOPY

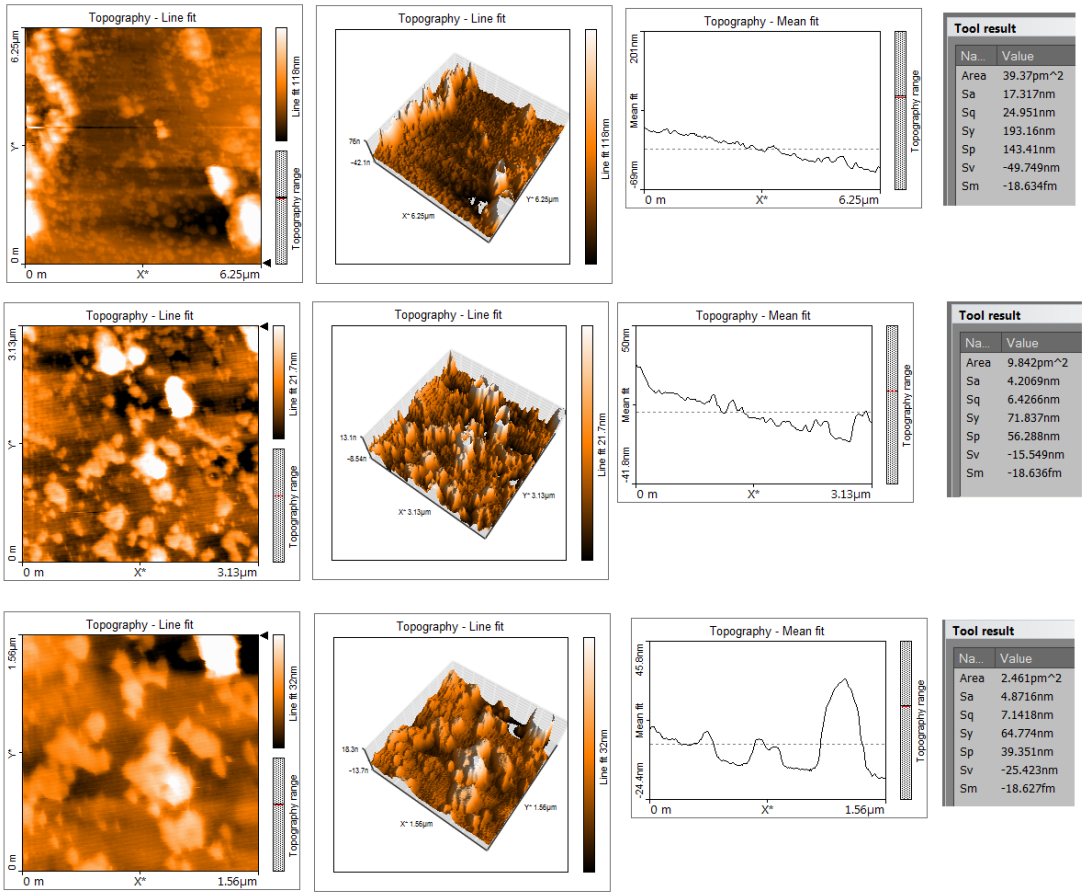


FIGURE 4.4 AFM IMAGES OF Te NANOPARTICLES USING *Conus betulinus*

AFM technique is the one of the best tools for measuring nano sized materials. This method analysis the particle surface using Tip, it is so high-pitched that as it is moved across something, the tip can feel the shape by measuring the forces between the atoms on the tip and the atoms on the object. An AFM topographical image of Te nanoparticles is shown in fig.4.4 which shows the agglomerated rock like structure. The average length of the rock structure is in 21.7 nm. It may be due to the Metal bindings.

4.4 SCANNING ELECTRON MICROSCOPY

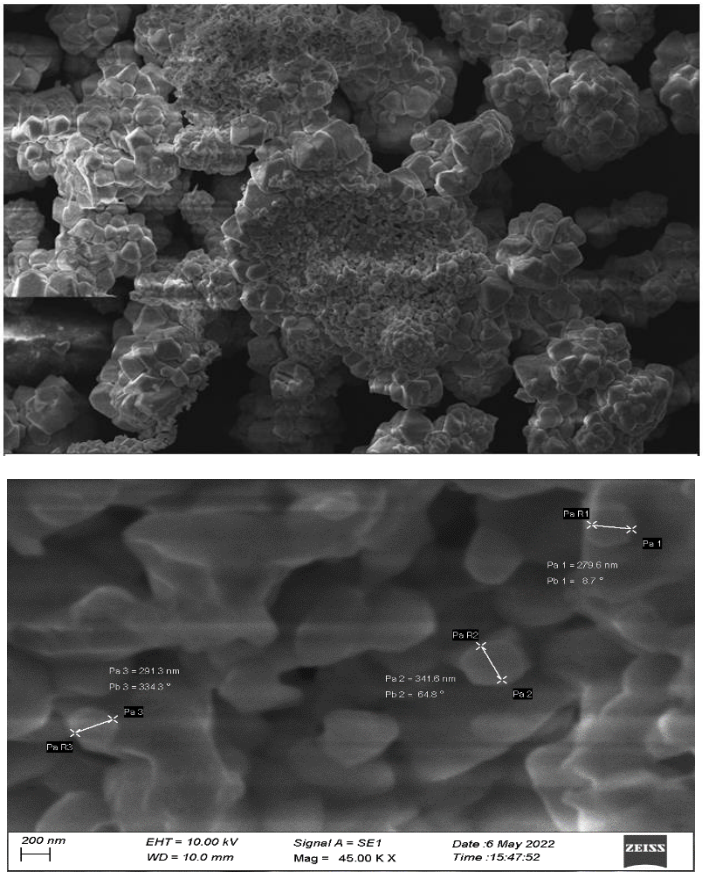


FIGURE 4.5 SEM SPECTRA OF Te NANOPARTICLES USING *Conus betulinus*

Scanning Electron Microscopy is one of the best technique to characterize the nanostructures. Electron –sample interactions reveal surface morphology of the synthesized nano particle images of Tellurium Nanoparticles is recorded. The image shows the presence of Tellurium Nanoparticles around 200nm.

4.5 APPLICATIONS OF TELLURIUM NANOPARTICLES

4.5.1 ANTI BACTERIAL ACTIVITY

4.5.1.1 AGAR WELL DIFFUSION METHOD

PRINCIPLE

The antimicrobials present in the given sample was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

MATERIALS REQUIRED

*(P. acnes and S. oralis)*was purchased from MTCC, Chandihar, India. Nutrient Agar medium, Nutrient broth, Gentamicin antibiotic solution was purchased from Himedia, India. Test samples, petri-plates, test tubes, beakers conical flasks were from Borosil, India. Spirit lamp, double distilled water.

AGAR- WELL DIFFUSION METHOD

a. Nutrient Agar Medium

The medium was prepared by dissolving 2.8 g of the commercially available Nutrient Agar Medium (HiMedia) in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

b. Nutrient broth

Nutrient broth was prepared by dissolving 2.8 g of commercially available nutrient medium (HiMedia) in 100ml distilled water and boiled to dissolve the medium completely. The

medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

PROCEDURE

Petri plates containing 20 ml nutrient agar medium were seeded with 24hr culture of bacterial strains *(P. acnes and S. oralis)* Wells were cut and different concentration of samples A,B and C (500µg/ml, 250µg/ml,100µg/ml and 50µg/ml) were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Gentamicin antibiotic was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA).[51]

4.6 RESULTS

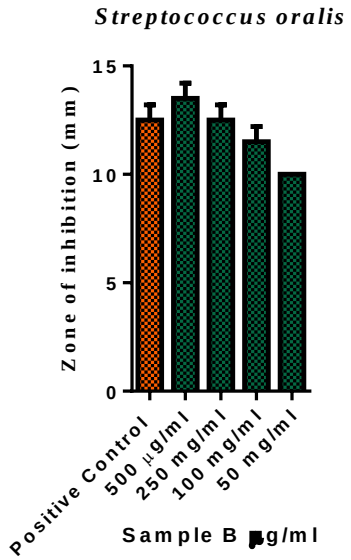
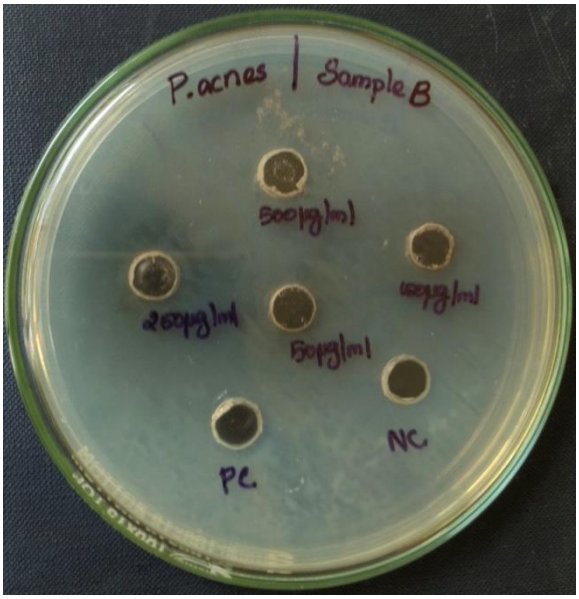


Figure 4.6 ANTIBACTERIAL ACTIVITY OF Te NANOPARTICLES AGAINST *Streptococcus oralis.*



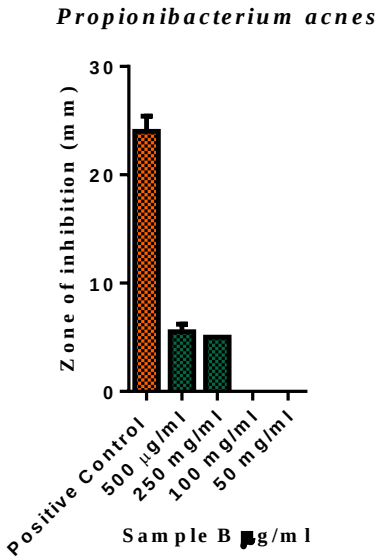


Figure 4.7 ANTIBACTERIAL ACTIVITY OF Te NANOPARTICLES AGAINST *Propionibacterium acnes*

Table 4.1 SD± Means of zone of inhibition obtained byTe Nanoparticles against *(P. acnes and S. oralis.).*

S.NO	NAME OF THE TEST ORGANISM	ZONE OF INHIBITION (mm) SD ± MEAN				
		PC	500 µg/µl	250 µg/µl	100 µg/µl	50 µg/µl
1.	*Streptococcus oralis*	24±1.4	6±0	5±0	0	0
2.	*Propionibacterium acnes*	12.5±0.7	13.5±0.7	12.5±0.7	11.5±0.7	10±0

4.7 ANTI FUNGAL ACTIVITY

4.7.1 AGAR WELL DIFFUSION METHOD

PRINCIPLE

The anti-fungal agent present in the given sample was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

MATERIALS REQUIRED

Potato dextrose agar medium, Amphotericin B antimycotic solution, test samples, test tubes, beakers conical flask, spirit lamp, double distilled water and petri-plates.

AGAR- WELL DIFFUSION METHOD

Potato Dextrose Agar Medium

The potato dextrose agar medium was prepared by dissolving 20 gm of potato influsion, 2 gm of dextrose and 1.5 gm of agar in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petri plates (25-30 ml/plate) while still molten.

PROCEDURE

Petri plates containing 20ml potato dextrose agar medium was seeded with 72 hr culture of fungal strain*(Cryptococcus neoformans and Aspergillusfumigatus)* wells were cut and different concentration of Tellurium nanoparticle (500, 250, 100 and 50 μg/ml) was added. The plates were then incubated at 28°C for 72 hours. The anti-fungal activity was assayed by measuring the

diameter of the inhibition zone formed around the wells. Amphotericin B was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA).

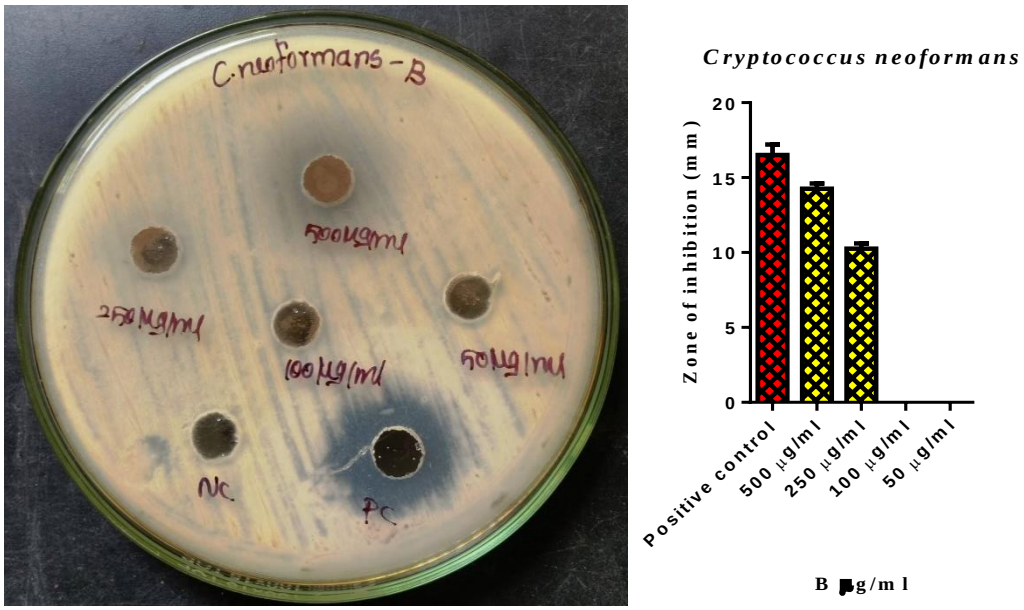
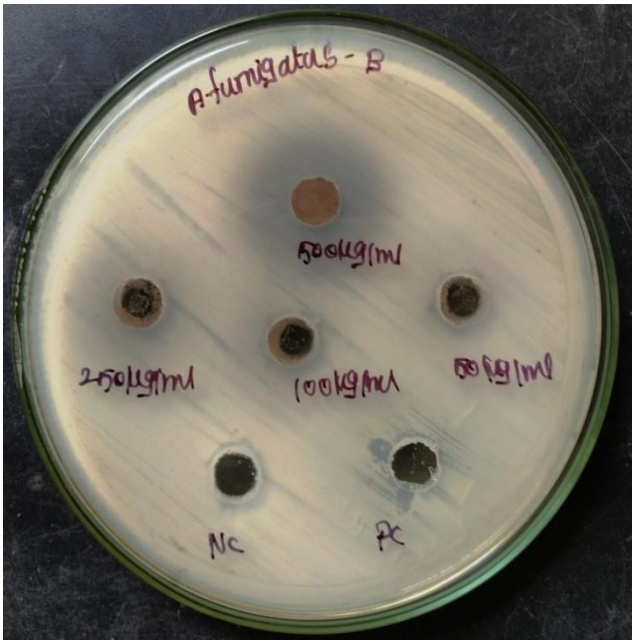


Figure 4.8 EFFECT OF Te NANO PARTICLES AGAINST *Cryptococcus neoformans.*



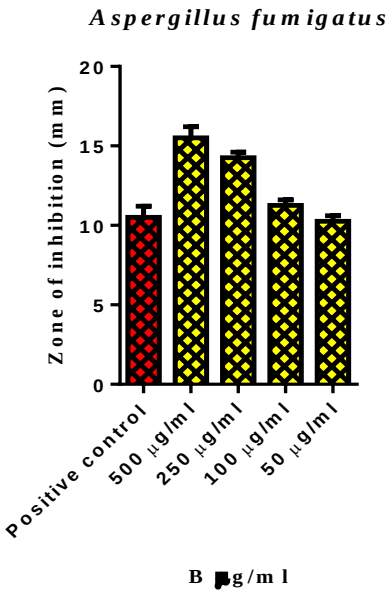


Figure 4.9 EFFECT OF Te NANOPARTICLES AGAINST *Aspergillus fumigatus.*

Table 4.2 Zone of inhibition of Te nanoparticles against *Cryptococcus neoformans and Aspergillusfumigatus*

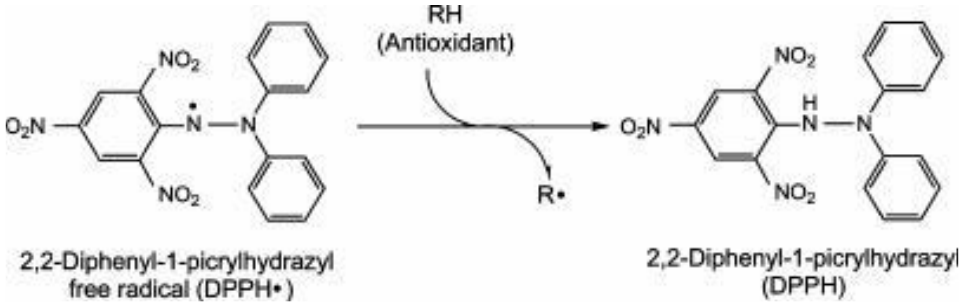
S.NO	Name of the test organism	Zone of inhibition (mm) SD ± Mean				
		500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	*Cryptococcus neoformans.*	14.25±0.35	10.25±0.35	0	0	16.5±0.7
2.	*Aspergillus fumigatus*	15.5±0.7	14.25±0.35	11.25±0.35	10.25±0.35	10.5±0.7

*Significance - p< 0.05

The inhibitory effect of Tellurium nano particles was studied against *Cryptococcus neoformans, Aspergillus fumigates* at various concentrations. Using Potato dextrose agar medium, the antifungal activity of Tellurium nanoparticles was studied. The results show that at 500 µg/ml, a high inhibitory activity was observed. Pathogens growth can be inhibited. Hence it can be used as excellent antifungal agent.[52]

4.8 DPPH Radical scavenging activity

The DPPH assay is popular in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers. Figure below, shows the mechanism by which DPPH accepts hydrogen from an antioxidant. DPPH is one of the few stable and commercially available organic nitrogen radicals (1). The antioxidant effect is proportional to the disappearance of DPPH in test samples. Monitoring DPPH with a UV spectrometer has become the most commonly used method because of its simplicity and accuracy. DPPH shows a strong absorption maximum at 517 nm (purple). The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant. This reaction is stoichiometric with respect to the number of hydrogen atoms absorbed. Therefore, the antioxidant effect can be easily evaluated by following the decrease of UV absorption at 517 nm.



MATERIALS REQUIRED

0.1mM DPPH solution, Ascorbic acid, Methanol

0.1 mM DPPH Solution

Dissolve 39 mg of DPPH in 100 ml of methanol and store at -20^{o} C until needed.

Ascorbic acid (Standard)

1mg/ ml of Ascorbic acid

Procedure

1. Briefly, prepare 0.1 mM of DPPH solution in methanol and add 100 µl of this solution to 300 µl of the solution of sample B at different concentration (500, 250, 100, 50 and 10 µg/mL).
2. The mixtures have to be shaken vigorously and allowed to stand at room temperature for 30 minutes.
3. Then the absorbance has to be measured at 517 nm using a UV-VIS spectrophotometer. (Ascorbic acid can be used as the reference).
4. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity.
5. The capability of scavenging the DPPH radical can be calculated by using the following formula.

6. DPPH scavenging effect (% inhibition) = [(absorbance of control- absorbance of reaction mixture)/absorbance of control] X 100

OD Value at 517 nm

Control Mean OD value: 1.31

TABLE 4.3 ABSORBANCE OF Te NANOPARTICLES AT VARIOUS CONCENTRATION BY DPPH SCAVENGING EFFECT

S. No	Tested sample concentration (µg/ml)	OD Value at 517 nm (in triplicates)		
1.	Control	1.23	1.37	1.335
2.	500 µg/ml	0.408	0.433	0.443
3.	250 µg/ml	0.449	0.453	0.45
4.	100 µg/ml	0.49	0.474	0.482
5.	50 µg/ml	0.483	0.489	0.492
6.	10 µg/ml	0.497	0.55	0.522
7.	Ascorbic acid	0.18	0.121	0.12

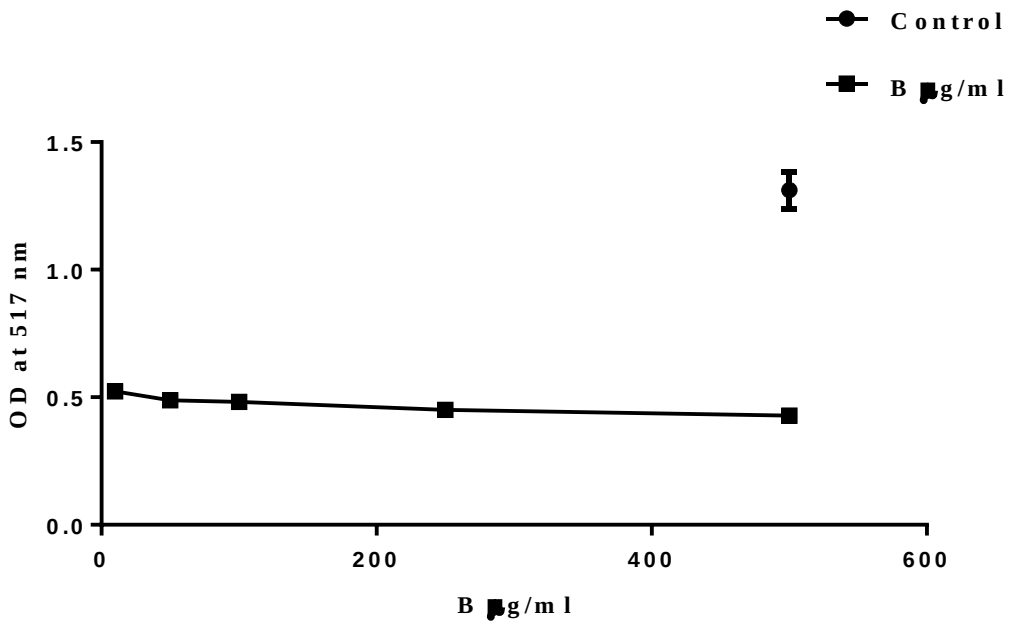


Figure 4.10 CONCENTRATION VS OD 517nm

TABLE 4.4 Percentage of Inhibition of synthesized Te Nanoparticles

S. No	Tested sample concentration (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
1.	Ascorbic acid	86.25	90.76	90.83	89.28
2.	500 µg/ml	68.85	66.94	66.18	67.32
3.	250 µg/ml	65.72	65.41	65.64	65.59
4.	100 µg/ml	62.59	63.81	63.21	63.21
5.	50 µg/ml	63.12	62.67	62.44	62.74
6.	10 µg/ml	62.06	58.01	60.15	60.07

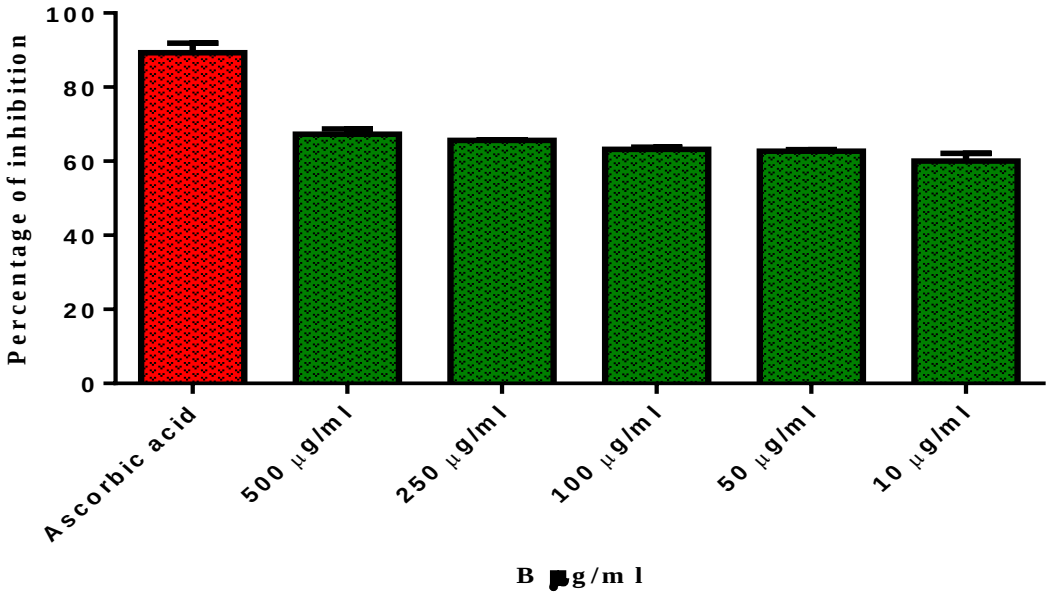


FIGURE 4.11 CONCENTRATION VERSUS PERCENTAGE OF INHIBITION

TABLE 4.5 IC50 VALUE OF TESTED SAMPLE: 97.55 µg/ml

log(inhibitor) vs. normalized response -- Variable slope		
Best-fit values		
LogIC50		1.989
HillSlope		-1.395
IC50		97.55
Std. Error		
LogIC50		0.07571
HillSlope		0.3445
95% Confidence Intervals		
LogIC50		1.826 to 2.153
HillSlope		-2.139 to -0.6505
IC50		66.94 to 142.1
Goodness of Fit		
Degrees of Freedom		13
R square		0.8411
Absolute Sum of Squares		3204
Sy.x		15.70
Number of points		
Analyzed	3	15

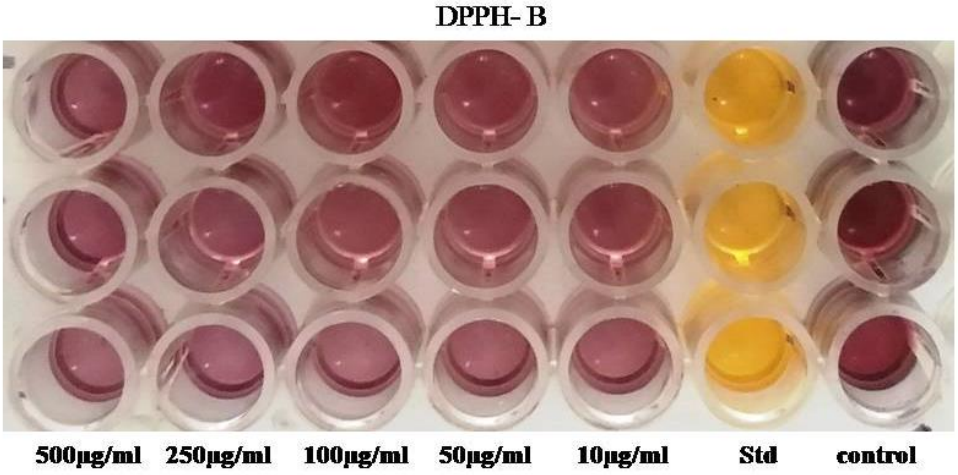


FIGURE 4.12 HYDROGEN PEROXIDE SCAVENGING ASSAY

The scavenging effect of Tellurium nanoparticles was studied by measuring the absorbance. The results show that at 500 µg/ml the percentage of inhibition is good. This shows

that the free radicals exist in our body will get scavenge using this Te nanoparticles. Hence it finds its application in anti-aging property. At higher concentration, excellent inhibition property is observed. The inhibitory concentration at 50% is found to be 97.55 µg/ml

4.9 HYDROGEN PEROXIDE SCAVENGING ASSAY

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate.

Material Required

Hydrogen peroxide solution and Sodium phosphate buffer.

Procedure

Ability of plant extracts to scavenge hydrogen peroxide was estimated according to the method reported by Ruch et al. with minor modification. A solution of hydrogen peroxide (43 mM) is prepared in phosphate buffer (1 M pH 7.4). Different concentration of tellurium nanoparticles (500, 250, 100, 50 and 10 µg/ml) was added to a hydrogen peroxide solution (0.6 ml, 43 mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as standard. The free radical scavenging activity was determined by evaluating % inhibition as above.

% inhibition = [(Control- Test)/control] ×100.

OD Value at 230 nm

Control Mean OD value: 1.214

TABLE 4.6 ABSORBANCE OF Te NANOPARTICLES AT VARIOUS CONCENTRATION BY DPPH SCAVENGING EFFECT

S. No	Tested sample concentration (µg/ml)	OD Value at 230 nm (in triplicates)		
1.	Control	1.53	1.104	1.008
2.	500 µg/ml	0.095	0.093	0.103
3.	250µg/ml	0.104	0.1	0.107
4.	100µg/ml	0.109	0.104	0.121
5.	50µg/ml	0.135	0.144	0.147
6.	10µg/ml	0.186	0.178	0.167
7.	Ascorbic acid	0.119	0.141	0.106

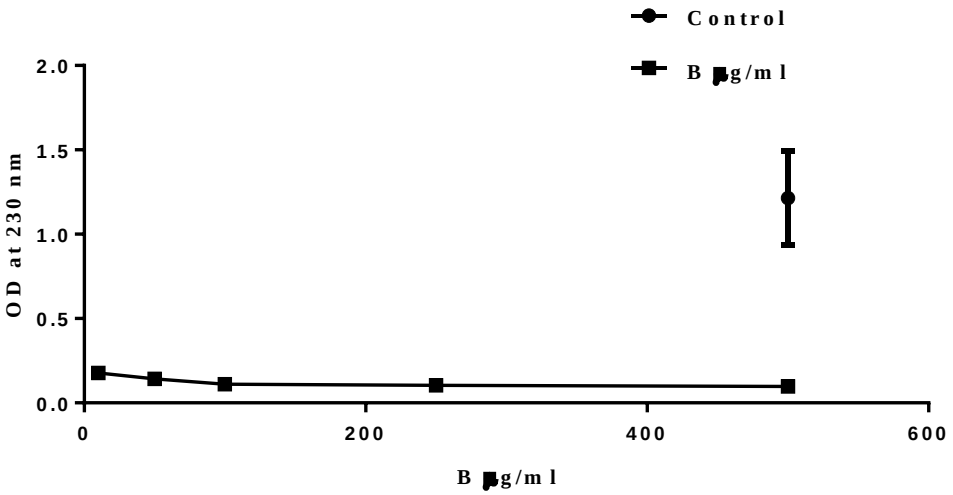


FIGURE 4.12PERCENTAGE OF INHIBITION

TABLE 4.7 PERCENTAGE OF INHIBITION

S. No	Tested sample concentration (µg/ml)	Percentage of inhibition (in triplicates)			Mean value
1	Ascorbic acid	90.19	88.38	91.26	89.95
2	500 µg/ml	92.17	92.33	91.51	92
3	250 µg/ml	91.43	91.76	91.18	91.46
4	100 µg/ml	91.02	91.43	90.03	90.82
5	50 µg/ml	88.87	88.13	87.89	88.30
6	10 µg/ml	84.67	85.33	86.24	85.42

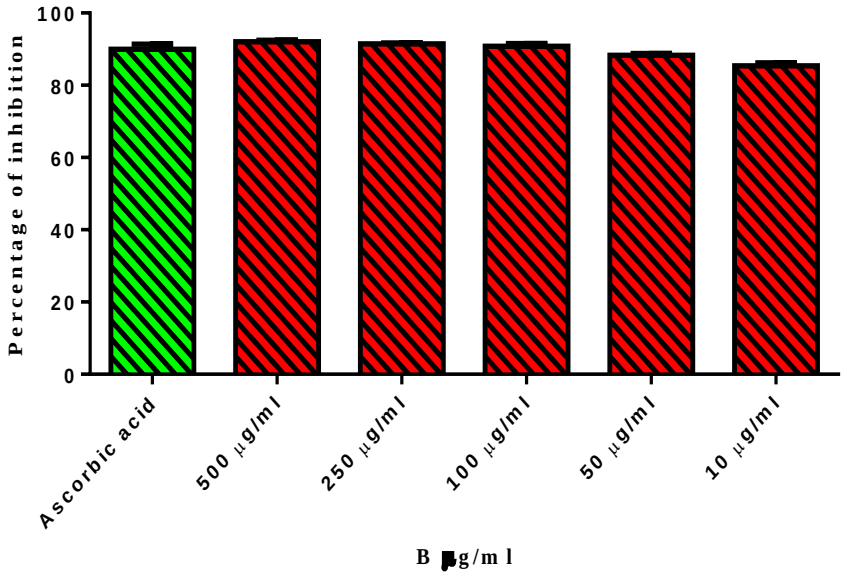


Figure 4.13 CONCENTRATION VERSUS PERCENTAGE OF INHIBITION

TABLE 4.8IC50 Value of tested sample: 55.22 µg/ml

log(inhibitor) vs. normalized response -- Variable slope		
Best-fit values		
LogIC50		1.742
HillSlope		-2.375
IC50		55.22
Std. Error		
LogIC50		0.03093
HillSlope		0.4567
95% Confidence Intervals		
LogIC50		1.675 to 1.809
HillSlope		-3.361 to -1.388
IC50		47.34 to 64.40
Goodness of Fit		
Degrees of Freedom		13
R square		0.9588
Absolute Sum of Squares		884.8
Sy.x		8.250
Number of points		
Analyzed	3	15

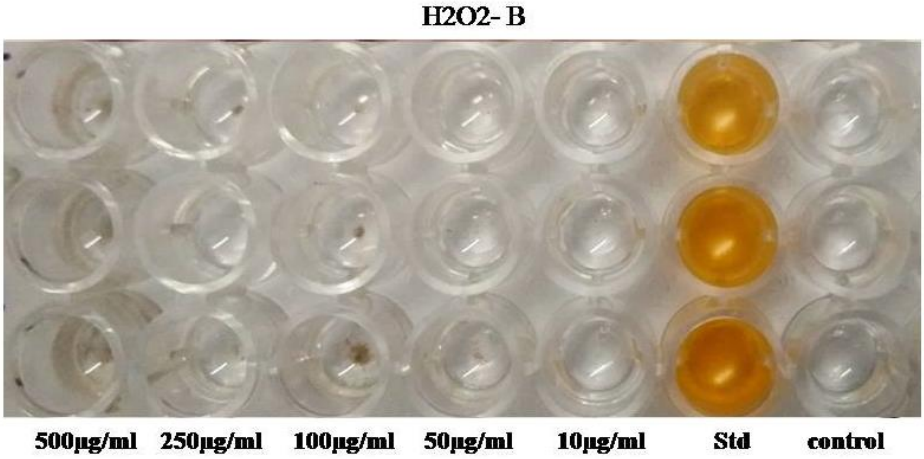


FIGURE 4.14 SCAVENGING EFFECT OF H_2O_2

Inhibitory concentration at 50 percentage was found to be 55.22 µg/ml. Dose dependent increase in the antibacterial activity was observed. At higher concentration (500 µg/ml), 92% excellent inhibition activity was observed [53].

CHAPTER V

CONCLUSIONS & REFERENCES

CONCLUSION

Researchers are keen to introduce nanoparticles in every field of life , Since it has various applications. Various methods such as physical, chemical and Biological methods are available to synthesis Nanoparticles. Green synthesis of Nanoparticles is an important method to synthesis, Since it is highly efficient for the Fabrication of Nanoparticles at Nanoscale without affecting our Environment.

By Eco-Friendly Method, using conus betulinus Te Nanoparticles can be synthesized. This method of Biogenically synthesized Te Nanoparticles is characterized using UV-visible Spectra, IR Spectra. Also, the surface morphology of the Nanoparticles is confirmed by SEM and AFM techniques.

The Extract of conus betulinus is successfully employed for the preparation of Te Nanoparticles. The nanoparticles synthesized shows good absorbance around 300 nm. The presence of Bio-active agents in the extract was confirmed from FTIR studies. The results of Atomic Absorption spectra showed the formation of Nanoparticles. Also, SEM images confirms the Flaky Nanoparticles.

Anti-Bacterial and Anti-Fungal applications showed on Excellent percentage of inhibition at higher concentration. Hence this Te Nanoparticles is used for Anti-Bacterial and Anti-Fungal application.

The scavenging effect of DPPH and Hydrogen Peroxide showed that the synthesized Nanoparticles can be employed for Anti-Aging application.

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Assessment of Soil Quality Parameters of Agricultural farmlands in Thoothukudi and Ramnad Districts using Digital Mini Soil Lab

Project in Chemistry

Submitted to St. Mary's college(Autonomous) in partial fulfillment for the award of the Degree of Bachelor of Science in Chemistry

DONE BY

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RE-ACCREDITED WITH "A^{+}" GRADE BY NAAC

THOOTHUKUDI-628001

2020-2021

DECLARATION

We hereby declare that the project entitled **"Assessment of Soil Quality Parameters of Agricultural farmlands in Thoothukudi and Ramnad Districts using Digital Mini Soil Lab"** submitted to St.Mary's college(Autonomous), Thoothukudi, affliated to Manonmaniam Sundaranar University, for the degree of Bachelor of Science is our original work and that, it has not previously formed on the basis of the award of any degree or similar title.

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May 2022
THOOTHUKUDI

CERTIFICATE

This is certify that project in chemistry **"Assessment of Soil Quality Parameters of Agricultural farmlands in Thoothukudi and Ramnad Districts using Digital Mini Soil Lab"** submitted to St.Mary's college (Autonomous) , Thoothukudi in partial fulfilment for the award of the degree of Bachelor of Science in Chemistry and is a record of the work done by following students during the year 2021-2022.

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CHAPTER-I

INTRODUCTION

Soil is a vital natural source and, at the same time, has an economic and eco-social potential.It allows the production of food and raw materials, recycles waste, creates forest agricultural land, filters and retains water, allows the usage and valorisation of sun energy, ensures the cycle and balance of substances in nature, maintains diversity of plant and animal species. It primarily shapes the quality of the environment; it is the resource and cultural heritage of the Earth; it ensures the life and social being of the population. Agricultural activities realised in landscape affect natural resources. A rational usage of renewable and non-renewable resources which are not retrieved in real time is an essential precondition. The farming system is the most widespread environmental technology with its positive and negative consequences. It utilises essential natural resources and, at the same time, influences other natural environments. Therefore, ecologisation of farming is a priority of farmers as well as environmentalists. Respecting the principles of soil sustainability and other components of environment is the basic precondition for life sustainability.

Sustainable agriculture is based on the principle of agriculture being a biological process which, in practice, should imitate key characteristics of the natural ecosystem. It strives to bring diversity into agro-ecosystem, recycle nutrients efficiently and maintain the priority of sunlight as a source of energy for agro-ecosystems. Specific manifestations of soil require different approaches. In soil protection, these must be ecological (biological) approaches, as this is the only way to achieve sustainable development of ground cover and the resulting economical and social development and environmental balance in society. Sustainable use of soil takes soil-ecological conditions into consideration and is realised in such a way and in such intensity, which gives rise to neither negative changes in soil, nor establishes trends for the

development of negative characteristics in soil. The essential principle of sustainable farming system is its protection from any degradation by natural or man-induced influences. Sustainable development of soil use also encompasses the protection of the soil acreage to such an extent which ensures that all soil functions are employed.

Soil parameters indicate the state of soil ecosystem characteristics, which especially reflect production, buffering, filter and other soil functions. From this view, the structure of soil profile (the soil class), soil type, soil depth, skeletal nature, the content and quality of humus substances, accessible nutrient supply, soil reaction, the content of foreign substances in soil, and soil edaphon seem to be of highest importance. Soil quality cannot be judged directly; it must be determined from the changes of its parameters. It is more accurate to evaluate the range of appropriate indicators rather than to use a single one. Soil quality is significantly affected by physical, chemical, biological and biochemical properties sensitive to changes in the environment and land management. With regard to physical properties, there are bulk density, porosity, water retention capacity, soil temperature, etc. In the group of chemical characteristics, total carbon and nitrogen content, soil reaction and content of available nutrients are observed. Evaluation of biological parameters focuses on microbial biomass and its activity, soil respiration, potentially mineralised nitrogen, the activity of soil enzymes, etc.

The results of exploiting land-use systems without consideration of the consequences on soil quality have been environmental degradation. Agricultural use and management systems have been generally adopted without recognizing consequences on soil conservation and environmental quality, and therefore significant decline in agricultural soil quality has occurred worldwide. Soil erosion and diffuse soil contamination are the major degradation processes on agricultural lands as a consequence of expansion and intensification of agriculture. Other non-agricultural uses, such as industrial and urban uses, also have important negative consequences on soil quality, due to local contamination, soil sealing, and changes in the

dynamics of the landscape systems. The concept of soil quality is useful to assess the condition and sustainability of soil and to guide soil research, planning, and conservation policy.

The importance of soil quality lies in achieving sustainable land use and management systems, to balance productivity and environmental protection. Unlike water and air quality, simple standards for individual soil-quality indicators do not appear to be sufficient because numerous interactions and trade-offs must be considered. For assessing soil quality a complex integration of static and dynamic chemical, physical, and biological factors need to be defined in order to identify different management and environmental scenarios. Also, the consequences of any decline in soil quality may not be immediately experienced. The soil system does not necessarily change as a result of changing external conditions or use, because soil has the capacity of resistance (or resilience) to the effects of potentially damaging conditions or misuse or to filter out harmful materials added to it. In part, this capacity of the soil in buffering the consequences of inputs and changes in external conditions arises because the soil is an exceedingly complex and varied material with many diverse properties and interactions between soil properties. It is this complex dynamic nature which often makes it difficult to distinguish between changes as a result of natural development and changes due to nonnatural external influences. Soil-quality assessment, based on inherent soil factors and focusing on dynamic aspects of soil system, is an effective method for evaluating the environmental sustainability of land use and management activities. However, the process of evaluating soil is not new, and agroecological land evaluation has much to offer. Land suitability is defined in land evaluation as “the fitness of a given land unit for a specified type of land use”. In a more operational sense, suitability expresses how well the biophysical potentialities and limitations of the land unit match the requirements of the land-use type. Therefore, new investigations must obviously be based on a solid understanding of past studies. Agroecological land evaluation predicts land behavior for each particular use, and soil-quality evaluation predicts

the natural ability of each soil to function. However, land evaluation is not the same as soil-quality assessment, because biological parameters of the soil are not considered in land evaluation. Soil surveys are the building blocks of the dataset needed to drive land evaluation. Soil surveys and soil taxonomy systems are used to define with precision specific soil types. Emerging technologies in data and knowledge engineering are providing excellent possibilities for the development and application processes of soil-quality assessment. As in land evaluation, the application phase of soil-quality assessment is a complex process of scaling-up from the representative areas of the development phase to implementation in unknown scenarios. This application phase can be executed with computer-assisted procedures. It involves the development and linkage of integrated components, the recently named decision or planning support tools. Decision support systems are computerized technology that can be used to support complex decision-making and problem-solving. Technically, decision support system comprises components for (i) sophisticated database management capabilities with access to internal and external data, information, and knowledge, (ii) powerful modeling functions accessed by model management system, and (iii) simple user interface designs that enable interactive queries, reporting, and graphing functions. By assessing soil quality, a land manager will be able to determine if a set of management practices is sustainable. For example, agricultural management systems located on the most suitable lands, according to their agroecological potentialities and limitations, are the best way to achieve sustainability.

There is a need to investigate coordinated and multidisciplinary approaches to assessing soil quality, evaluating long-term potential and limitations (inherent soil aspects), and monitoring the short-term changes (dynamic soil aspects) in response to sustainable soil use and management. This chapter presents a wide perspective on soil quality and the complex task of its assessment, considering the inherent and dynamic aspects of soil system. It focuses on the possibilities for applying and integrating accumulated knowledge on land-evaluation

modeling, in order to predict soil-quality indexes. Advanced information technologies, which enable the integration of large and complex databases, models, tools and techniques, are proposed to improve the decision-making process in soil-quality assessment application.

1.1.Soil Forming Processes

A soil forming process may be defined as a complex or sequence of events including both complicated reactions and simple rearrangement of matter which intimately affect the soil. These processes are also known as soil building processes or pedogenic processes.

The basic soil forming processes involved in soil formation includes the following.

- Gains or Additions of water, mostly as rainfall, organic and mineral matter to the soil.
- Losses of the above materials from the soil.
- Transformation of mineral and organic substances within the soil.
- Translocation or the movement of soil materials from one point to another within the soil. It is usually divided into two aspects.
  - Movement in solution (leaching)
  - Movement in suspension (eluviation) of clay, organic matter and hydrous oxides

1.2.Fundamental Soil Forming Processes

1.2.1.Humification:

- It is the process of transformation or decomposition of raw organic matter in to humus.
- In this process the soluble organic substances regroup themselves in to large molecules by polymerization and become poorly soluble.
- The characteristics are influenced by the nature of vegetation residue and the way it becomes decomposed and synthesized in to new organic compounds.

1.2.2. Eluviation (Latin, ex or e,out and lavere, to wash):

- Eluviation means washing out. It is the process of removal of constituents in suspension or solution (Clay, Fe_2O_3, Al_2O_3, SiO_2, humus, $CaCO_3$, other salts etc) by the percolating water

from the upper to lower layers. The Eluviation process involves mobilization and translocation of mobile soil constituents resulting in textural differences. Translocation depends upon relative mobility of elements and depth of percolation.

• The horizon formed by the process of eluviation is termed as eluvial horizon.

1.2.3.Illuviation (Latin- il, in, and lavere, to wash):

• The process of deposition of soil materials (removed from the eluvial horizon) in the lower layer is termed as Illuviation.

• This is the region of maximum accumlation of materials such as iron and aluminium oxides and silicate clays.

• The horizon formed by this process is termed as illuvial horizon (B-horizon, especially Bt).

• The process leads to horizon of gains and textural contrast between E and Bt horizons.

1.3.Specific Soil Forming Processes

The fundamental processes provide a framework for more specific processes

1.3.1.Podzolization (Russian, pod means under and zola means ash):

• It is the process of eluviation of oxide of iron and aluminium (sesqui oxides) and also humus under acidic condition (pH 4-5), removal of carbonates by organic acids formed by organic matter and illuviation of the silicon in surface horizon.

• Abudant organic matter, commonly found under forest, cold and humid climate are favourable for the formation of such soils.

• The eluiviated horizon assumes a bleached grey colour and is left in highly acid, siliceous condition and, the term podzol has been used for such soils.

1.3.2.Laterization (Latin, later-a brick):

• The term laterite is derived from the word later meaning brick or tile and was originally applied to a group of high clay Indian soils found in Malabar hills of Kerala, Tamil Nadu, Karnataka , Madya Pradesh and Maharashtra.

• Laterization is inverse process to that of podzolization i.e. the process that removes silica, instead of sesquioxides from the upper layers and thereby leaving sesquioxides to concentrate in the solum.

• The process operates under rain forests of tropical areas, warm and humid (tropical) climate and basic parent materials are favourable for such soils.

• It refers specifically to a particular cemented horizon in certain soils which when dried, become very hard, like a brick.

• Such soils (in tropics) when massively mixed with sesquioxides (iron and aluminium oxides) to an extent of 70 to 80 per cent of the total mass, are called laterites or latosols (Oxisols).

1.3.3.Salinization

• It is the process of accumulation of salts, such as sulphates and chlorides of calcium, magnesium, sodium and potassium in soils in the form of a salty (salic) horizon.

• The intensity and depth of accumulation vary with the amount of water available for leaching.

• It is quite common in arid and semi arid regions.

• It may also take place through capillary rise of saline ground water and by inundation with seawater in marine and coastal soils.

• Salt accumulation may also result from irrigation or seepage in areas of impeded drainage.

1.3.4.Desalinization

• It is the process of removal of excess soluble salts from horizons that contained enough soluble salts to impair the plant growth.

• Drainage is essential for desalinization.

1.3.5.Alkalization (Solonization) :

• The process by which soils with high exchangeable sodium and pH > 8.5 are formed; often sodium carbonate and sodium bicabonate are formed in extreme cases.

• The soil colloids become dispersed and tend to move downward. The dispersion results in poor physical condition of the soil.

1.3.6.Dealkalization (Solodization):

• The process refers to the removal of Na+ from the exchange sites. This process involves dispersion of clay. Dispersion occurs when Na+ ions become hydrated.

• The process is effected by intensive leaching and degradation which takes place in older soils.

1.3.7.Calcification

• The process operates in arid and semi-arid regions and refers to precipitation and accumulation of calcium carbonate (CaCO3) in some part of the profile. The accumulation of CaCO3 may result in the development of a calcic horizon.

• Calcium is readily soluble in acidic soil water and/or when CO2 concentration is high in root zone as:

$$CO_2 + H_2O = H_2CO_3$$

$$H_2CO_3 + Ca = Ca\ (HCO3)_2 \text{ (soluble)}$$

$$Ca\ (HCO_3)_2 = CaCO_3 + H_2O + CO_2 \text{ (precipitates)}$$

1.3.8.Decalcification

• In regions where some water percolates through the soil profile , decalcification takes place leading to the formation of calcic horizon down below.

• In humid regions, calcium cabonate reacts with water containing dissolved carbon dioxide to form soluble bicarbonate which may be completely leached out of the soil profile.

$$CaCO_3 + CO_2 + H_2O \text{ (insoluble)} = Ca(HCO3)_2 \text{ (soluble)}$$

1.3.9.Carbonation

• It occurs when carbon dioxide interacts chemically with minerals. When carbon dioxide is dissolved in water, it forms weak carbonic acid.

• When carbonic acid comes in contact with the surface of the earth it dissolves large masses of limestone, creating caves and caverns.

1.3.10.Gleization:

• The term glei is of Russian origin means blue, grey or green clay.

• The gleization is a process of reduction, due to anaerobic condition, of iron in waterlogged soils with the formation of mottles and concretions. Such soils are called as hydromorphic soils.

• The process is not dependent on climate (high rainfall as in humid regions) but often on drainage conditions.

1.3.11. Pedoturbation:

• It is the process of mixing of the soil.

1. Faunal pedoturbation: It is the mixing of soil by animals such as ants, earthworms, moles, rodents, and man himself

2. Floral pedoturbation : It is the mixing of soil by plants as in tree tipping that forms pits and mounds

3. Argillic pedoturbation: It is the mixing of materials in the solum by the churning process caused by swell-shrink clays as observed in deep Black cotton soils.

1.4.Soil Quality

As suggested in the early 1990s, soil quality is “the capacity of a soil to function”. More specifically, soil quality has been defined by a committee for the Soil Science Society of America as “the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation”. Also, soil quality can be considered as the ability of a soil to fulfill its functions in the ecosystem, which are determined by the

integrated actions of different soil properties. With respect to agriculture, soil quality would be the soil's fitness to support crop growth without becoming degraded or otherwise harming the environment.

1.5. Soil Health

Soil health is the other principle for sustainable soil management used by some soil scientists. soil health is defined as the continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries; to sustain biological productivity; promote the quality of air and water environments; and maintain plant, animal, and human health. In this sense, the soil is considered as a living system, address all essential functions of soil in the landscape, compare the condition of a given soil against its own unique potential within climatic, landscape, and vegetation patterns, and somehow enable meaningful assessments to trends. Although some authors consider the terms soil quality and soil health as synonymous, the integrated concept of soil quality can be defined, including the inherent soil quality, traditionally named soil suitability, and the dynamic soil quality or soil health.

1.6. Soil Functions

The soil system can perform many functions, and often simultaneously. the soil must provide the following basic functions:

(i) a physical, chemical, and biophysical setting for living organisms;

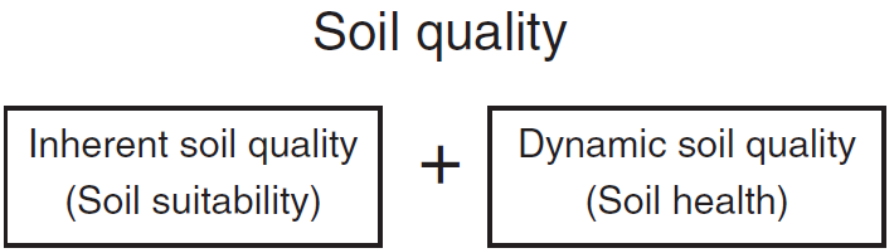
(ii) the regulation and partition of water flow, storage, and recycling of nutrients and other elements;

(iii) support for biological activity and diversity for plant growth and animal productivity;

(iv) the capacity to filter, buffer, degrade, immobilize, and detoxify organic and inorganic substances; and

(v) provide mechanical support for living organisms and their structures.

Specific soil functions can be defined with respect to issues like particular crop growth, and soil erosion or soil contamination hazard (Table 1). Several soil physical functions, such as water retention and infiltration or soil aeration, are directly connected to the biological status of soil system, as also are the kinds of organisms and nutrient supply. Soil quality is therefore a multifunctional concept. It is well known that overuse or exploitation of some functions (e.g., production function for crops) can lead to the damage of other ones. The spatial and temporal variation in the provision of functions should be incorporated in evaluations or assessments.



Soil-quality issue	Soil function
Crop growth	Plant root penetration
	Plant water-use efficiency
	Water- and air-filled pore space
	Water infiltration
Natural fertility	Nutrient availability
	Cation-exchange capacity
	Acidity
	Salinity/alkalinity
	Toxicity
Erosion risk	Runoff potential
	Erodibility
	Cover protection
	Subsoil compaction
	Workability
Compaction risk	Water retention
	Water infiltration
	Cohesion
	Workability/trafficability
Contamination risk	Leaching potential
	Toxic absorption
	Toxic mobility
	Chemicals degradation

Table 1. Specific soil functions considered for several soil quality issues

1.7. Soil Threats

Consideration of soil threats is crucial for assessing the quality of the soil system. These are the major threats faced by soils:

(i) soil erosion,

(ii) soil contamination,

(iii) decline in organic matter and biodiversity,

(iv) soil compaction,

(v) salinization,

(vi) floods and landslides, and

(vii) soil sealing.

In many places, soil erosion is the most severe consequence of soil degradation with respect to restoration of soil quality, and controlling erosion is a prerequisite for a healthy soil. However, most of the soil degradation processes are interlinked, and are often linked by similar causative factors. The risk of these soil threats can be monitored by use of indicators such as trends in yields on soils under irrigation to monitor risk of salinity. Actions to protect soil quality necessitate tackling collectively the different threats.

1.8. Assessment Procedures

Any evaluations of soil quality must consider the multiple soil uses (e.g., agricultural production, forest, rangeland, nature conservation, recreation, or urban development). However, the most widely accepted concept of soil quality and the most significant in a global context concerns agro-ecosystems. In soil-quality evaluation or assessment, the two main questions that must be answered are: (i) how does the soil function; and (ii) what procedures are appropriate for making the evaluation. After answering those questions, a range of parameter values or indexes that indicate a soil is functioning at full potential can be calculated using landscape characteristics, knowledge of pedogenesis, and a more complete understanding

of the dynamic processes occurring within a soil. Soil-quality assessment focuses on dynamic aspects to evaluate the sustainability of soil management practices, but it must be based on the inherent soil factors.

1.9. Soil-Quality Indicators

A soil-quality indicator is a simple attribute of the soil which may be measured to assess quality with respect to a given function. It is important to be able to select attributes that are appropriate for the task, given the complex nature of the soil and the exceptionally large number of soil parameters that may be determined, as exemplified in the table 2.

Indicator	Relationship to Soil Health
Soil organic matter (SOM)	Soil fertility, structure, stability, nutrient retention, soil erosion, and available water capacity
PHYSICAL	
Soil structure	Retention and transport of water and nutrients, habitat for microbes, and soil erosion
Depth of soil and rooting	Estimate of crop productivity potential, compaction, and plow pan
Infiltration and bulk density	Water movement, porosity, and workability
Water holding capacity	Water storage and availability
CHEMICAL	
pH	Biological and nutrient availability
Electrical conductivity	Plant growth, microbial activity, and salt tolerance
Extractable nitrogen (N), phosphorus (P), and potassium (K)	Plant available nutrients and potential for N and P loss
BIOLOGICAL	
Microbial biomass carbon (C) and N	Microbial catalytic potential and repository for C and N
Potentially mineralizable N	Soil productivity and N supplying potential
Soil respiration	Microbial activity measure

Table 2. Soil Quality Indicators

1.10. Soil conditions and plant growth

Plants can respond to soil conditions in ways that cannot readily be explained in terms of the ability of the roots to take up water and nutrients. Roots may sense difficult conditions in the soil and thence send inhibitory signals to the shoots which harden the plants against the

consequences of a deteriorating or restrictive environment, especially if the plants' water supply is at risk. Generally, this behaviour can be interpreted as feedforward responses to the soil becoming too dry or too hard, or to the available soil volume being very small as with bonsai plants, or to roots' becoming infected with pathogens. However, soil that is too soft or in which the roots are forced to grow in very large pores can also induce large conservative responses, the significance of which is unclear. The inhibitory signals may affect stomatal conductance, cell expansion, cell division and the rate of leaf appearance. Their nature is still under debate, and the debate is becoming increasingly complex, which probably signifies that a network of hormonal and other responses is involved in attuning the growth and development of a plant to its environment.

1.11.Physico-Chemical Properties in Soil Quality

1.11.1.pH

The most significant property of soil is its pH level, Its effects on all other parameters of soil. Therefore, pH is considered while analysing any kind of soil. If the pH is less than 6 then it is said to be an acidic soil, the pH range from 6-8.5 it's a normal soil and greater than 8.5 then it is said to be alkaline soil.

1.11.2.Texture

Soil texture is a qualitative classification tool used in both the field and laboratory to determine the classes for agricultural soils based on their physical texture. Soil in different regions shows different texture, the texture of the soil is mostly depends upon the size of particles. Soil texture shows its effect on aeration and root penetration. It also effect on the nutritional status of soil. Soil texture can be expressed significantly by its electrical conductivity.

1.11.3. Moisture

Water content or moisture content is the quantity of water contained in a material, such as soil called soil moisture, Moisture is one of the most important properties of soil. Absorption of the nutrient by soil is largely depends on moisture content of the soil moisture of soil also shows its effect on the texture of soil.

1.11.4. Soil temperature

Soil temperature depends on the ratio of the energy absorbed to that lost. Soil has a temperature range between -20 to 60 °C. The temperature of the soil is the most important property because it shows its effect on the chemical, physical and biological processes related to growth of plants. Soil temperature changes with season, time of day, and local conditions of climate.

1.11.5. Electrical conductivity

Electrical conductivity is also a very important property of the soil, it is used to check the quality of the soil. It is a measure of ions present in solution. The electrical conductivity of a soil solution increases with the increased concentration of ions. Electrical conductivity is a very quick, simple and inexpensive method to check health of soils. It is a measure of ions present in solution. The electrical conductivity of a soil solution increases with the increased concentration of ions.

1.11.6.Nitrogen

Nitrogen is the most critical element obtained by plants from the soil and is a bottleneck in plant growth. About 80% of the atmosphere is nitrogen gas. Nitrogen gas diffuses into water where it can be “fixed” (converted) by blue-green algae to ammonia for algal use. Nitrogen can also enter lakes and streams as inorganic nitrogen and ammonia. Because nitrogen can enter

aquatic systems in many forms, there is an abundant supply of available nitrogen in these systems.

1.11.7. Phosphorus

Phosphorus is a most important element present in every living cell. It is one of the most important micronutrient essential for plant growth. Phosphorus most often limits nutrients remains present in plant nuclei and act as an energy storage.

1.11.8. Potassium

Potassium plays an important role in different physiological processes of plants, it is one of the important element for the development of the plant [15]. It is involved in many plant metabolism reactions, ranging from lignin and cellulose used for the formation of cellular structural components, for regulation of photosynthesis and production of plant sugars that are used for various plant metabolic needs.

1.11.9. Soil organic matter

It is also a valuable property of soil. If the soil is poor in organic matter, then it enhances the process of soil erosion. If the soil organic matter is present in soil, then this soil is useful for the agricultural practices. Organic matter may be added in the soil in the form of animal manures, compost, etc. The presence of the higher content of organic matter in the soil can be another possible reason for lowering of the pH. Soil organic matter content has decreased from surface to subsoil due to levelling.

CHAPTER-II

LITERATURE SURVEY

Soil quality is the competence of soil to perform necessary functions that are able to maintain animal and plant productivity of the soil. Soil consists of various physical, chemical, and biological parameters, and all these parameters are involved in the critical functioning of soil. There is a need for continuous assessment of soil quality as soil is a complex and dynamic constituent of Earth's biosphere that is continuously changing by natural and anthropogenic disturbances. Any perturbations in the soil cause disturbances in the physical (soil texture, bulk density, etc.), chemical (pH, salinity, organic carbon, etc.), and biological (microbes and enzymes) parameters. These physical, chemical, and biological parameters can serve as indicators for soil quality assessment. However, soil quality assessment cannot be possible by evaluating only one parameter out of physical, chemical, or biological. So, there is an emergent need to establish a minimum dataset (MDS) which shall include physical, chemical, and biological parameters to assess the quality of the given soil. This review attempts to describe various physical, chemical, and biological parameters, combinations of which can be used in the establishment of MDS (1)

Soil quality is one of the three components of environmental quality, besides water and air quality (2). Water and air quality are defined mainly by their degree of pollution that impacts directly on human and animal consumption and health, or on natural ecosystems. In contrast, soil quality is not limited to the degree of soil pollution, but is commonly defined much more broadly as "the capacity of a soil to function within ecosystem and land-use boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health". This definition reflects the complexity and site-specificity of the belowground part of

terrestrial ecosystems as well as the many linkages between soil functions and soil-based ecosystem services. Indeed, soil quality is more complex than the quality of air and water, not only because soil constitutes solid, liquid and gaseous phases, but also because soils can be used for a larger variety of purposes. This multi-functionality of soils is also addressed when soil quality is defined from an environmental perspective as "the capacity of the soil to promote the growth of plants, protect watersheds by regulating the infiltration and partitioning of precipitation, and prevent water and air pollution by buffering potential pollutants such as agricultural chemicals, organic wastes, and industrial chemicals". Soil quality can be assessed both for agro-ecosystems where the main, though not exclusive ecosystem service is productivity, and for natural ecosystems where major aims are maintenance of environmental quality and biodiversity conservation. Soil quality is the basis for the development of sustainable agriculture and may be used for evaluating the sustainability of soil management practices (3) . A vital resource of the ecosystem, soil, is assessed as the significant and nonrenewable pillar and plays a key role to regulate nutrient absorption, utilizing water and increasing productivity (4). Soil pH is an important consideration for farmers and gardeners for several reasons, including the fact that many plants and soil life forms prefer either alkaline or acidic conditions or the pH can affect the availability of nutrients in the soil. Physico chemical soil analysis of Muthannankulam of Coimbatore district, Tamilnadu is reported by Manimegalai et al., (5). Assesment of soil physico-chemical quality indicators in rice soils of Cuddalore district was reported by Seevagan et al., (6). Assessment of Soil quality indicators to maximize sugarcane productivity in Theni district of Tamil Nadu was reported by Jeevika et al., (7) and concluded that application of organic matter, amendments rich in calcium and magnesium in acidic soil and application of gypsum and other amendments rich in sulphur in alkali soils can maximize productivity of sugarcane.

The indicators of a soil cover the whole range of physico-chemical and biological properties that reflect soil functions to measure under various field conditions and response to changes in climate, soil and crop management practices (8). The nature of soil and water tested from Puliyanthangal and Kathiyavadi villages of Ranipet district and reported that the water parameters in the study area were fluctuating, within, in par and above the permissible limits (9). High-quality soils maintain natural ecosystems with increased air and water quality for improved food and fiber production (10). The physicochemical properties of soils are important to prevent soil from degradation and for increasing farm productivity. Physical properties like texture, structure, and porosity affect the chemical behavior of soil. Nutrient availability to plants is affected by so many chemical properties including soil pH. If the soil solution is basic, plants cannot utilize nitrogen, phosphorus, potassium, and other nutrients they need for growth. In acidic soils, plants are more likely to take up toxic metals and some plants eventually die of toxicity. Soil color, pH, and electric conductivity (EC) are interlinked and controlled by the organic matter content of soil. Soil organic matter is one of the most important determinants of soil quality and has a close association with soil productivity and fertility. There is an enormous amount of carbon stored in organic matter. It contains 58 % of organic carbon while soil is the largest terrestrial reservoir containing at least 1500 gigaton (Gt) of organic carbon. Soil organic matter contains different kinds of organic compounds with variable chemical composition and turnover rates. Soil organic matter being a reservoir of carbon is subjected to variations due to different management practices under varying land use types. The impact due to land use changes on soil organic matter content depends on a number of factors such as old and new land use types, the soil type, management, and climate. Organic matter stores energy for plants and helps to improve soil fertility by increasing the amount of available nutrients like nitrogen, phosphorous, and sulfur. Sulfur and phosphorus in the form of SO_4 and H_2PO_4 are absorbed by plant roots. A substantial amount of nitrogen and phosphorus

is required while sulfur is required in less quantity. Sulfur deficiency is not very obvious as compared to nitrogen and phosphorus. Phosphorus is not present in free form in nature. It is present in compound form. It is present in phosphate rock formation such as apatite that is calcium phosphate. Normally, agricultural soils need fertilizer asthese soils cannot fulfill the need of phosphorus for crops. Fertilizers are applied to increase the yield and quality of products. Fertilizers usually have phosphate, nitrate, and some other nutrients. For nitrogen-deficient soils, manure or chemical fertilizers are applied to enhance nutrients in soil. Widespread nutritional deficiencies occur from relatively fertile soil layers by water erosion and overgrazing coupled with continuous nutrient mining by crops.

2.1. Water holding capacity

Soil water holding capacity is the amount of water that a given soil can hold and then make available for crop use. Water holding capacity is largely determined by soil texture and by the amount of pore spaces in the soil, where water and air can be found. Sandy soils have lower water holding capacity, while silt and clay soils tend to have higher water holding capacity. A crop grown in a sandy soil will need to be irrigated more frequently, but with less total water, than a crop grown in a clay or silty soil. A clay or silty soil will hold more water for the crop to use, so can be irrigated less frequently. Compacted soils have less pore space for the water, and therefore have lower water holding capacity.

2.2. Soil biological activity

Healthy soils are teeming with living organisms: bacteria, fungi, insects, earthworms, etc. As these living things go through their life cycles, they perform many functions that help improve the quality of soil. Soil organisms decompose fresh organic matter such as crop residues and animal manures. In the process, they help soil particles stick together into stable aggregates. They also create humus, a form of organic matter that doesn’t decompose further, that helps soils hold water and nutrients. Soils with higher biological activity tend to have fewer

plant disease organisms. Earthworms tunnel through soils, opening up pathways for air and water to move into the soil.

2.3. Soil conservation

When water from rainfall or irrigation washes over bare soil, or wind blows over bare soil, soil particles may be washed or blown away, out of the field. This process is called soil erosion; the farming practices used to stop erosion are known as soil conservation practices. Healthy soil is a very valuable natural resource. Soil particles that erode from fields can cause environmental problems, such as polluting creeks, rivers, lakes and even oceans. Airborne soil particles can lower air quality, and cause respiratory illnesses. Farmers can protect soils from erosion by limiting the time when there is bare soil in the field, improving soil structure, and by managing tillage, irrigation and crop rotation.

2.4. pH

Soil pH is a measure of the acidity or basicity (alkalinity) of a soil. pH is defined as the negative logarithm (base 10) of the activity of hydronium ions (H^+ or, more precisely, H_3O^+) in a solution. In soils, it is measured in a slurry of soil mixed with water (or a salt solution, such as 0.01 M $CaCl_2$), and normally falls between 3 and 10, with 7 being neutral. Acid soils have a pH below 7 and alkaline soils have a pH above 7. Ultra-acidic soils ($pH < 3.5$) and very strongly alkaline soils ($pH > 9$) are rare.

Soil pH is considered a master variable in soils as it affects many chemical processes. It specifically affects plant nutrient availability by controlling the chemical forms of the different nutrients and influencing the chemical reactions they undergo. The optimum pH range for most plants is between 5.5 and 7.5, however, many plants have adapted to thrive at pH values outside this range.

2.4.1. Classification of soil pH ranges

The United States Department of Agriculture Natural Resources Conservation Service classifies soil pH ranges as follows:

Denomination	pH range
Ultra acidic	< 3.5
Extremely acidic	3.5–4.4
Very strongly acidic	4.5–5.0
Strongly acidic	5.1–5.5
Moderately acidic	5.6–6.0
Slightly acidic	6.1–6.5
Neutral	6.6–7.3

2.5. Nutrients in soil

Soil is a major source of nutrients needed by plants growth. The three main nutrients are nitrogen (N), phosphorus (P),and potassium (K). Together they make up the trio known as NPK. Other important nutrients are calcium, magnesium and sulfur.

2.5.1. Primary nutrients:

The primary nutrients are nitrogen, phosphorus,and potassium.

The intermediate nutrients are sulfur, magnesium,and calcium.

The remaining essential elements are the micronutrients and are required in very small quantities.

2.5.2. Secondary nutrients

Calcium, Magnesium, Sulphur are essential plant nutrients. They are called" Secondary" nutrients because plants require them in smaller quantities than nitrogen, phosphorus, and potassium. Calcium and magnesium both increase soil pH , but sulfur from some sources reduces soil pH.

2.5.3.Trace elements in soil

Some trace elements of potential concern as soil contaminants are: arsenic(As),boron(B) cadmium (Cd), chromium (Cr), copper (Cu), fluorine (F),lead(Pb), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se),and Zinc(Zn).

The main objective of the present study is

- ✓ Collect soil samples from the farmlands of Thoothukudi and Ramanad districts.
- ✓ To determine the physio-chemical properties of the soil such as pH, electrical conductivity, Organic Carbon, Nitrogen, Potassium, Sulphur, Boron, Iron, Manganese, Zinc and copper using “MRIDAPARIKSHAK” digital soil testing mini lab.
- ✓ To suggest a suitable fertilizer for the specified farmlands

3.1. Soil sampling

The soil samples were collected from the farmlands of Thoothukudi and Ramnad districts in March 2022. The selected farmlands were tabulated as below. For each sample, soil material in the layer was collected from five points (North, South, East, West, and Center) inside a circle with a radius of 25m then mixed together as a soil sample. The soil samples were collected, air-dried, and passed through a 2mm sieve to remove stones, grass, litter, and any material on the soil surface.

Sample No	Marked as	Name of the place
Sample 1	1	 **Thinaikulam, Ramnad District**

Sample 2	2	 **Kurukkusalai, Thoothukudi District**
Sample 3	3	 **Thaliyarenthal, Ramanad District**
Sample 4	4	 **Tharuvaikulam , Thoothukudi District**

Sample 5	5	 **Sawyerpuram, Thoothukudi District**
Sample 6	6	 Vaagaikulam, Thoothukudi District

3.2. Instrumentation:

Mridaparikshak is a minilab developed by ICAR-Indian Institute of Soil Science (IISS), Bhopal, an institute that comes under the Division of Natural Resource Management of Indian Council of Agricultural Research. *Mridaparikshak* has been developed in technical collaboration with M/s Nagarjuna Agrochemicals Pvt. Ltd., Bhopal. With *Mridaparikshak* one can determine the available quantities of soil nutrients and prescribe fertilizer doses for nitrogen (N), phosphorus (P), potassium (K), sulphur (S), Iron (Fe), zinc (Zn), boron (B), copper (Cu) and Manganese(Mn)based on the measures soil test values.

Mridaparikshak lets you know quantitatively the status of soil pH, soil electrical conductivity (EC), and organic carbon, available N, available P, available K, available S, available Zn, B and Fe. The results as given by *Mridaparikshak*correspond to the results obtained by soil test laboratories. The results are comparable with the results obtained by Walkley and Black procedure for organic C, Subbaiah and Asija method for available N, Olsen and Bray methods for available P, neutral 1 N ammonium acetate method of available K, DTPA extraction method for available Fe and Zn, and hot water soluble method for available B.

3.3. Components of MRIDAPARIKSHAK

3.3.1.Manual

A mini-lab manual is provided with software and training video CD'S, that gives the details on, how to use *Mridaparikshak*.

3.3.2. Equipments

1. **Smart Soil Pro**: An instrument that measures available forms of nutrients and prescribes fertilizer nutrient doses.
2. **Shaker:** A mechanical device that is used for homogeneous shaking of the solution prepared for analysis of soil sample.
3. **Weighing Balance:** A pocket balance to give precise quantity of chemicals and soil.
4. **Hot plate:** A hot plate which is used for heating the solution to get required temperature for analysis of soil sample.

3.3.4. Chemicals

1. The chemicals to be used are provided in reagent bottles that are numbered from 1 to 42.

3.3.5. Glass-wares and plastic-wares

1. Glass Measuring cylinder.
2. Glass Beaker.
3. Plastic Beaker

4. Thermometer

3.3.6. Sieve

1. Two sieves, one with 2 mm opening and another with 0.5 mm opening.

3.3.7. Other

1. Filter paper
2. Tissue paper
3. Rod for stirring
4. Gloves
5. Goggles
6. Notebook for recording the soil data
7. cleaning solution
8. Distilled water.

MRIDAPARIKSHAK

4.1. Soil Organic Carbon and Nitrogen

Soil organic matter is any material produced originally by living organisms (plant or animal) that is returned to the soil and goes through the decomposition process . At any given time, it consists of a range of materials from the intact original tissues of plants and animals to the substantially decomposed mixture of materials known as humus. Most soil organic matter originates from plant tissue. As soil organic matter is derived mainly from plant residues, it contains all of the essential plant nutrients. Therefore, accumulated organic matter is a storehouse of plant nutrients. The stable organic fraction (humus) adsorbs and holds nutrients in a plant-available form.

Nutrient	Low	Medium
Organic carbon	< 0.5 %	0.5 - 7.5%
Available nitrogen (N)	< 240Kg/ha	240-480kg/ha

Table 3 : Low and medium levels of SOC and Nitrogen

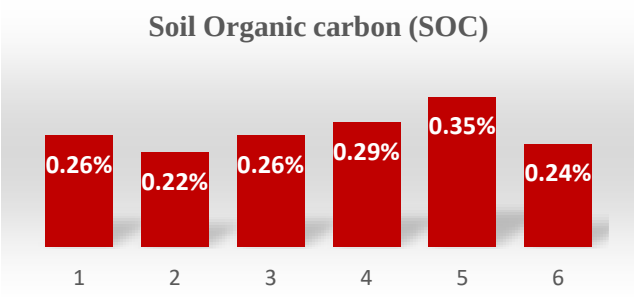
From Table 4 it is clear that organic carbon and available nitrogen is in the lower level for all the six samples.

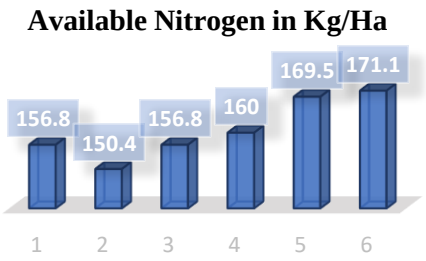
Sample No.	Soil Organic carbon	Nitrogen
1	0.26%	156.8kg/ha

2	0.22%	150.4kg/ha
3	0.26%	156.8kg/ha
4	0.29%	160kg/ha
5	0.35%	169.5kg/ha
6	0.24%	171.1kg/ha

Table 4: Soil Organic carbon and available Nitrogen in the soil Samples

Graphical Representation





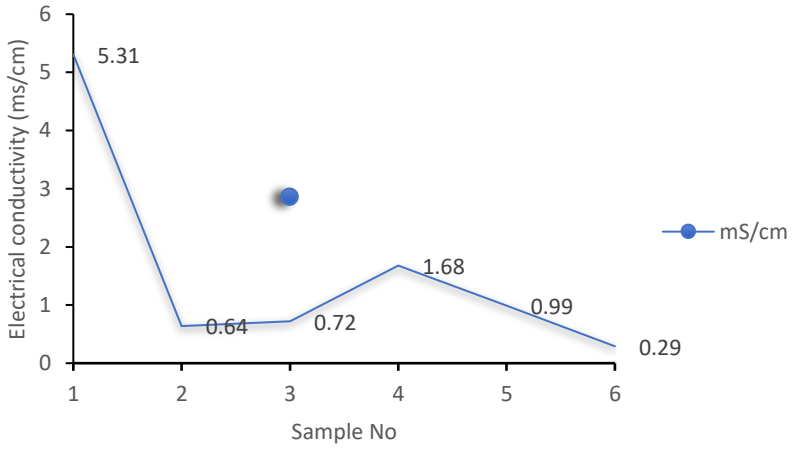
4.2. Electrical Conductivity

Sand does not hold onto moisture well, so it has a lower conductivity. Silty soil, similar in texture to the wet mud on a river bank, has a middling base conductivity. This type of soil is able to hold onto water relatively well. Soils rich in clay have a higher conductivity due to how well they are able to hold onto moisture, and ones with a middling conductivity tend to have the greatest crop yield. They are able to hold in just enough water, while at the same time draining away excess. Another property that relates to EC and soil texture is called cation exchange capacity (CEC). CEC relates to the amount of clay and organics in soil. Clay has higher electrical conductivity, so the higher the CEC, the higher the conductivity is. Salts are very conductive and will raise the EC of the soil. Water used to irrigate crops will directly affect

the quality of the soil by either increasing or diluting available salts and nutrients. This in turn affects the electrical conductivity. Natural rains will dilute the amount of salt near the roots of plants. This helps to keep the plant from getting “burned” by excess salts and nutrients. This means that the plant's roots are essentially clogged by the salts and nutrients. They become unable to take up salts, which can stunt its growth. If irrigation water has a high salt content it can accumulate in fields, increasing the salinity and electrical conductivity.

Optimal EC levels in the soil usually range from 1.10-5.70 milli Siemens per cm (mS/cm). Too low EC levels indicate low available nutrients, and too high EC levels indicate an excess of nutrients.

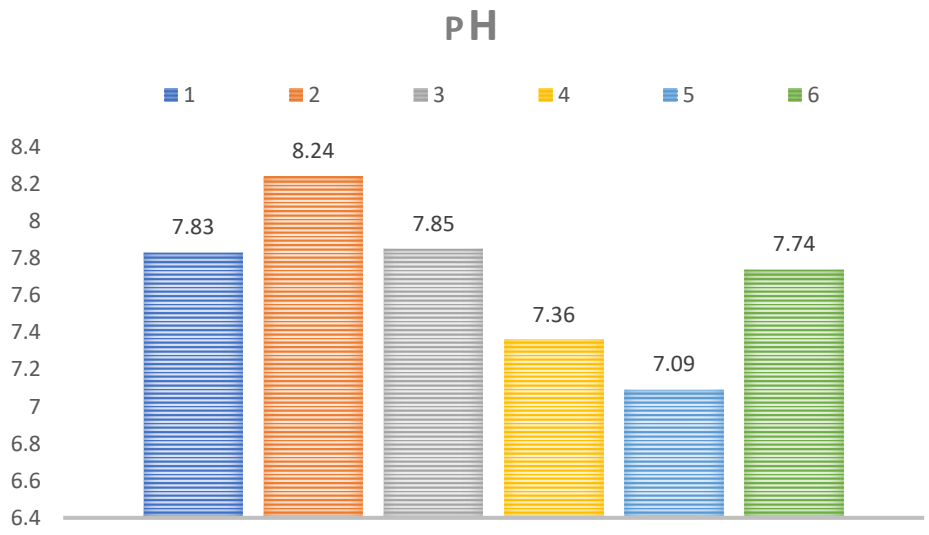
From the graph it is clear that the EC values range from 0.29-5.73 milli Siemens per centi meter. This indicates that sample 1 have high electrical conductivity and high excess of nutrients and samples 6, sample 2 and sample 3 have lower levels indicating the lower percentage of nutrients.



4.3. Soil pH

The soil pH measures active soil acidity or alkalinity. A pH of 6.9 or less is acid. Soils with a pH of 7.0 are neutral, values higher than 7.0 are alkaline. Under normal conditions, the most desirable pH range for mineral soil is 6.0 to 7.0 and 5.0 to 5.5 for organic soil. The level of

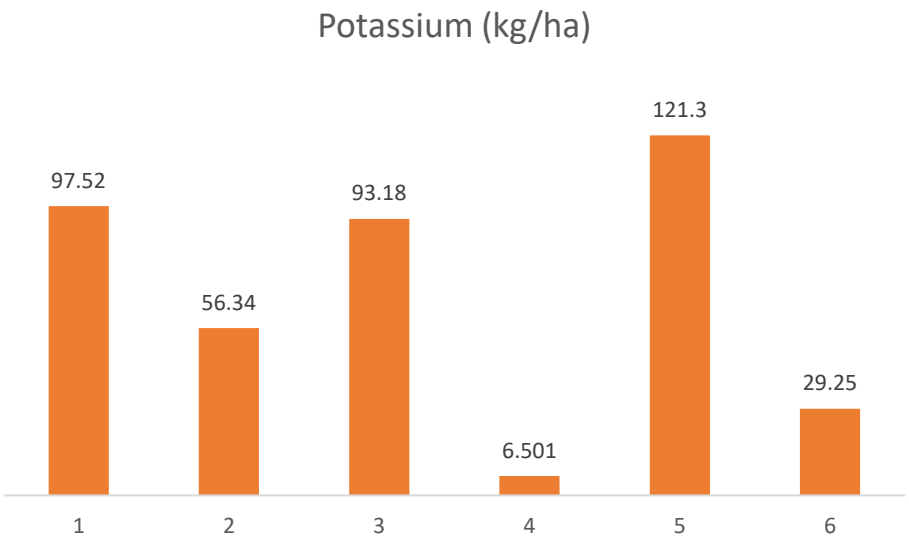
acidity will specify the amount of soil amendment that is needed to bring it up or down to the appropriate level. Acidic ("sour") soil is counteracted by applying finely ground limestone or wood ash, and alkaline ("sweet") soil is typically treated with gypsum (calcium sulfate), ground sulfur, or compost. From the graph its clear that sample 2 is alkaline and whereas all the other soil samples are found to be neutral.



4.4. Potassium

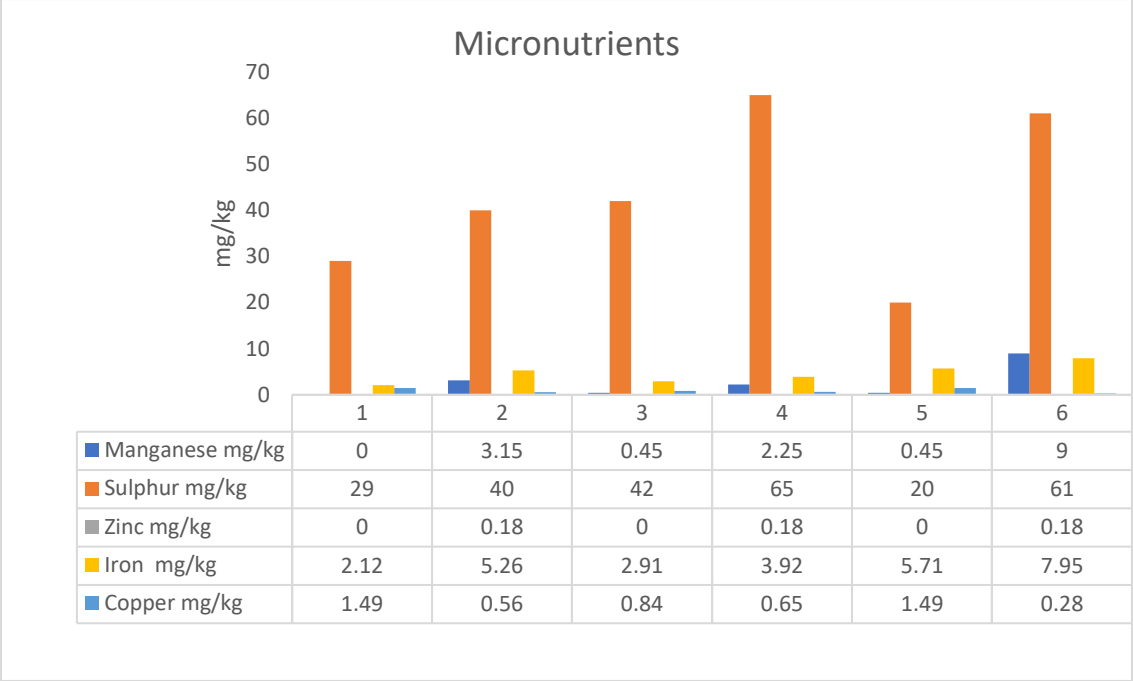
It is an essential nutrient for plant growth. It's classified as a macronutrient because plants take up large quantities of K during their life cycle. From the graph its clear that sample 5 alone have medium level of potassium all the other samples have low level of potassium. The table shows the optimum potassium level needed for the crop production.

Nutrient	Low	Medium	High
Available potassium (K)	< 110Kg/ha	110-280Kg/ha	> 280Kg/ha



4.5. Micronutrients

Micronutrients are essential plant nutrients that are found in trace amounts in tissue, but play an imperative role in plant growth and development. As per the reported literature (11) the soil critical levels established for Zn, Cu, Fe, Mn, B, Mo and S were 0.6, 0.2, 4.0, 2.0, 0.5, 0.2 and 10.0 mg kg^{-1}, respectively. From the graph its clear that all the samples have very low zinc levels. In case of copper all the soil samples except sample no.6 have high levels. Samples 1, 3 and 4 have low levels of iron. Most of the soil samples show high concentration for Sulphur and manganese.



	1	2	3	4	5	6
Manganese mg/kg	0	3.15	0.45	2.25	0.45	9
Sulphur mg/kg	29	40	42	65	20	61
Zinc mg/kg	0	0.18	0	0.18	0	0.18
Iron mg/kg	2.12	5.26	2.91	3.92	5.71	7.95
Copper mg/kg	1.49	0.56	0.84	0.65	1.49	0.28

Conclusion

The soil quality parameters of soil samples collected from the farmlands were successfully assessed. It was found that in all the soil samples the Soil Organic Carbon (SOC) is below the critical value 0.5% hence the soil was unable to hold most of the nutrients. The very low level of organic carbon may be due to erosion and nutrient leaching. It was suggested to maintain these farmlands by using more animal manures and slurries, digestates (material left after anaerobic digestion of biodegradable materials, like domestic food waste), manures, composts, biosolids, paper crumble and wood and food processing by-products.

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PHYTOCHEMICAL ANALYSIS AND STUDY OF EXTRACTION RATE OF LAWSONE FROM LAWSONIA INERMIS

Project in Chemistry

Submitted to St.Mary's College (Autonomous) in partial fulfillment for the award of the degree of **BACHELOR OF SCIENCE** in Chemistry.

By

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Thoothukudi – 628001

2021-2022

DECLARATION

We hereby declare that the project entitled **"PHYTOCHEMICAL ANALYSIS AND STUDY OF EXTRACTION RATE OF LAWSONE FROM LAWSONIA INERMIS "** submitted to St.Mary's college (Autonomous) Thoothukudi affiliated to Manonmaniam Sundaranar University for the Degree of Bachelor of science is our original work and that it has not previously formed the basis for the award of any degree, Diploma or Similar title .

Maria Selvi A.

Muthu Durga L.

Sagaya Kanute J.

Sheran Rose M.

Suriya C.S.

May 2022

Thoothukudi

CERTIFICATE

This is to certify that the report of the project in chemistry entitled "**PHYTOCHEMICAL ANALYSIS AND STUDY OF EXTRACTION RATE OF LAWSONE FROM LAWSONIA INERMIS** " is submitted to St. Mary's College (Autonomous), in partial fulfillment for the award of the Degree of Bachelor of Science in Chemistry and is a record of the work done by the following students during the year 2021-2022.

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CONTENTS

INTRODUCTION

1.1 Introduction

Henna is a reddish-brown or reddish-orange dye made from the old world shrub *Lawsonia inermis*, which produces henna with small pink, red, or white flowers. Henna is used to colour the hair and temporary body art known as mehndi. The dye is extracted from the dried leaf and petioles of the plant [1].

The bark of the henna is gray, brown in color and is smooth. The leaves of the henna plant are medium green in color and can vary a great deal, even on the same plant. The leaves when young, are quite smooth and flat whereas when their life is about to end, they begin to curl and are quite long. Leaves under the flowers are always small and young looking. The leaves are in pairs and differ in sizes from approximately 2-4cm.

The flowers are quite small, (about 1/4th inch), grow in grape formations and are extremely fragrant. It grows best in heat up to 120F levels and stains better in these conditions. It grows better in dry soil and withers in temperatures below 50F degrees [2].

Humans have used henna extracts containing lawsone as hair and skin dyes for more than 5000 years. Lawsone reacts chemically with the protein keratin in skin and hair via Michael addition, resulting in a strong permanent stain that lasts until the skin or hair is shed. The darker colored ink is due to more lawsone-keratin interactions occurring, which break down as the concentration of lawsone decreases and the tattoo fades [3].

Figure 1.1 Henna Plant

Lawsone (2-hydroxy-1,4-naphthoquinone), also known as hennotannic acid, is a red-orange dye present in the leaves of the henna plant (*Lawsonia inermis*), for which it is named, as well as in the flower of water hyacinth (*Eichhornia crassipes*) [4].

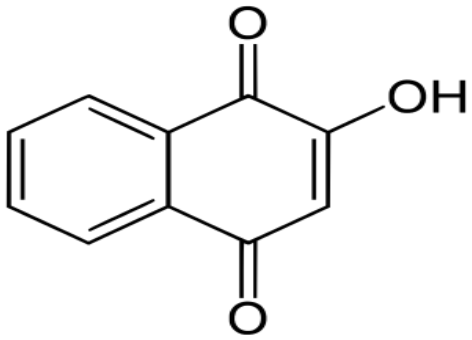

Figure 1.2 Structure of Lawsone

Lawsone is hypothesized to undergo a reaction similar to Strecker synthesis in reactions with amino acid. Recent research has been conducted on lawsone's potential applications in the forensic science field. Since lawsone shows many similarities

with ninhydrin, the current reagent for latent fingerprint development, studies have been conducted to see if lawsone can be used in this field. It has a characteristic purple/brown coloration as opposed to the purple associated with ninhydrin [5]. Lawsone shows promise as a reagent for fingerprint detection because of its photoluminescence maximized at 640 nm, which is high enough that it avoids background interference common for ninhydrin [6].

1.2 **Classification**

Lawsonia inermis, also known as henna , henna tree, mignonette tree, and the Egyptian privet [7].

Kingdom: Plantae

Unranked: Angiosperms

Eudicots

Rosids

Order: Myrtales

Family: Lythraceae

Genus: *Lawsonia*

Species: *L.inermis*

1.3 Types of Henna

1.3.1 Natural Henna or Red henna

This is the real form of henna that leaves a reddish-orange stain on the hair and body. This is pure henna and is green in color. It is used to make a paste by adding natural oils and water into it. Red henna leaves the hair strong and lustrous.

Figure 1.3 Red Henna

1.3.2 Black Henna

Black henna is a black henna dye that consists of Paraphenylenediamine and is very unsafe to use on the skin. It stains black in no time. It is being widely used for dying hair and body art. The adulteration of chemicals can be harmful to the skin and hair [2].

Figure 1.4 Black Henna

1.4 Phytochemicals

Almost a hundred phytoconstituents, representing a variety of classes, have been identified from all parts of *Lawsonia inermis*. Phenolic compounds, including coumarins, flavonoids and naphthoquinones, are particularly prevalent in henna extracts. This abundance of biologically active compounds implies that henna has throughout the millennia diversified its chemical armory to withstand a range of threats [8].

Phytochemical screening of Henna leaf extracts using standard procedures of water, methanol and ethyl acetate revealed the presence of Tannins, Saponins, Flavonoids, Alkaloids, Glycoside, Phenol and Anthraquinones [9, 10].

1.5 MEDICINAL VALUE OF *LAWSONIA INERMIS*

The plant is famous for its anticancer and anti-inflammatory activities. Its bark and seeds are used in the Unani and Ayurveda. The health benefits of Henna are described below:

- The mehndi plant adds nutrition to the hair and clears dandruff.
- *Lawsonia inermis* is useful against heat stroke and used against a headache.
- Henna bark and root are used for the treatment of liver enlargement and jaundice.
- Mehndi leaves are soaked in water and drunken to cure cracking of nails and adds nutrition to the body.
- *Lawsonia inermis* powder with butter cures scabies, mange and swelling. It acts against hair loss and strengthens hair. Hence, henna is used as a natural agent for baldness.

- Because of the astringent nature of mehndi leaves, it is used to cure sunburn and other rashes in the body.
- People suffering from fever or high-temperature use as a home remedy for the treatment.
- It acts as a cooling agent when applied on the burns and wounds. It is also an effective sun block.
- It is used for treatment of arthritis. Massage of *Lawsonia inermis* oil for a month can give relief.
- The bark can help in the treatment of skin diseases.
- Massage of *Lawsonia inermis* oil for a month can give relief.
- The bark can help in the treatment of skin diseases.
- It is useful for the treatment of hemorrhoids or piles.
- It also used against a sore throat.
- Leprosy in early stages can be treated by *Lawsonia inermis.*
- It clears eczema and also kills ringworm.
- It used for the treatment of fungal infection [11]

1.6 Colorimeter

1.6.1 Principles

Colorimeters are used to detect color and determine the solutions concentration, i.e. when a wavelength is passed through a sample, some of the light is absorbed and some passes through. It is the wavelengths of light that pass through that are detected. By knowing which wavelengths have passed through, the detector can also work out which colored wavelengths were absorbed. If the solution to be tested is colorless, a common

procedure is to introduce a reagent that reacts with the solution to produce a colored solution. The results are compared against known standards.

The colorimeter uses the Beer-Lambert law to detect the absorbance of the wavelength. Beer-Lamberts law is written as

$$A = \varepsilon cl$$

Where, A is the absorbance, ε is the molar absorptivity, c is the concentration of the solution and l is the length that the light passes through. Aside from this, if there is a continual changing of the solution, i.e. it is a reaction, and then percentage of transmittance against time is generally used.

To measure concentrations, the amount of light absorbed is dependent upon the amount of solute in the solution- a higher concentration of dissolved solute means that more light will be absorbed, and vice versa, hence, the concentration can be backed out from the absorption of specific wavelengths [12].

Figure 1.5 Colourimeter

LITERATURE REVIEW

Wasim Raja et.al investigated antimicrobial and photochemical properties of *Lawsonia inermis* leaf extract. Antimicrobial activity was evaluated by disk diffusion method by using Gram positive, *B. subtilius, S. aureus and S. epidermidis* and Gram negative; *E. coli, S. flexneri, P. aeruginosa* bacteria. This study showed that methanolic extracts of *Lawsonia inermis* inhibit the growth of micro organisms dose dependently. Phytochemical analysis of the extracts showed the presence of glycosides, phytosterol, steroids, saponins, tannins and flavonoids. The presence of flavonoids and glycosides are commonly known to possess antimicrobial activity. These results confirm the antibacterial activity of *Lawsonia inermis* leaves can be used in therapy of bacterial infection [13].

In Ayurveda and Unani medicine, henna is considered as a source of non-toxic therapeutic agent for blood tonic, cancer, infectious disease, inflammation, tuberculosis, tumors and wounds [14].

L. C. Chuku et.al. studied qualitative phytochemical screening and anti-inflammatory properties of *Lawsonia inermis* leaves extracted with N-butanol and ethyl acetate. Results revealed the presence of flavonoids, terpenes, tannins, cyanogenic and cardiac glycosides. Anti-inflammatory activity was observed on a week old cockerel chicks induced carrageenan inflammation post extract administration and aspirin tablet was used as control. The anti-inflammatory activity of the extract as portrayed due to the plants rich phytochemical potential [15].

Powdered seeds of henna were effective against dysentery and liver disorders. The bark is used in a variety conditions, such as burns, jaundice, spleen enlargement, leprosy, and skin disorders. Roots of *L. inermis* were considered as a potent medicine for some sexually transmitted infections such as gonorrhea and herpes [16].

Lawsone (2-hydroxy-1, 4-naphthoquinone) a key molecules of henna is used as starting material in the synthesis of variety of clinically valuable anticancer drugs such as Atovaquone, Lapachol and Dichloroallyl lawsone [17].

The Phytochemical, proximate and sedative properties of the aqueous crude leaf extract of *Lawsonia inermis* using standard procedures were studied by Audu et.al. The qualitative phytochemical analyses of *L. inermis* revealed the presence of varying proportions of alkaloid, tannin, saponins, cardiac glycosides phenolic and resins, while, the proximate composition includes moisture content (33.2%), , ash (29.9%), crude fibre (21%), crude lipid (12.0%), crude protein (3.38%) and nitrogen free extracts (0.52%). Results revealed that *L. inermis* aqueous crude leaf extract contained diverse phytochemical constituents that caused sedation with adverse consequences on fish opercula ventilation [18].

Using *Lawsonia inermis* seeds has been frequently recommended for the improvement of memory in Iranian Traditional Medicine. In this respect, different fractions of the plant were prepared and evaluated for their in vitro biological assays related to Alzheimer's disease, including acetylcholinesterase and butyrylcholinesterase inhibitory activity as well as metal chelating ability and antioxidant activity. The dichloromethane and ethyl acetate fractions were able to inhibit the BChE selectively with IC50 values of

113.47 and 124.90 μg/mL, respectively, compared with donepezil as the reference drug (IC50 = 1.52 μg/mL). However, all fractions were inactive toward AChE. Phytochemical analysis of the dichloromethane fraction indicated the presence of β-sitosterol [19].

Henna is known to be dangerous to people with glucose-6-phosphate dehydrogenase deficiency, which is more common in males than females. Infants and children of particular ethnic groups, mainly from the Middle East and North Africa, are especially vulnerable [20].

SCOPE

Henna is used for beautifying hands, legs and dyeing of hairs all across the world and especially in Indian Subcontinent. The henna plant is used for various medicinal purposes like dysentery, liver disorders, antimicrobial properties, anti-inflammatory properties. There is immense scope for cultivating henna in India. The plant is also hardy in nature and facilitates easy and low-risk cultivation. Worldwide, there is increasing demand for its leaves and powder, making its cultivating profitable. Literature shows that the presence of alkaloids, flavonoids, glycosides, saponins, tannins, quinines, resins and sterols in the leaf extracts of *Lawsonia inermis* [21].

Henna is mainly used in celebration of special occasions such as weddings and birthdays in the joyous gathering of people. The Henna paste symbolizes good health and prosperity in marriage, and in some cultures, the darker the henna stain, the deeper the love between two individuals.

Objectives

- To prepare different types of henna extracts
- To study the phytochemicals present in these extract
- To study the rate of extraction of the dye in pH solutions from 1 to 13

IV. EXPERIMENTAL SECTION

4.1 Collection of plant leaves

The plant leaves were collected in the month of April from Nazareth, Thoothukudi district, Tamilnadu.

4.2 Preparation of the extract

4.2.1 Hot extract

The henna leaves of about 10 g was taken, boiled with 200mL of distilled water and filtered while hot. It is further used for phytochemical analysis.

4.2.2 Cold extract

The henna leaves of about 10g was taken and it is soaked in 200mL of water for one week. After one week the contents were shaken well with distilled water and filtered. It is further used for phytochemical analysis.

4.2.3 Grind extract

About 10g of the henna leaves were taken and it was grinded well with a morter and a pestle. About 200 ml of water was added and filtered. This extract was used for further analysis

4.3 Test for phytochemicals

4.3.1 Test for phenol compounds

To 2ml of the extract, a few drops of 10% ferric chloride solution is added. A bluish-black or brownish green precipitate indicated the presence of phenol compounds.

4.3.2 Test for saponins

3ml of the extract was diluted to 10ml with distilled water and shaken vigorously for 2minutes. Formation of froth indicated the presence of saponin of the extract.

4.3.3 Test for carbohydrates

The small portion of the extract was mixed with 2ml of Molisch‘s reagent and the mixture was shaken well. Then conc. Sulphuric acid was poured carefully along the side of the test tube. Violet ring at the junction indicated the presence of carbohydrate.

4.3.4 Test for carotenoids

20mL of the extract was mixed 10 ml of ether in a separating funnel and shaken well. After some time the ether layer was taken and con. Sulphuric acid was added. A blue colour showed the presence of carotenoids.

4.3.5 Test for reducing compounds

To the extract equal volumes of Fehling's A and Fehling's B solution was added and warmed on a water bath. The formation of a brick red precipitate depicted the presence of reducing compound.

4.3.6 Test for free anthraquinones

20mL of the extract was mixed 10 ml of ether in a separating funnel and shaken well. Ether layer is separated after some time. To the ether layer con. Sulphuric acid was added. This was then shaken with equal volume of 10% ammonia solution. The presence of a bright pink colour in the aqueous layer indicated the presence of free anthraquinones.

4.3.7 Test for alkaloids

To the filtrate ammonia and stannous chloride was added. Formation of coloured precipitates or turbidity indicates the presence of alkaloids.

4.3.8 Test for flavonoids

To the extract a few drops of 20% sodium hydroxide solution were added. A change to yellow colour which on addition of acid changed to colourless solution depicted the presence of flavonoids.

4.3.9 Test for steroids

10 ml of the ether was mixed with 2ml of extract and conc. Sulphuric acid was added to form lower layer. A reddish yellow colour at the interface was an indicative of the presence of steroids

4.3.10 Test for Glycosides

To the aqueous extract from each plant sample was mixed with 2ml of glacial acetic acid and containning 1 drop of ferric chloride. The above mixture was carefully added to 1ml of conc. Sulphuric acid in another test tube. If cardiac glycoside in present in the

sample, appearance of a brown ring indicates the presence of the cardiac glycoside constituent.

4.3.11 Test for phlobatannins

Then to each plant extract, hydrochloric acid was added and each plant sample was then boiled with the help of Hot plate stirrer. Formation of red coloured precipitate confirmed a positive result.

4.3.12 Test for xanthoprotein

To the extract add 2ml of conc. Nitric acid followed by adding excess of ammonia . The presnce of red orange precipitate indicates the presence of xanthoprotein.

4.4 Study of extraction rate in different pH solutions

Study of extraction rate was carried out at different pH solutions. pH solutions of 1-6 were prepared by using hydrochloric acid. pH solutions from to 13 were prepared by using sodium hydroxide.

About 10 mL of the extract was taken and it was boiled in 100mL of pH 1 solution. After 5 minutes, initial absorbance was measured using colourimeter. Then after 2 minutes, the absorbance was again noted down using colourimeter. This is continued up to 23 minutes.

Similarly extraction rate was studied using colourimeter by using pH solutions from 2 to 13

RESULTS AND DISCUSSION

5.1 Phytochemical Analysis

Phytochemical analysis was carried out with three different extracts and the results are given in Table 5.1.

Table 5.1 Results of the phytochemical analysis

S.No.	PHYTOCHEMICALS ANALYSED	Types of extract		
		Hot Extract	Cold Extract	Grind Extract
1.	Tannins	+	+	+
2.	Saponins	+	+	+
3.	Carbohydrate	+	+	-
4.	Antraquinone	-	-	-
5.	Alkaloid	+	+	+
6.	Flavonoid	+	+	+
7.	Xanthoprotein	+	+	+
8.	Reducing Compound	-	-	-
9.	Phenolic Compound	+	-	+
10.	Glycosides	+	+	+
11.	Phlobatannins	-	-	-
12.	Carotenoid	-	-	-
13.	Triterpenoid	-	-	-
14.	Steroids	+	+	+

Observations

All the *Lawsonia inermis* analysed showed the presence of carbohydrate in both hot and cold extracts. Anthroquinone, Reducing compound, phlobatannins, carotenoid, triterpenoid were not shown in all the extracts. Tannins ,alkaloid, xanthoprotein, flavonoid, glycosides, Saponins and steroids were present in all the three extracts. Alkaloids have anti-inflammatory, anticancer, analgesics, local anesthetic and pain relief, neuropharmacologic, antimicrobial, antifungal, and many other activities. Phytonutrients like flavonoids have beneficial anti-inflammatory effects and they protect your cells from oxidative damage that can lead to disease. These dietary antioxidants can prevent the development of cardiovascular disease, diabetes, cancer, and cognitive diseases like Alzheimer's and dementia. Anthraquinones are not shown in all three extracts of *Lawsonia inermis*. Phenolic Compounds are shown in both hot and grind extract. Tannin components were observed to be anticarcinogenic. Many tannin molecules have also been shown to reduce the mutagenic activity of a number of mutagens. The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation. steroids have so many benefits such that they help to reduce many health problems, such as high blood pressure, allergies, depression, anxiety, osteoporosis, cancer, and diabetes .They also work to increase energy and concentration to help users maintain their maximum daily performance. Saponins exhibit antimicrobial properties, guarding your body against fungi, bacteria and viruses. At the same time, they improve immune function by stimulating the production of T-cells.

5.2 Study of extraction of the dye

The dye from the henna plant was extracted in pH solutions of 1 to 13, there absorbacne values are noted with time and the results are tabulated, and graphs were plotted between absorbance and time.

Table 5.2 Absorbance values in pH 1

Time (minutes)	Absorbance
5	0.15
7	0.24
9	0.43
11	0.59
13	0.6
15	0.66
17	0.73
19	0.84
21	0.97
23	0.99

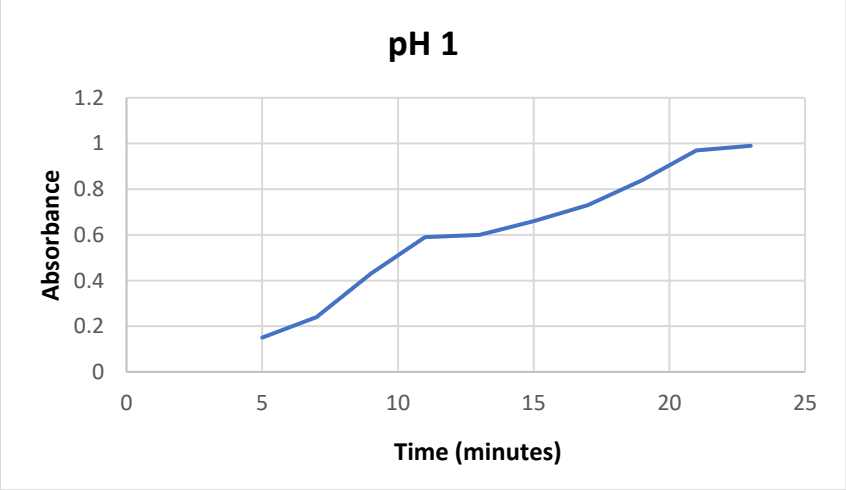

Figure 5.1 Plot of Absorbance vs time (minutes) in pH 1

In this graph we can observe that the absorbance increases in 9 minutes with the absorbance value 0.43 and decreases with 0.6 at 13 minutes. This graph shows a sudden increase and decrease in absorbance**.**

Table 5.3 Absorbance values in pH 2

Time (minutes)	Absorbance
5	0.68
7	0.7
9	0.72
11	0.88
13	0.89
15	0.93
17	0.96
19	0.95
21	0.94
23	0.93

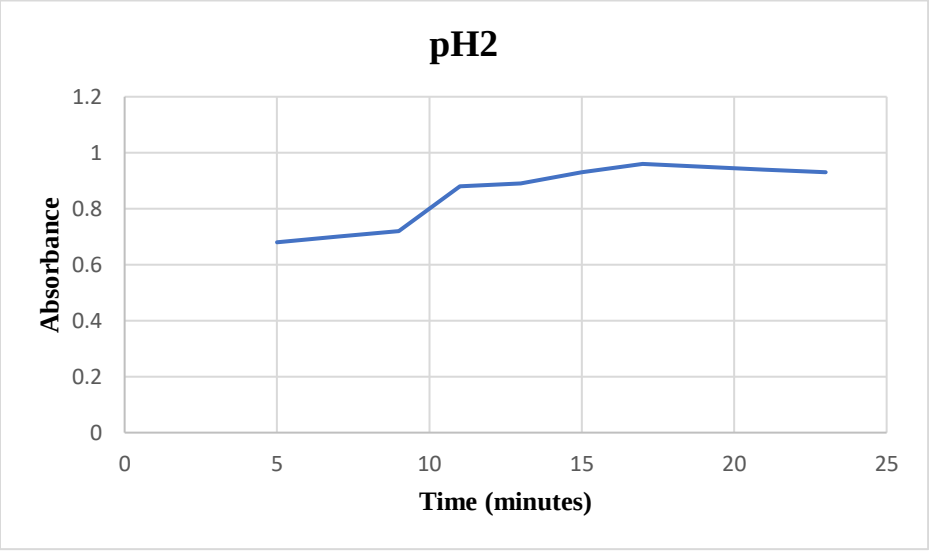

Figure 5.2 Plot of Absorbance vs time (minutes) in pH 2

In this graph the pH value gives a sudden decrease in absorbance value is seen. As we observe the reading of 0.7 in 7 minutes and it increases in the reading of 0.72 in 9 minutes. This graph makes a big difference in the absorbance.

Table 5.4 Absorbance values in pH 3

Time (minutes)	Absorbance
5	0.74
7	0.91
9	0.96
11	0.98
13	0.99
15	0.91
17	0.91
19	1
21	0.84
23	0.9

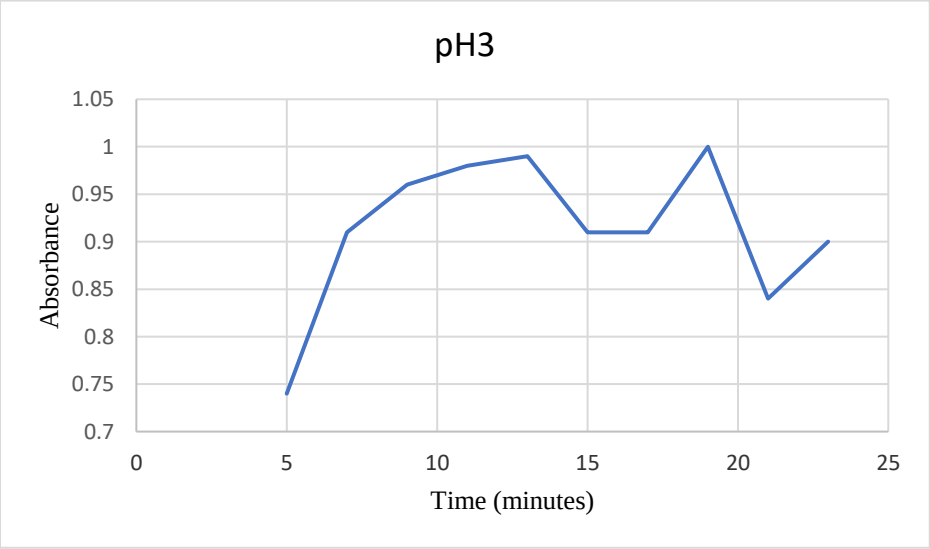

Figure 5.3 Plot of Absorbance vs time (minutes) in pH 3

In this graph we observed that at 15 and 17 min absorbance gives the same value as 0.91 and it increases in the minute of 19 with the reading as 1 and it suddenly decreases in the minute of 21 with the reading of 0.84.

Table 5.5 Absorbance values in pH 4

Time (minutes)	Absorbance
5	0.97
7	0.98
9	1.04
11	1
13	1.01
15	1.07
17	1.12
19	1.11
21	1.12
23	1.15

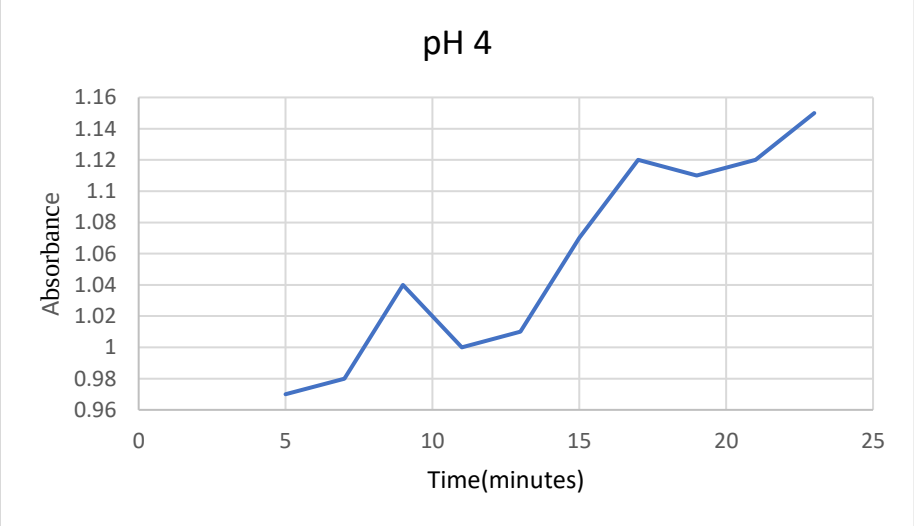

Figure 5.4 Plot of Absorbance vs time (minutes) in pH 4

In this graph we observe that at 13 and 15 min the absorbance gives the literally same and it increases in the minute 17 with the reading as 1.12. It shows the improper curve of increasing and decreasing in absorbance values.

Table 5.6 Absorbance values in pH 5

Time (minutes)	Absorbance
5	0.7
7	0.73
9	0.89
11	0.91
13	0.92
15	0.85
17	0.9
19	0.92
21	0.98
23	1

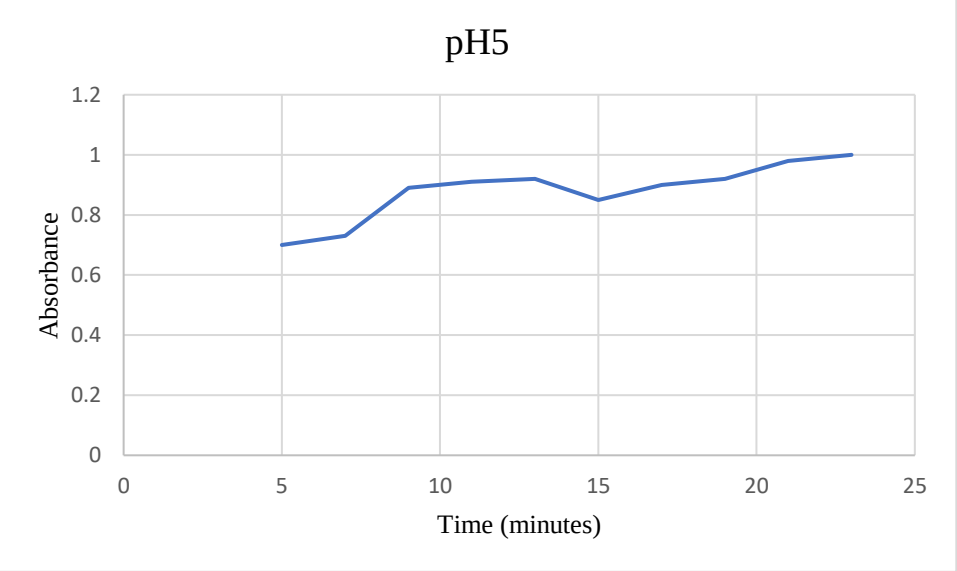

Figure 5.5 Plot of Absorbance vs time (minutes) in pH 5

In this graph we observe the minute of 11 and 13 gives the literally same and it decreases in the minute 15 with the reading as 0.85.

Table 5.7 Absorbance values in pH 6

Time (minutes)	Absorbance
5	0.89
7	0.95
9	1.01
11	1.11
13	1.12
15	1.01
17	1.1
19	1.12
21	1.13
23	1.14

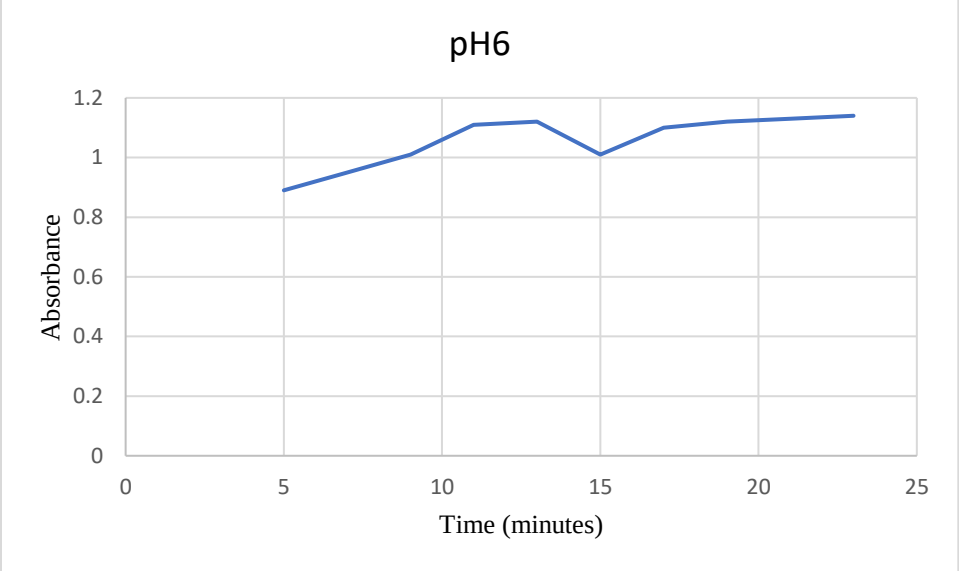

Figure 5.6 Plot of Absorbance vs time (minutes) in pH 6

In this graph we observe the minute of 11 and 13 gives the literally same absorbance value and it decreases in the minute 15 with the reading as 1.01.

Table 5.8 Absorbance values in pH 7

Time (minutes)	Absorbance
5	0.93
7	0.94
9	0.95
11	0.96
13	0.93
15	0.91
17	0.9
19	0.87
21	0.88
23	0.89

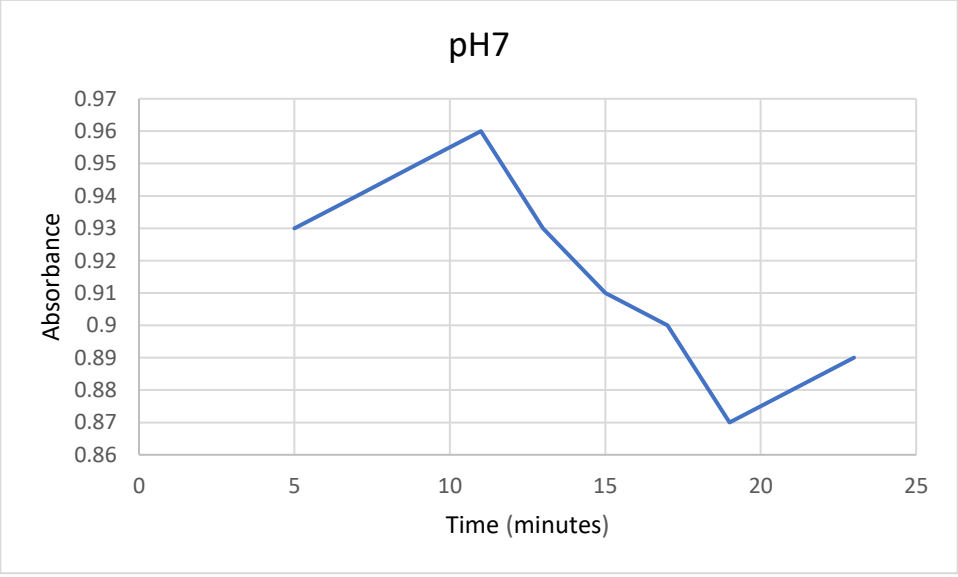

Figure 5.7 Plot of Absorbance vs time (minutes) in pH 7

In this graph we observe the minute of 11 with the reading as 0.96 and it decreases in the minute 19 with the reading as 0.87 and again increases in the minute 23 with the reading as 0.89.

Table 5.9 Absorbance values in pH 8

Time (minutes)	Absorbance
5	0.79
7	0.9
9	0.92
11	0.94
13	0.95
15	0.93
17	0.91
19	0.9
21	0.9
23	0.83

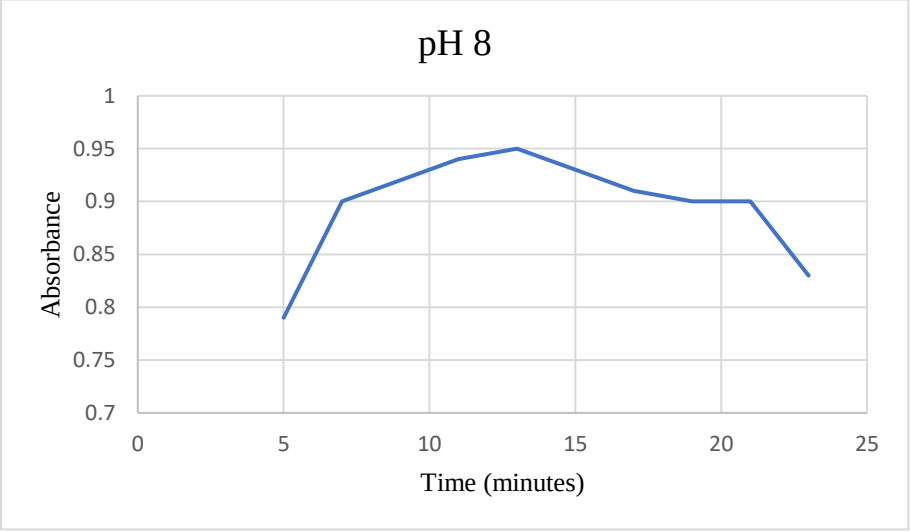

Figure 5.8 Plot of Absorbance vs time (minutes) in pH 8

In this graph we can observe the same reading at the minute of 19 and 21.The reading increases at the minute of 5 to 13 and it decreases at the minute of 15 to 23 of absorbance in relation to time.

Table 5.10 Absorbance values in pH 9

Time (minutes)	Absorbance
5	0.8
7	0.81
9	0.89
11	0.96
13	1
15	1.05
17	0.95
19	0.93
21	0.9
23	0.89

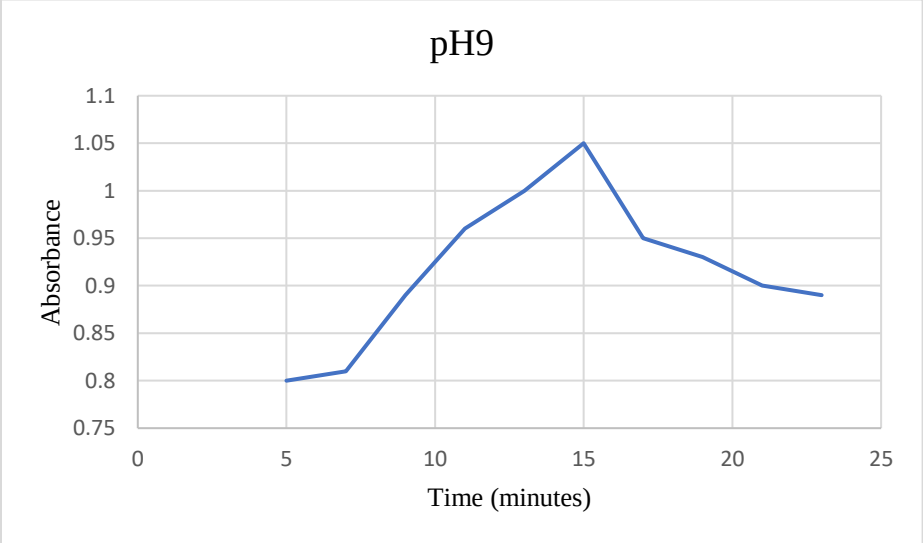

Figure 5.9 Plot of Absorbance vs time (minutes) in pH 9

In this graph there is a great peak at the minute of 15 which has the reading as 0.95 and decreases of all. It increases at the minute of 13 and 15 which gives the reading as 1 and 1.05 and it decreases at the minute of 17 to 23.

Table 5.11 Absorbance values in pH 10

Time (minutes)	Absorbance
5	0.83
7	0.92
9	0.93
11	0.9
13	0.89
15	0.88
17	0.86
19	0.85
21	0.83
23	0.79

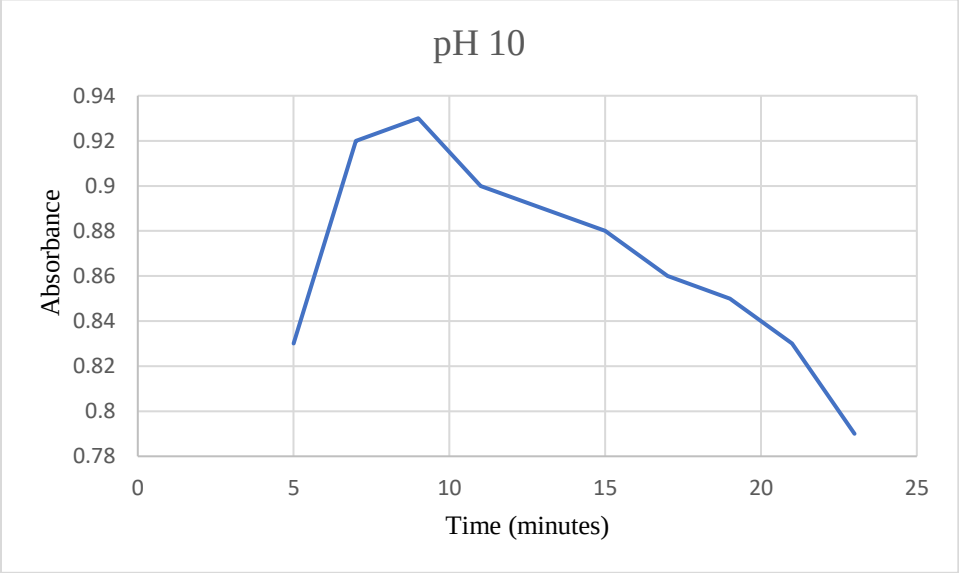

Figure 5.10 Plot of Absorbance vs time (minutes) in pH 10

In this graph we observe the minute of 5 and 7 gives the literally same absobance value and it decreases in the minute 9 with the reading as 0.9. It shows the decreasing in pH values as well.

Table 5.12 Absorbance values in pH 11

Time (minutes)	Absorbance
5	0.97
7	1.04
9	1.06
11	1.05
13	1.03
15	1.01
17	1.02
19	0.99
21	0.97
23	0.95

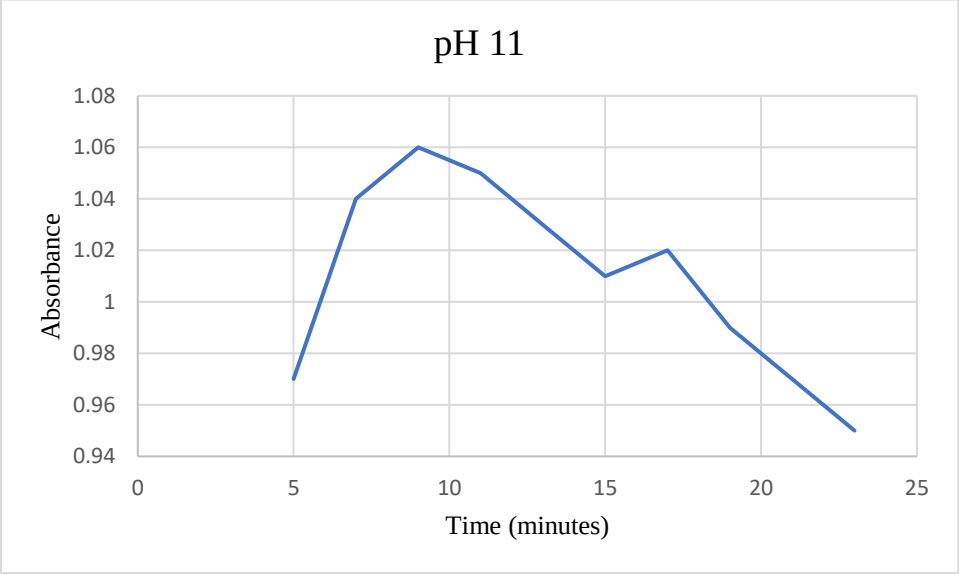

Figure 5.11 Plot of Absorbance vs time (minutes) in pH 11

This graph shows an improper curve as we observe at the minute of, 7, 11 to 15 at the last at the minute of 21.It doesn't represent any proper curve of absorbance in relation to time.

Table 5.13 Absorbance values in pH 12

Time (minutes)	Absorbance
5	0.54
7	1.15
9	1.19
11	1.29
13	1.23
15	1.22
17	1.18
19	1.13
21	1.12
23	1.02

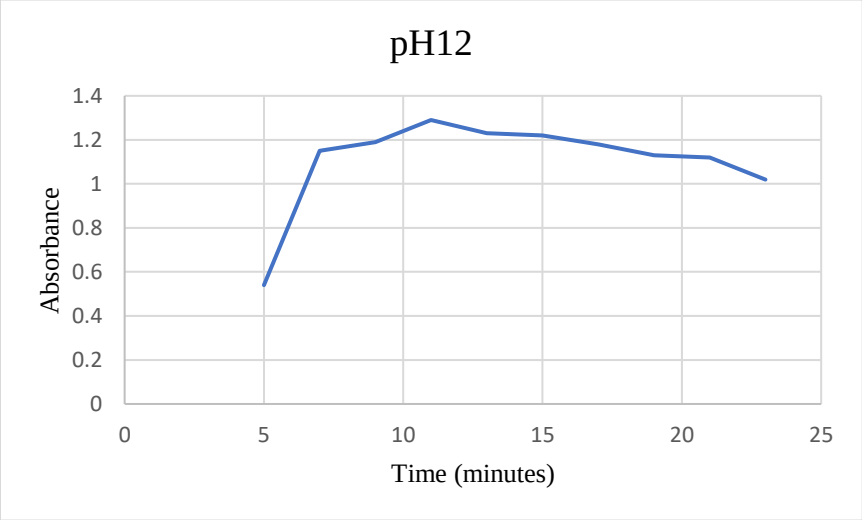

Figure 5.12 Plot of Absorbance vs time (minutes) in pH 12

Graph 12 shows a steady increase at the minute of 5 to 11 with the readings as 0.54, 1.15, 1.19, 1.29 and it decreases at the minute of 13 to 23, which shows the reading as 1.23, 1.22, 1.18, 1.13, 1.12 and 1.04.

Table 5.14 Absorbance values in pH 13

Time (minutes)	Absorbance
5	0.95
7	1.18
9	1.17
11	1.03
13	1.14
15	1.26
17	1.36
19	1.4
21	1.44
23	1.52

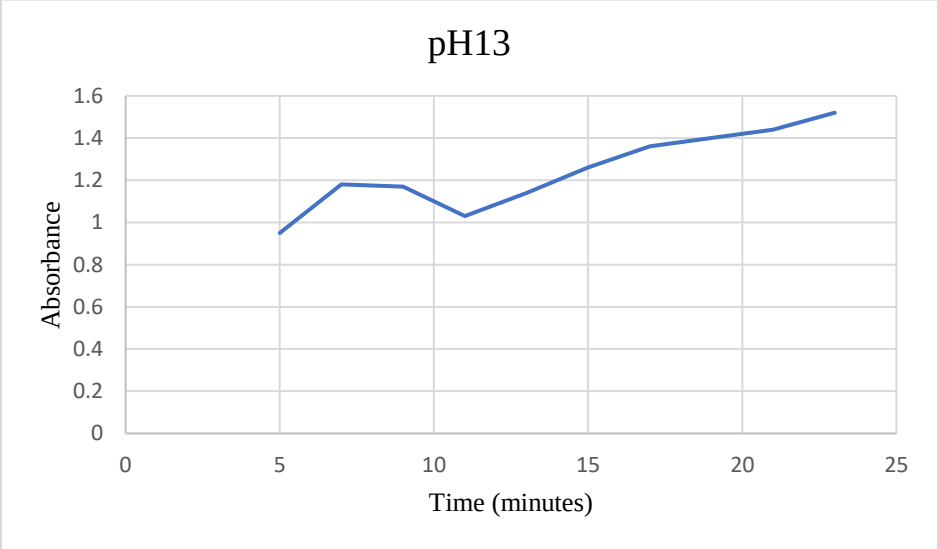

Figure 5.13 Plot of Absorbance vs time (minutes) in pH 13

Graph 13 indicates a sudden increase at the minute of 13 to 23. It increases at a minute of 9 to 11 and increases of all with the reading as 1.14, 1.26, 1.36 and it decreases at the minute of 19 with the readings as 1.4 and it increases at a minute of 21 and 23 with the reading as 1.44 and 1.52.

Overall observations

The above graph shows the absorption of *Lawsonia inermis* in different pH solutions. In pH 1 solution, the absorbance value increases steadily with time. In pH 2 the absorbance values increase much faster than pH 1. The graph from pH 4 to pH 6 (acidic) does not show steady absorbance but at the last readings all the graph shows high absorbance. The pH 7 (neutral) the rate of absorbance first increase and then decrease with time and again increases. The pH 8 to pH 11 does not give any definite pattern. pH 4, pH 10 and pH 11. It shows a steady increase and decreases of the reading. In pH 12 and 13, the absorption value increases faster in the beginning and after it decreases and then increase. Thus, they give us a proper increase in absorbance in relation to time. So, we would recommend that pH 12 and pH 13 gives us a best result in the extraction of dyes.

CONCLUSIONS

Phytochemical analysis were done in three different types of extract from the *Lawsonia inermis* plant collected from Nazareth ,Thoothukudi district in April 2022 and it is found that phytochemicals like saponins, tannins, alkaloid, xanthoprotein are present in the extracts.

Extraction studies showed that in acidic pHs the extraction of dye is more. And in basic pHs, first the extraction of dye was more than acidic pH and difference in the colour was noted. With the increase in the pH of the medium, the rate of extraction increased.

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Investigation of colour intensity of henna extracts with natural and chemical substances by colourimetry

Project in chemistry

Submitted to St. Mary's College (Autonomous), Thoothukudi in partial fulfilment for the award of the degree of **Bachelor of Science** in Chemistry.

Project done by

T. Alphonse Rajam

J. Crossinie

K. Divya Tharshini

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St. Mary's College (Autonomous), Thoothukudi
(Re-accrediated by 'A+' Grade by NAAC)
Thoothukudi- 628001.

2021-2022

DECLARATION

We hearby declare that the project entitled **" Investigation of colour intensity of henna extracts with natural and chemical substances by colourimetry"** submitted to St. Mary's college (Autonomous), Thoothukudi, affiliated to Manonmaniam Sundaranar University, for the Degree of Bachelor of science is our original work and that, it has not previously formed the basis for the award of any Degree, Diploma or similar title.

T.Alphonse Rajam

C.Crossinie

K.Divya Tharshini

S.Maria Sudhanci

S.Varsha Antanitta

May 2022
Thoothukudi

CERTIFICATE

This is to certify that project in chemistry entitled **"Investigation of colour intensity of henna extracts with natural and chemical substances by colourimetry"** is submitted to St. Mary's College (Autonomous), Thoothukudi in partial fulfilment for the award of the Degree of Bachelor of Science in Chemistry and is a record of the work done by the following students during the year 2021-2022.

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CONTENTS

INTRODUCTION

1.1 Introduction

Henna is a reddish-brown or reddish-orange dye made from the old world shrub *Lawsonia inermis*, which produces henna with small pink, red, or white flowers. Henna is used to colour the hair and temporary body art known as mehndi. The dye is extracted from the dried leaf and petioles of the plant [1].

The bark of the henna is gray, brown in color and is smooth. The leaves of the henna plant are medium green in color and can vary a great deal, even on the same plant. The leaves when young, are quite smooth and flat whereas when their life is about to end, they begin to curl and are quite long. Leaves under the flowers are always small and young looking. The leaves are in pairs and differ in sizes from approximately 2-4cm.

The flowers are quite small, (about 1/4th inch), grow in grape formations and are extremely fragrant. It grows best in heat up to 120F levels and stains better in these conditions. It grows better in dry soil and withers in temperatures below 50F degrees [2].

Humans have used henna extracts containing lawsone as hair and skin dyes for more than 5000 years. Lawsone reacts chemically with the protein keratin in skin and hair via Michael addition, resulting in a strong permanent stain that lasts until the skin or hair is shed. The darker colored ink is due to more lawsone-keratin interactions occurring, which break down as the concentration of lawsone decreases and the tattoo fades [3].

Figure 1.1 Henna Plant

Lawsone (2-hydroxy-1,4-naphthoquinone), also known as hennotannic acid, is a red-orange dye present in the leaves of the henna plant (*Lawsonia inermis*), for which it is named, as well as in the flower of water hyacinth (*Eichhornia crassipes*) [4].

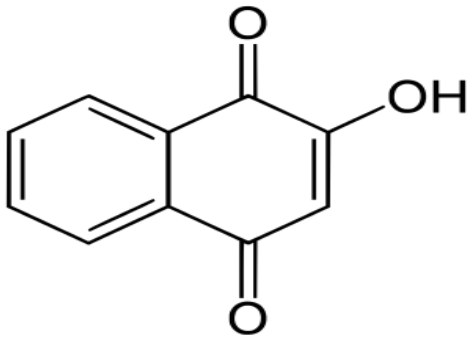

Figure 1.2 Structure of Lawsone

Lawsone is hypothesized to undergo a reaction similar to Strecker synthesis in reactions with amino acid. Recent research has been conducted on lawsone's potential applications in the forensic science field. Since lawsone shows many similarities

with ninhydrin, the current reagent for latent fingerprint development, studies have been conducted to see if lawsone can be used in this field. It has a characteristic purple/brown coloration as opposed to the purple associated with ninhydrin [5]. Lawsone shows promise as a reagent for fingerprint detection because of its photoluminescence maximized at 640 nm, which is high enough that it avoids background interference common for ninhydrin [6].

1.2 **Classification**

Lawsonia inermis, also known as henna , henna tree, mignonette tree, and the Egyptian privet [7].

Kingdom: Plantae

Unranked: Angiosperms

Eudicots

Rosids

Order: Myrtales

Family: Lythraceae

Genus: *Lawsonia*

Species: *L.inermis*

1.3 Types of Henna

1.3.1 Natural Henna or Red henna

This is the real form of henna that leaves a reddish-orange stain on the hair and body. This is pure henna and is green in color. It is used to make a paste by adding natural oils and water into it. Red henna leaves the hair strong and lustrous.

Figure 1.3 Red Henna

1.3.2 Black Henna

Black henna is a black henna dye that consists of Paraphenylenediamine and is very unsafe to use on the skin. It stains black in no time. It is being widely used for dying hair and body art. The adulteration of chemicals can be harmful to the skin and hair [2].

Figure 1.4 Black Henna

1.4 Phytochemicals

Almost a hundred phytoconstituents, representing a variety of classes, have been identified from all parts of *Lawsonia inermis*. Phenolic compounds, including coumarins, flavonoids and naphthoquinones, are particularly prevalent in henna extracts. This abundance of biologically active compounds implies that henna has throughout the millennia diversified its chemical armory to withstand a range of threats [8].

Phytochemical screening of Henna leaf extracts using standard procedures of water, methanol and ethyl acetate revealed the presence of Tannins, Saponins, Flavonoids, Alkaloids, Glycoside, Phenol and Anthraquinones [9, 10].

1.5 MEDICINAL VALUE OF *LAWSONIA INERMIS*

The plant is famous for its anticancer and anti-inflammatory activities. Its bark and seeds are used in the Unani and Ayurveda. The health benefits of Henna are described below:

- The mehndi plant adds nutrition to the hair and clears dandruff.
- *Lawsonia inermis* is useful against heat stroke and used against a headache.
- Henna bark and root are used for the treatment of liver enlargement and jaundice.
- Mehndi leaves are soaked in water and drunken to cure cracking of nails and adds nutrition to the body.
- *Lawsonia inermis* powder with butter cures scabies, mange and swelling. It acts against hair loss and strengthens hair. Hence, henna is used as a natural agent for baldness.

- Because of the astringent nature of mehndi leaves, it is used to cure sunburn and other rashes in the body.
- People suffering from fever or high-temperature use as a home remedy for the treatment.
- It acts as a cooling agent when applied on the burns and wounds. It is also an effective sun block.
- It is used for treatment of arthritis. Massage of *Lawsonia inermis* oil for a month can give relief.
- The bark can help in the treatment of skin diseases.
- Massage of *Lawsonia inermis* oil for a month can give relief.
- The bark can help in the treatment of skin diseases.
- It is useful for the treatment of hemorrhoids or piles.
- It also used against a sore throat.
- Leprosy in early stages can be treated by *Lawsonia inermis.*
- It clears eczema and also kills ringworm.
- It used for the treatment of fungal infection [11]

1.6 Colorimeter

1.6.1 Principles

Colorimeters are used to detect color and determine the solutions concentration, i.e. when a wavelength is passed through a sample, some of the light is absorbed and some passes through. It is the wavelengths of light that pass through that are detected. By knowing which wavelengths have passed through, the detector can also work out which colored wavelengths were absorbed. If the solution to be tested is colorless, a common

procedure is to introduce a reagent that reacts with the solution to produce a colored solution. The results are compared against known standards.

The colorimeter uses the Beer-Lambert law to detect the absorbance of the wavelength. Beer-Lamberts law is written as

$$A = \varepsilon cl$$

Where, A is the absorbance, ε is the molar absorptivity, c is the concentration of the solution and l is the length that the light passes through. Aside from this, if there is a continual changing of the solution, i.e. it is a reaction, and then percentage of transmittance against time is generally used.

To measure concentrations, the amount of light absorbed is dependent upon the amount of solute in the solution- a higher concentration of dissolved solute means that more light will be absorbed, and vice versa, hence, the concentration can be backed out from the absorption of specific wavelengths [12].

Figure 1.5 Colourimeter

LITERATURE REVIEW

Wasim Raja et.al investigated antimicrobial and photochemical properties of *Lawsonia inermis* leaf extract. Antimicrobial activity was evaluated by disk diffusion method by using Gram positive, *B. subtilius, S. aureus and S. epidermidis* and Gram negative; *E. coli, S. flexneri, P. aeruginosa* bacteria. This study showed that methanolic extracts of *Lawsonia inermis* inhibit the growth of micro organisms dose dependently. Phytochemical analysis of the extracts showed the presence of glycosides, phytosterol, steroids, saponins, tannins and flavonoids. The presence of flavonoids and glycosides are commonly known to possess antimicrobial activity. These results confirm the antibacterial activity of *Lawsonia inermis* leaves can be used in therapy of bacterial infection [13].

In Ayurveda and Unani medicine, henna is considered as a source of non-toxic therapeutic agent for blood tonic, cancer, infectious disease, inflammation, tuberculosis, tumors and wounds [14].

L. C. Chuku et.al. studied qualitative phytochemical screening and anti-inflammatory properties of *Lawsonia inermis* leaves extracted with N-butanol and ethyl acetate. Results revealed the presence of flavonoids, terpenes, tannins, cyanogenic and cardiac glycosides. Anti-inflammatory activity was observed on a week old cockerel chicks induced carrageenan inflammation post extract administration and aspirin tablet was used as control. The anti-inflammatory activity of the extract as portrayed due to the plants rich phytochemical potential [15].

Powdered seeds of henna were effective against dysentery and liver disorders. The bark is used in a variety conditions, such as burns, jaundice, spleen enlargement, leprosy, and skin disorders. Roots of *L. inermis* were considered as a potent medicine for some sexually transmitted infections such as gonorrhea and herpes [16].

Lawsone (2-hydroxy-1, 4-naphthoquinone) a key molecules of henna is used as starting material in the synthesis of variety of clinically valuable anticancer drugs such as Atovaquone, Lapachol and Dichloroallyl lawsone [17].

The Phytochemical, proximate and sedative properties of the aqueous crude leaf extract of *Lawsonia inermis* using standard procedures were studied by Audu et.al. The qualitative phytochemical analyses of *L. inermis* revealed the presence of varying proportions of alkaloid, tannin, saponins, cardiac glycosides phenolic and resins, while, the proximate composition includes moisture content (33.2%), , ash (29.9%), crude fibre (21%), crude lipid (12.0%), crude protein (3.38%) and nitrogen free extracts (0.52%). Results revealed that *L. inermis* aqueous crude leaf extract contained diverse phytochemical constituents that caused sedation with adverse consequences on fish opercula ventilation [18].

Using *Lawsonia inermis* seeds has been frequently recommended for the improvement of memory in Iranian Traditional Medicine. In this respect, different fractions of the plant were prepared and evaluated for their in vitro biological assays related to Alzheimer's disease, including acetylcholinesterase and butyrylcholinesterase inhibitory activity as well as metal chelating ability and antioxidant activity. The dichloromethane and ethyl acetate fractions were able to inhibit the BChE selectively with IC50 values of

113.47 and 124.90 μg/mL, respectively, compared with donepezil as the reference drug (IC50 = 1.52 μg/mL). However, all fractions were inactive toward AChE. Phytochemical analysis of the dichloromethane fraction indicated the presence of β-sitosterol [19].

Henna is known to be dangerous to people with glucose-6-phosphate dehydrogenase deficiency, which is more common in males than females. Infants and children of particular ethnic groups, mainly from the Middle East and North Africa, are especially vulnerable [20].

SCOPE

Henna is used for beautifying hands, legs and dyeing of hairs all across the world and especially in Indian Subcontinent. The henna plant is used for various medicinal purposes like dysentery, liver disorders, antimicrobial properties, anti-inflammatory properties. There is immense scope for cultivating henna in India. The plant is also hardy in nature and facilitates easy and low-risk cultivation. Worldwide, there is increasing demand for its leaves and powder, making its cultivating profitable. Literature shows that the presence of alkaloids, flavonoids, glycosides, saponins, tannins, quinines, resins and sterols in the leaf extracts of *Lawsonia inermis* [21].

Henna is mainly used in celebration of special occasions such as weddings and birthdays in the joyous gathering of people. The Henna paste symbolizes good health and prosperity in marriage, and in some cultures, the darker the henna stain, the deeper the love between two individuals.

Objectives

- To prepare different types of henna extracts
- To study the phytochemicals present in these extract
- To study the rate of extraction of the dye in pH solutions from 1 to 13

IV. EXPERIMENTAL SECTION

4.1 Collection of plant leaves

The plant leaves were collected in the month of April from Nazareth, Thoothukudi district, Tamilnadu.

4.2 Preparation of the extract

4.2.1 Hot extract

The henna leaves of about 10 g was taken, boiled with 200mL of distilled water and filtered while hot. It is further used for phytochemical analysis.

4.2.2 Cold extract

The henna leaves of about 10g was taken and it is soaked in 200mL of water for one week. After one week the contents were shaken well with distilled water and filtered. It is further used for phytochemical analysis.

4.2.3 Grind extract

About 10g of the henna leaves were taken and it was grinded well with a morter and a pestle. About 200 ml of water was added and filtered. This extract was used for further analysis

4.3 Test for phytochemicals

4.3.1 Test for phenol compounds

To 2ml of the extract, a few drops of 10% ferric chloride solution is added. A bluish-black or brownish green precipitate indicated the presence of phenol compounds.

4.3.2 Test for saponins

3ml of the extract was diluted to 10ml with distilled water and shaken vigorously for 2minutes. Formation of froth indicated the presence of saponin of the extract.

4.3.3 Test for carbohydrates

The small portion of the extract was mixed with 2ml of Molisch‘s reagent and the mixture was shaken well. Then conc. Sulphuric acid was poured carefully along the side of the test tube. Violet ring at the junction indicated the presence of carbohydrate.

4.3.4 Test for carotenoids

20mL of the extract was mixed 10 ml of ether in a separating funnel and shaken well. After some time the ether layer was taken and con. Sulphuric acid was added. A blue colour showed the presence of carotenoids.

4.3.5 Test for reducing compounds

To the extract equal volumes of Fehling's A and Fehling's B solution was added and warmed on a water bath. The formation of a brick red precipitate depicted the presence of reducing compound.

4.3.6 Test for free anthraquinones

20mL of the extract was mixed 10 ml of ether in a separating funnel and shaken well. Ether layer is separated after some time. To the ether layer con. Sulphuric acid was added. This was then shaken with equal volume of 10% ammonia solution. The presence of a bright pink colour in the aqueous layer indicated the presence of free anthraquinones.

4.3.7 Test for alkaloids

To the filtrate ammonia and stannous chloride was added. Formation of coloured precipitates or turbidity indicates the presence of alkaloids.

4.3.8 Test for flavonoids

To the extract a few drops of 20% sodium hydroxide solution were added. A change to yellow colour which on addition of acid changed to colourless solution depicted the presence of flavonoids.

4.3.9 Test for steroids

10 ml of the ether was mixed with 2ml of extract and conc. Sulphuric acid was added to form lower layer. A reddish yellow colour at the interface was an indicative of the presence of steroids

4.3.10 Test for Glycosides

To the aqueous extract from each plant sample was mixed with 2ml of glacial acetic acid and containning 1 drop of ferric chloride. The above mixture was carefully added to 1ml of conc. Sulphuric acid in another test tube. If cardiac glycoside in present in the

sample, appearance of a brown ring indicates the presence of the cardiac glycoside constituent.

4.3.11 Test for phlobatannins

Then to each plant extract, hydrochloric acid was added and each plant sample was then boiled with the help of Hot plate stirrer. Formation of red coloured precipitate confirmed a positive result.

4.3.12 Test for xanthoprotein

To the extract add 2ml of conc. Nitric acid followed by adding excess of ammonia . The presnce of red orange precipitate indicates the presence of xanthoprotein.

4.4 Study of extraction rate in different pH solutions

Study of extraction rate was carried out at different pH solutions. pH solutions of 1-6 were prepared by using hydrochloric acid. pH solutions from to 13 were prepared by using sodium hydroxide.

About 10 mL of the extract was taken and it was boiled in 100mL of pH 1 solution. After 5 minutes, initial absorbance was measured using colourimeter. Then after 2 minutes, the absorbance was again noted down using colourimeter. This is continued up to 23 minutes.

Similarly extraction rate was studied using colourimeter by using pH solutions from 2 to 13

RESULTS AND DISCUSSION

5.1 Phytochemical Analysis

Phytochemical analysis was carried out with three different extracts and the results are given in Table 5.1.

Table 5.1 Results of the phytochemical analysis

S.No.	PHYTOCHEMICALS ANALYSED	Types of extract		
		Hot Extract	Cold Extract	Grind Extract
1.	Tannins	+	+	+
2.	Saponins	+	+	+
3.	Carbohydrate	+	+	-
4.	Antraquinone	-	-	-
5.	Alkaloid	+	+	+
6.	Flavonoid	+	+	+
7.	Xanthoprotein	+	+	+
8.	Reducing Compound	-	-	-
9.	Phenolic Compound	+	-	+
10.	Glycosides	+	+	+
11.	Phlobatannins	-	-	-
12.	Carotenoid	-	-	-
13.	Triterpenoid	-	-	-
14.	Steroids	+	+	+

Observations

All the *Lawsonia inermis* analysed showed the presence of carbohydrate in both hot and cold extracts. Anthroquinone, Reducing compound, phlobatannins, carotenoid, triterpenoid were not shown in all the extracts. Tannins ,alkaloid, xanthoprotein, flavonoid, glycosides, Saponins and steroids were present in all the three extracts. Alkaloids have anti-inflammatory, anticancer, analgesics, local anesthetic and pain relief, neuropharmacologic, antimicrobial, antifungal, and many other activities. Phytonutrients like flavonoids have beneficial anti-inflammatory effects and they protect your cells from oxidative damage that can lead to disease. These dietary antioxidants can prevent the development of cardiovascular disease, diabetes, cancer, and cognitive diseases like Alzheimer's and dementia. Anthraquinones are not shown in all three extracts of *Lawsonia inermis*. Phenolic Compounds are shown in both hot and grind extract. Tannin components were observed to be anticarcinogenic. Many tannin molecules have also been shown to reduce the mutagenic activity of a number of mutagens. The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation. steroids have so many benefits such that they help to reduce many health problems, such as high blood pressure, allergies, depression, anxiety, osteoporosis, cancer, and diabetes .They also work to increase energy and concentration to help users maintain their maximum daily performance. Saponins exhibit antimicrobial properties, guarding your body against fungi, bacteria and viruses. At the same time, they improve immune function by stimulating the production of T-cells.

5.2 Study of extraction of the dye

The dye from the henna plant was extracted in pH solutions of 1 to 13, there absorbacne values are noted with time and the results are tabulated, and graphs were plotted between absorbance and time.

Table 5.2 Absorbance values in pH 1

Time (minutes)	Absorbance
5	0.15
7	0.24
9	0.43
11	0.59
13	0.6
15	0.66
17	0.73
19	0.84
21	0.97
23	0.99

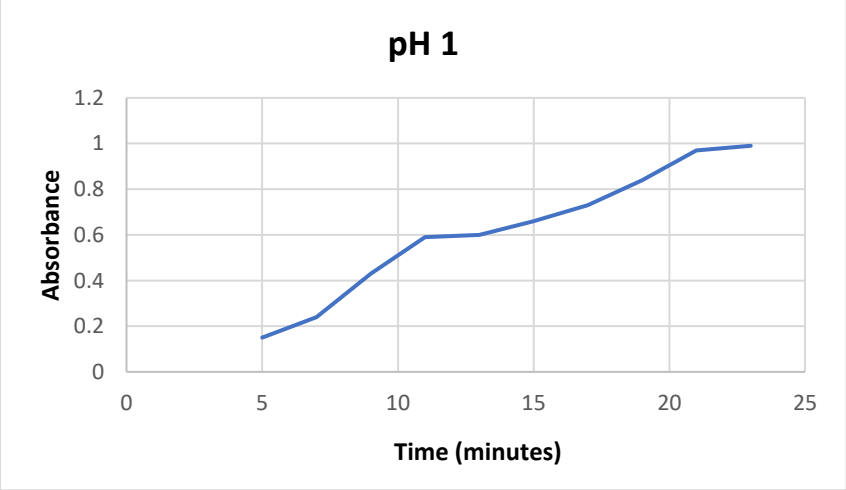

Figure 5.1 Plot of Absorbance vs time (minutes) in pH 1

In this graph we can observe that the absorbance increases in 9 minutes with the absorbance value 0.43 and decreases with 0.6 at 13 minutes. This graph shows a sudden increase and decrease in absorbance**.**

Table 5.3 Absorbance values in pH 2

Time (minutes)	Absorbance
5	0.68
7	0.7
9	0.72
11	0.88
13	0.89
15	0.93
17	0.96
19	0.95
21	0.94
23	0.93

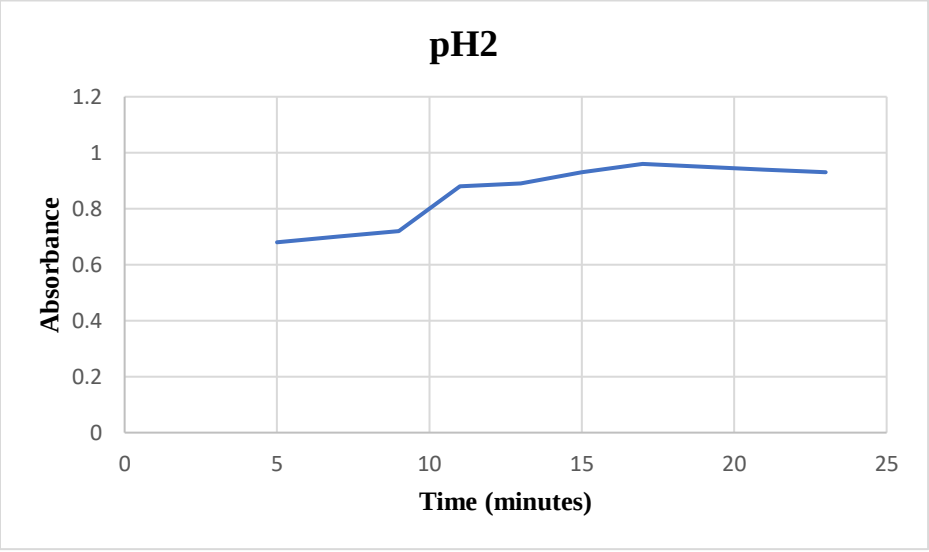

Figure 5.2 Plot of Absorbance vs time (minutes) in pH 2

In this graph the pH value gives a sudden decrease in absorbance value is seen. As we observe the reading of 0.7 in 7 minutes and it increases in the reading of 0.72 in 9 minutes. This graph makes a big difference in the absorbance.

Table 5.4 Absorbance values in pH 3

Time (minutes)	Absorbance
5	0.74
7	0.91
9	0.96
11	0.98
13	0.99
15	0.91
17	0.91
19	1
21	0.84
23	0.9

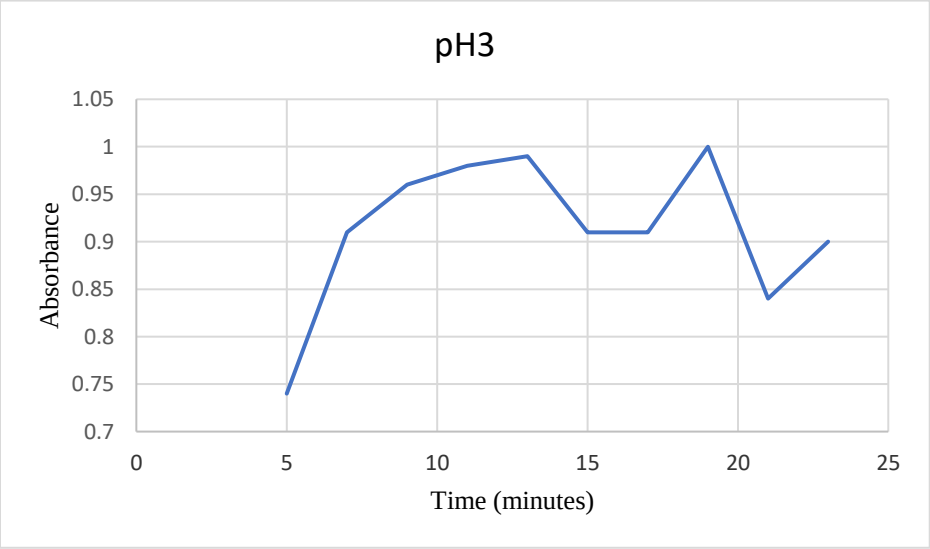

Figure 5.3 Plot of Absorbance vs time (minutes) in pH 3

In this graph we observed that at 15 and 17 min absorbance gives the same value as 0.91 and it increases in the minute of 19 with the reading as 1 and it suddenly decreases in the minute of 21 with the reading of 0.84.

Table 5.5 Absorbance values in pH 4

Time (minutes)	Absorbance
5	0.97
7	0.98
9	1.04
11	1
13	1.01
15	1.07
17	1.12
19	1.11
21	1.12
23	1.15

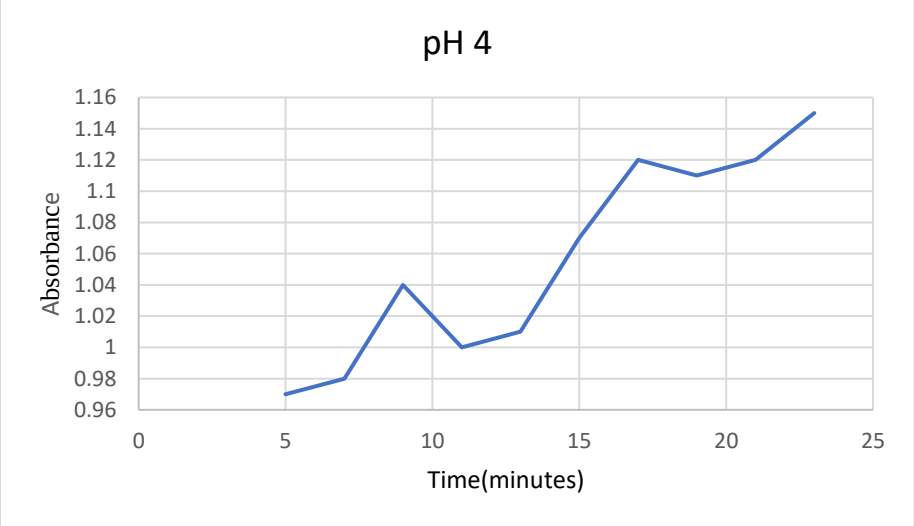

Figure 5.4 Plot of Absorbance vs time (minutes) in pH 4

In this graph we observe that at 13 and 15 min the absorbance gives the literally same and it increases in the minute 17 with the reading as 1.12. It shows the improper curve of increasing and decreasing in absorbance values.

Table 5.6 Absorbance values in pH 5

Time (minutes)	Absorbance
5	0.7
7	0.73
9	0.89
11	0.91
13	0.92
15	0.85
17	0.9
19	0.92
21	0.98
23	1

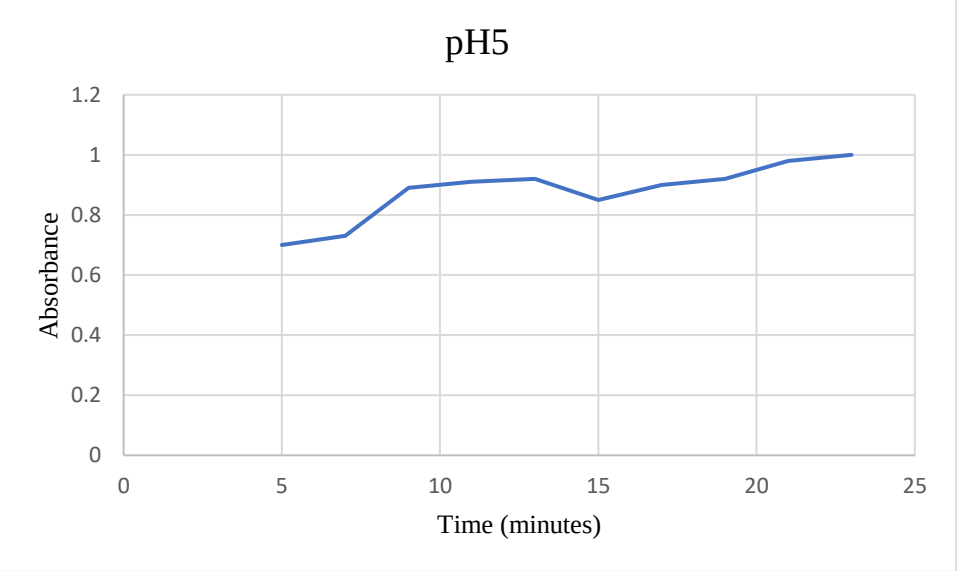

Figure 5.5 Plot of Absorbance vs time (minutes) in pH 5

In this graph we observe the minute of 11 and 13 gives the literally same and it decreases in the minute 15 with the reading as 0.85.

Table 5.7 Absorbance values in pH 6

Time (minutes)	Absorbance
5	0.89
7	0.95
9	1.01
11	1.11
13	1.12
15	1.01
17	1.1
19	1.12
21	1.13
23	1.14

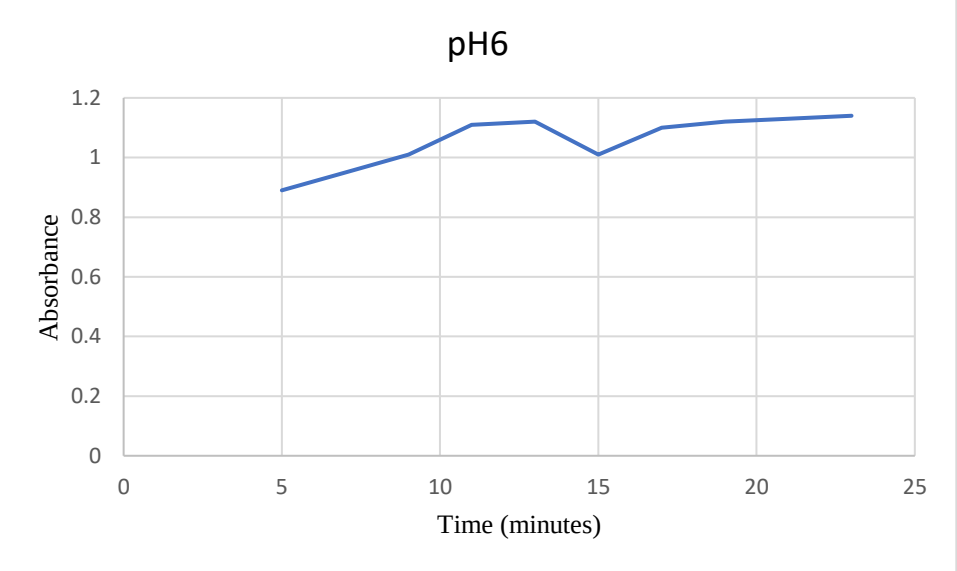

Figure 5.6 Plot of Absorbance vs time (minutes) in pH 6

In this graph we observe the minute of 11 and 13 gives the literally same absorbance value and it decreases in the minute 15 with the reading as 1.01.

Table 5.8 Absorbance values in pH 7

Time (minutes)	Absorbance
5	0.93
7	0.94
9	0.95
11	0.96
13	0.93
15	0.91
17	0.9
19	0.87
21	0.88
23	0.89

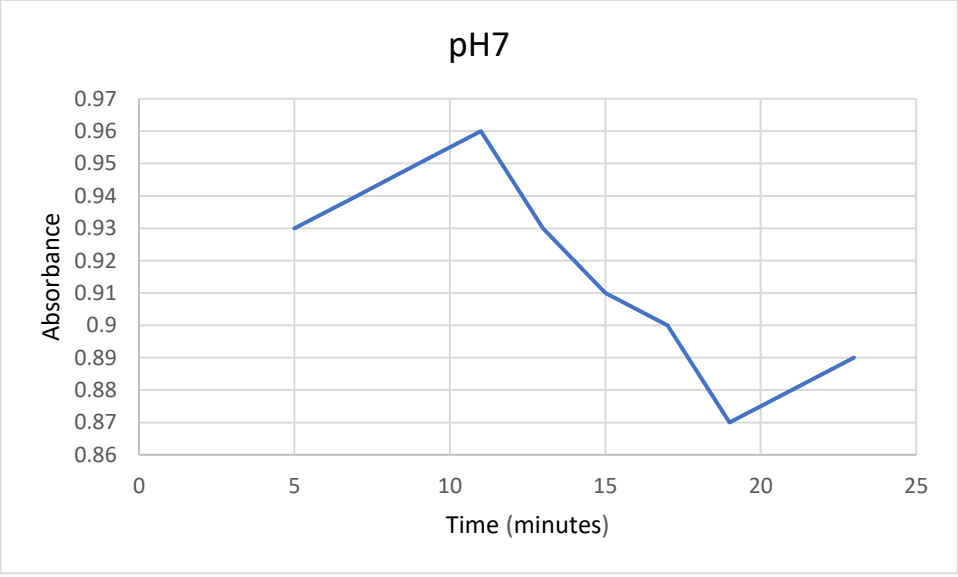

Figure 5.7 Plot of Absorbance vs time (minutes) in pH 7

In this graph we observe the minute of 11 with the reading as 0.96 and it decreases in the minute 19 with the reading as 0.87 and again increases in the minute 23 with the reading as 0.89.

Table 5.9 Absorbance values in pH 8

Time (minutes)	Absorbance
5	0.79
7	0.9
9	0.92
11	0.94
13	0.95
15	0.93
17	0.91
19	0.9
21	0.9
23	0.83

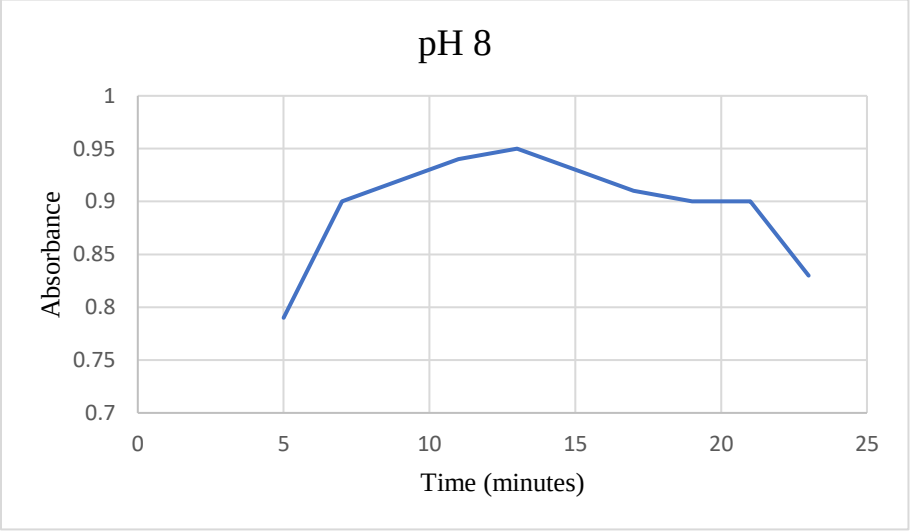

Figure 5.8 Plot of Absorbance vs time (minutes) in pH 8

In this graph we can observe the same reading at the minute of 19 and 21.The reading increases at the minute of 5 to 13 and it decreases at the minute of 15 to 23 of absorbance in relation to time.

Table 5.10 Absorbance values in pH 9

Time (minutes)	Absorbance
5	0.8
7	0.81
9	0.89
11	0.96
13	1
15	1.05
17	0.95
19	0.93
21	0.9
23	0.89

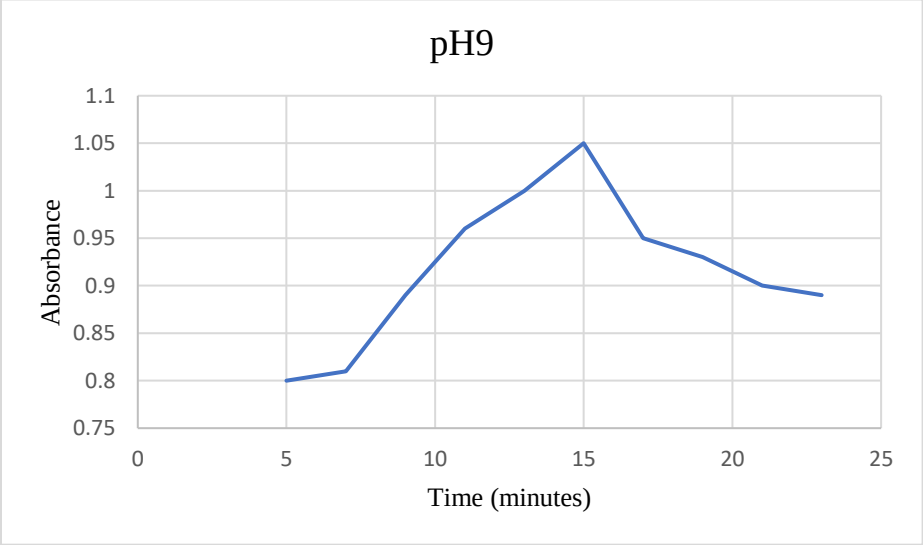

Figure 5.9 Plot of Absorbance vs time (minutes) in pH 9

In this graph there is a great peak at the minute of 15 which has the reading as 0.95 and decreases of all. It increases at the minute of 13 and 15 which gives the reading as 1 and 1.05 and it decreases at the minute of 17 to 23.

Table 5.11 Absorbance values in pH 10

Time (minutes)	Absorbance
5	0.83
7	0.92
9	0.93
11	0.9
13	0.89
15	0.88
17	0.86
19	0.85
21	0.83
23	0.79

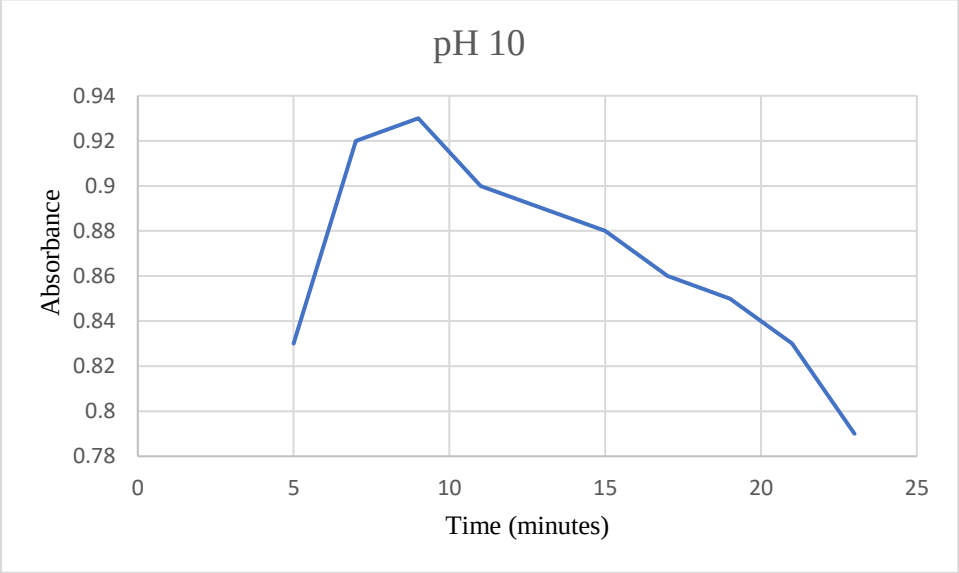

Figure 5.10 Plot of Absorbance vs time (minutes) in pH 10

In this graph we observe the minute of 5 and 7 gives the literally same absobance value and it decreases in the minute 9 with the reading as 0.9. It shows the decreasing in pH values as well.

Table 5.12 Absorbance values in pH 11

Time (minutes)	Absorbance
5	0.97
7	1.04
9	1.06
11	1.05
13	1.03
15	1.01
17	1.02
19	0.99
21	0.97
23	0.95

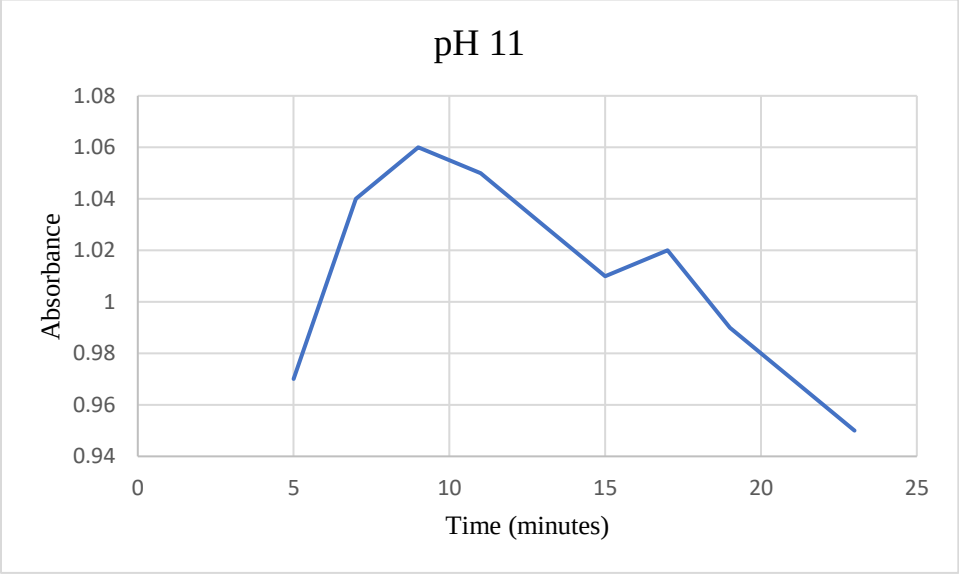

Figure 5.11 Plot of Absorbance vs time (minutes) in pH 11

This graph shows an improper curve as we observe at the minute of, 7, 11 to 15 at the last at the minute of 21.It doesn't represent any proper curve of absorbance in relation to time.

Table 5.13 Absorbance values in pH 12

Time (minutes)	Absorbance
5	0.54
7	1.15
9	1.19
11	1.29
13	1.23
15	1.22
17	1.18
19	1.13
21	1.12
23	1.02

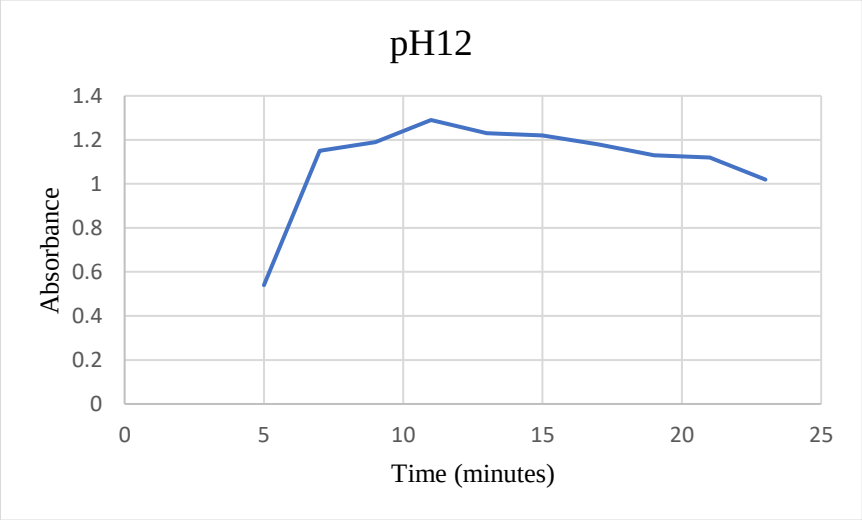

Figure 5.12 Plot of Absorbance vs time (minutes) in pH 12

Graph 12 shows a steady increase at the minute of 5 to 11 with the readings as 0.54, 1.15, 1.19, 1.29 and it decreases at the minute of 13 to 23, which shows the reading as 1.23, 1.22, 1.18, 1.13, 1.12 and 1.04.

Table 5.14 Absorbance values in pH 13

Time (minutes)	Absorbance
5	0.95
7	1.18
9	1.17
11	1.03
13	1.14
15	1.26
17	1.36
19	1.4
21	1.44
23	1.52

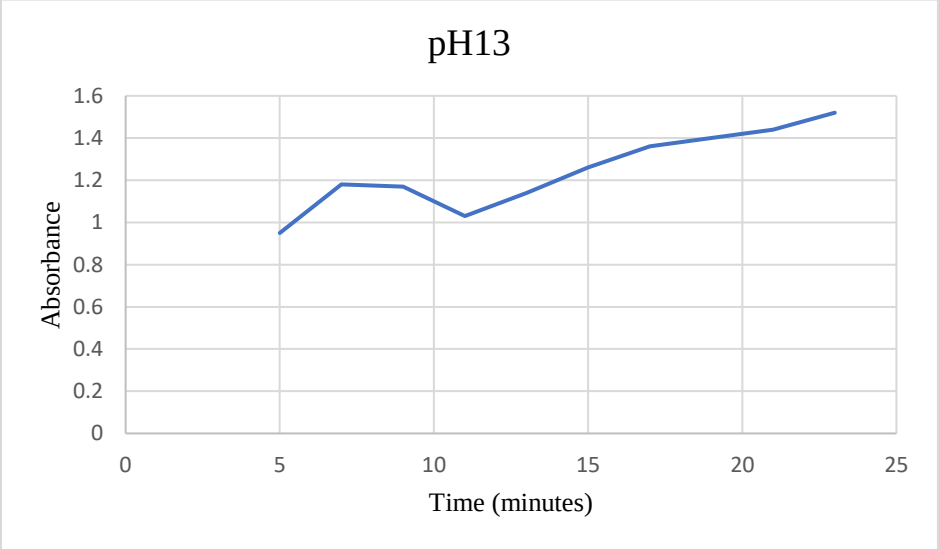

Figure 5.13 Plot of Absorbance vs time (minutes) in pH 13

Graph 13 indicates a sudden increase at the minute of 13 to 23. It increases at a minute of 9 to 11 and increases of all with the reading as 1.14, 1.26, 1.36 and it decreases at the minute of 19 with the readings as 1.4 and it increases at a minute of 21 and 23 with the reading as 1.44 and 1.52.

Overall observations

The above graph shows the absorption of *Lawsonia inermis* in different pH solutions. In pH 1 solution, the absorbance value increases steadily with time. In pH 2 the absorbance values increase much faster than pH 1. The graph from pH 4 to pH 6 (acidic) does not show steady absorbance but at the last readings all the graph shows high absorbance. The pH 7 (neutral) the rate of absorbance first increase and then decrease with time and again increases. The pH 8 to pH 11 does not give any definite pattern. pH 4, pH 10 and pH 11. It shows a steady increase and decreases of the reading. In pH 12 and 13, the absorption value increases faster in the beginning and after it decreases and then increase. Thus, they give us a proper increase in absorbance in relation to time. So, we would recommend that pH 12 and pH 13 gives us a best result in the extraction of dyes.

CONCLUSIONS

Phytochemical analysis were done in three different types of extract from the *Lawsonia inermis* plant collected from Nazareth ,Thoothukudi district in April 2022 and it is found that phytochemicals like saponins, tannins, alkaloid, xanthoprotein are present in the extracts.

Extraction studies showed that in acidic pHs the extraction of dye is more. And in basic pHs, first the extraction of dye was more than acidic pH and difference in the colour was noted. With the increase in the pH of the medium, the rate of extraction increased.

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Synthesis and Characterisation of L-Arginine and L-Cysteine Doped Nickel Sulphate Single Crystals

PROJECT IN CHEMISTRY

Submitted to St. Mary's College (Autonomous), Thoothukudi, in partial fulfilment for the award of the degree of **Bachelor of Science** in Chemistry.

Project done by

J. Arockiya Mashina

R. Mandhira Priya

A. Mercy

A. Panimaya Rosy

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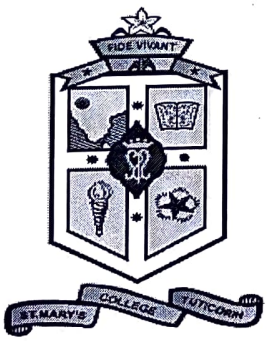

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2021-2022

DECLARATION

We hereby declare that the project entitled **"Synthesis and Characterisation of L-Arginine and L-Cysteine Doped Nickel Sulphate Single Crystals"** submitted to St. Mary's college (Autonomous), Thoothukudi, affiliated to Manonmaniam Sundaranar University, for the Degree of Bachelor of Science is our original work and that, it has not previously formed the basis for the award of any Degree, Diploma or similar title.

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CERTIFICATE

This is to certify that the project in Chemistry entitled **"Synthesis and Characterisation of L-Arginine and L-Cysteine Doped Nickel Sulphate Single Crystals"** is submitted to St.Mary's College (Autonomous),Thoothukudi, in partial fulfilment for the award of the Degree of Bachelor of Science in Chemistry and is a record of the work done by the following students during the year 2021-2022.

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CONTENTS

INTRODUCTION

1.Solid

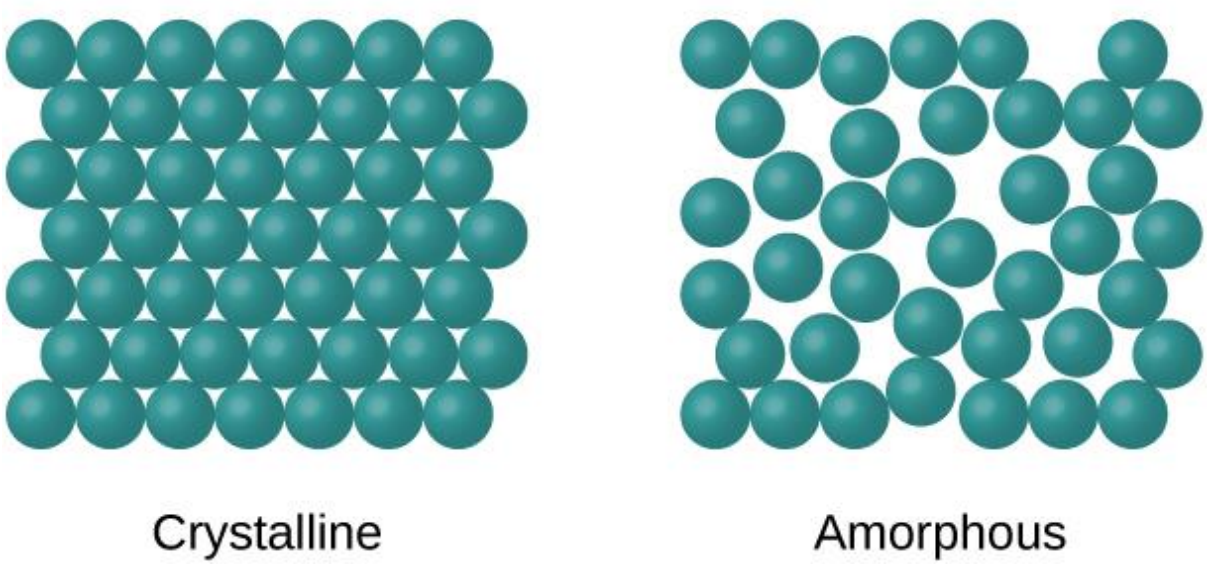
Solid is one of the four fundamental states of matter. It is characterized by structural rigidity and resistance to change shape or volume.

1.1 Classification of solid

Solid can be divided into two types

(i) Crystalline solids

(ii) Amorphous solids



(i) Crystalline solids

It is three dimensional analogs of a brick wall. They have a regular structure in which the particles pack in repeating pattern from one edge of the solid to the other.

Ex. Metals and ordinary ice

(ii) Amorphous solid

In amorphous solids atom are arranged in an irregular fashion.

Ex. Glass, rubber and plastics.

1.2 Types of crystalline material

Generally crystalline materials can be classified as

(i) Single crystals

(ii) Poly crystalline materials

(iii) Liquid crystals

(i) Single crystal

When the periodicity of the pattern extends throughout a certain piece of material one speaks of a single crystal.

(ii) Poly crystalline materials

In poly crystalline material the periodicity of structure is interrupted at grain boundaries. The size of the grain in which the structure is periodic may vary atom macroscopic dimension to several in any system. When the size of the grains or crystalline becomes comparable to the size of the pattern unit it is amorphous substances.

(iii) Liquid crystals

In another class of crystals, there is only two or more dimensional regularity via the "liquid crystals" such substance actually flow and will rise in a capillary tube.

1.3 Single-crystal

A single crystal is defined as a crystal consisting of a noninterrupted repetition of the unit cell in three dimensions. The single-crystal XRD method provides information on the structure of the unit cell, bond lengths and angles, molecular conformation, molecular packing, hydrogen bonding pattern, density, and crystal disorder. To acquire data from a single crystal, the sample is mounted, and a narrow beam of X-rays is passed through it and diffracts against the sample, and this diffracted beam is collected on the detector. Calculated powder-diffraction patterns from single-crystal data provide information on preferred orientation and phase purity. Phase purity refers to the presence of a single dominant crystalline phase, which is a desirable attribute for an API. Phase impurities are typically low levels of other forms that can be present along with the desired form and impact the stability and potentially the performance of the DP. Single-crystal X-ray determination offers a powerful tool to assess the phase purity.

Determining the crystal structure using this approach is the most common and accurate way of understanding the three-dimensional structure, and it is important to understand the physical properties of one crystalline form versus another. It also shows the intermolecular interactions of the atoms within the crystal. The XRD pattern obtained using the single-structure analysis serves as a reference diffractogram The technique does rely on obtaining high-quality crystals of adequate size and hence needs crystals grown to be able to get crystal structure.

1.3.1 Types of crystal system

In general seven crystal systems are available and they are explained as follows

i) Cubic crystal system

In this crystal system, all the unit cell edge lengths are equal and are at right angles to one another i.e., $a = b = c$ and $\alpha = \beta = \gamma = 90°$. In cubic system, there are three Bravais lattices;

they are simple (primitive); body centered and face centered. Examples for cubic system are Au, Cu, Ag, NaCl, diamond, etc. In simple cubic lattice, lattice points or atoms are present at the corners of the cube. In body-centered cube, atoms are present at the corners and one atom is completely present at the centre of the cube. In the case of face-centered cube, atoms are present at corners and at the centres of all faces of cube

ii) Tetragonal crystal system

In this crystal system, two lengths of the unit cell edges are equal where as the third length is different. The three edges are perpendicular to one another i.e., $a = b \neq c$ and $\alpha = \beta = \gamma = 90°$. In tetragonal system, there are two bravais lattices; they are simple and body-centered. Examples for tetragonal crystal systems are TiO_2, SnO_2, etc.,

iii) Orthorhombic crystal system

In this crystal system, unit cell edge lengths are different and they are perpendicular to one another i.e., $a \neq b \neq c$ and $\alpha = \beta = \gamma = 90°$. There are four Bravais lattices in this system they are simple, face centered, body centered and base centered. Examples for orthorhombic crystal system are $BaSO_4$, K_2SO_4, $SnSO_4$, etc.

iv) Monoclinic crystal system

In this crystal system, the unit cell edge lengths are different. Two unit cell edges are not perpendicular, but they are perpendicular to the third edge i.e., $a \neq b \neq c$; $\alpha=\gamma=90 \neq \beta$. This crystal system has two Bravais lattices; they are simple and base centered. Examples for Monoclinic crystal system are $CaSO_4.2H_2O$ (gypsum), Na_3AlF_6 (cryolite), etc.

v) Triclinic crystal system

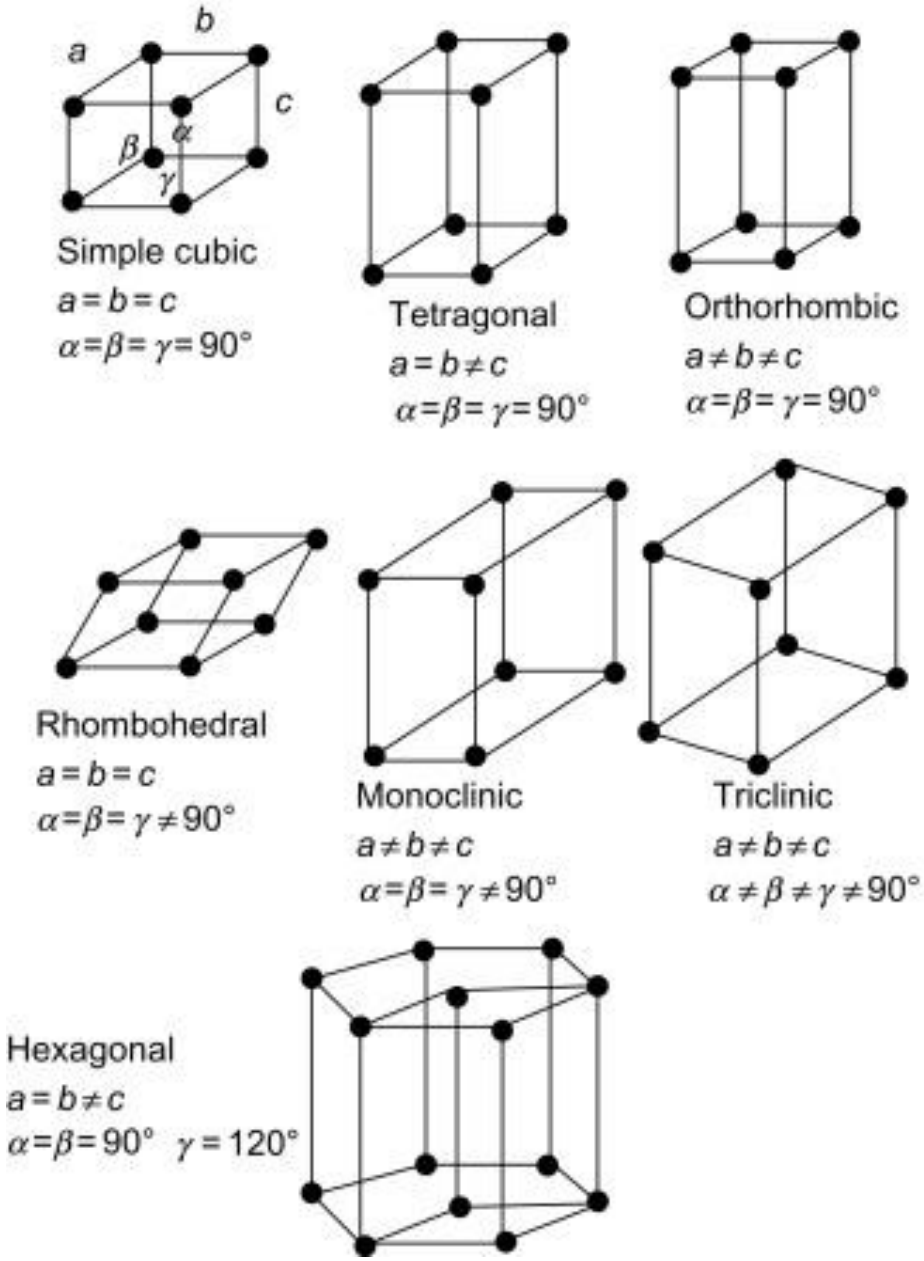
In this crystal system, the unit cell edge lengths are different and are not perpendicular i.e., $a \neq b \neq c$ and $\alpha \neq \beta \neq \gamma \neq 90°$ and all the angles are different. This crystal exists in primitive cell only. Examples for triclinic crystal system are $K_2Cr_2O_7$, CuSO4. 5H2O etc

vi) Rhombohedral crystal system

In this crystal system, all the lengths of unit cell edges are equal. The angles between the axes are equal but other than 90° i.e., $a = b = c$ and $\alpha = \beta = \gamma \neq 90°$. Examples for rhombohedral crystal system are As, Bi, Sb, etc.

vii) Hexagonal crystal system

In this crystal system, two sides of the unit cell edge lengths are equal and the angle between these edges is 120°. These two edges are perpendicular to the third edge, and not equal in length i.e., $a = b \neq c$ and $\alpha = \beta = 90°$; $\gamma = 120°$. The Bravais lattice is primitive only. The atoms in this crystal system are arranged in the form of a hexagonal close pack.



1.4 Classification of methods of crystal growth

For the growth of crystal required the laboratory and industrial application a great variety of techniques have been employed. The growth methods are generally classified into four major groups.

➢ Solid Growth - solid to solid phase transition

➢ Melt Growth - liquid to solid phase transition

➢ Vapour Growth - vapour to solid phase transition

➢ Solution Growth - liquid to solid phase transition

1.4.1 Solid growth method

Most of the solid state growth techniques require atomic diffusion at normal temperature. Such diffusion is very slow except in the case of super ionic materials. Thus solid state growth techniques are seldom used growth techniques for the production of single crystals of very small significance. Most of these techniques were known and used before 1900.

1.4.2 Melt growth method

The most important method to produce bulk single crystals of a given material by solidification is melt. Some methods for growing single crystal from melt are :

a) Czochralski method

b) Kyropoulos method

c) Bridgman- stockbarger method

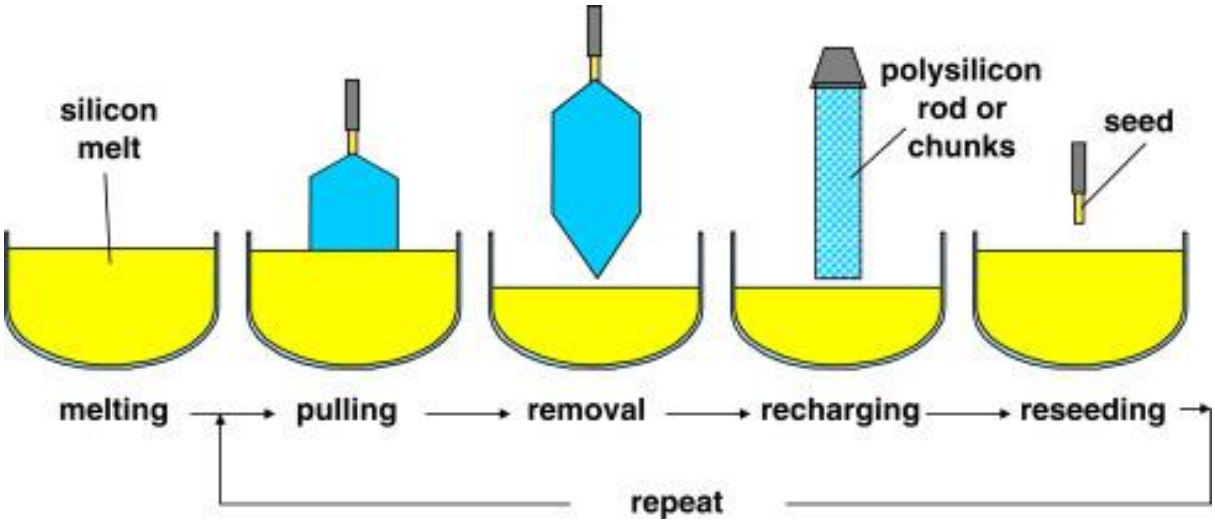
d) Floating zone technique method

e) Verneuil method

a) Czochralski method

In Czochralski method, commonly known as puling technique, the material is melted by induction or resistance heating in a suitable non reactive seed crystal is dipped into the melt. After thermal equilibrium is attained, the seed is slowly lifted from the melt. As the seed is pulled, continuous growth occurs at the interface. The diameter of growing crystal can be controlled by adjusting the rate of pulling, rate of melt level drop and the heat fluxes into and

out of the system.



Advantages

➢ This method is used to grow large single crystals. Thus it is used extensively in the semiconductor industry.

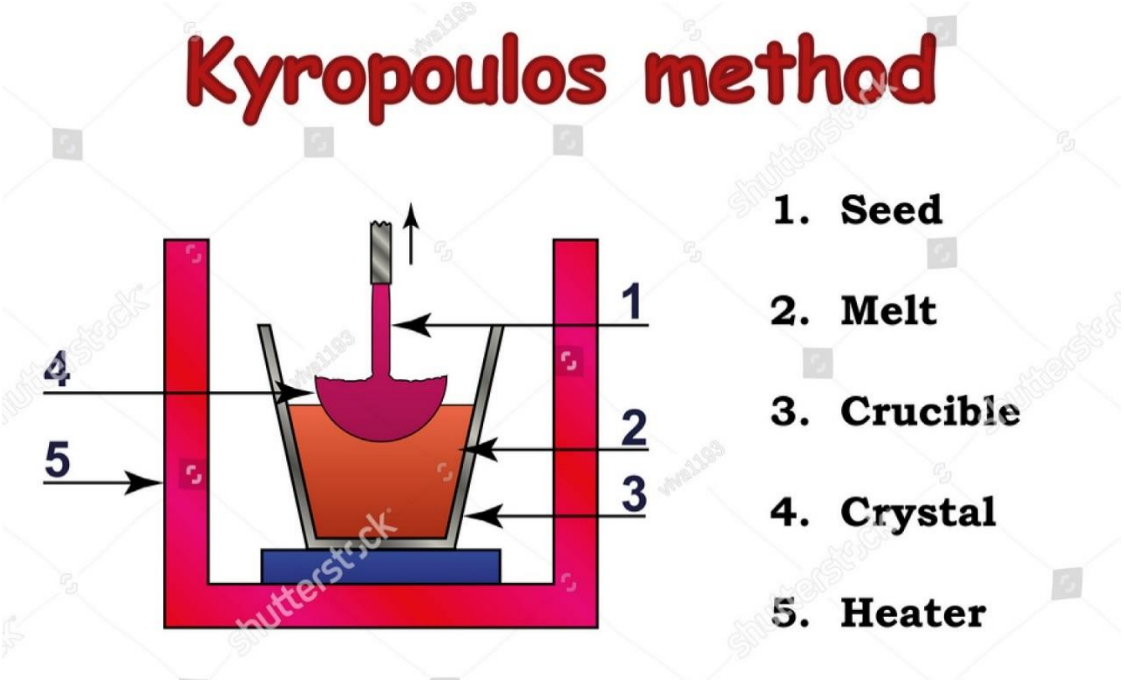
➢ There is no direct contact between the crucible walls and the crystal which helps to produce unstressed single crystal.

Disadvantage

In general this method is not suitable for incongruently melting compounds and of course the need for a seed crystal of the same composition limits is used as tool for exploratory synthetic research.

(b) Kyropoulos method

This method is similar to czochralski method, but instead of pulling the seed crystal, the crystal liquid interface moves into the melt as crystallization proceeds.



Advantage

➢ The crystal is grown in a larger diameter.

➢ With the large diameter crystal we can make prisms, lenses and other optical components

(c) Bridgman - Stockbarger method

In this method, a short temperature gradient is provided across the melt which results in nucleation in the colder region. The conical geometry of the crucible at bottom limits the number of nuclei formed.

Advantage

➢ This method is technically simple.

➢ Cost efficient

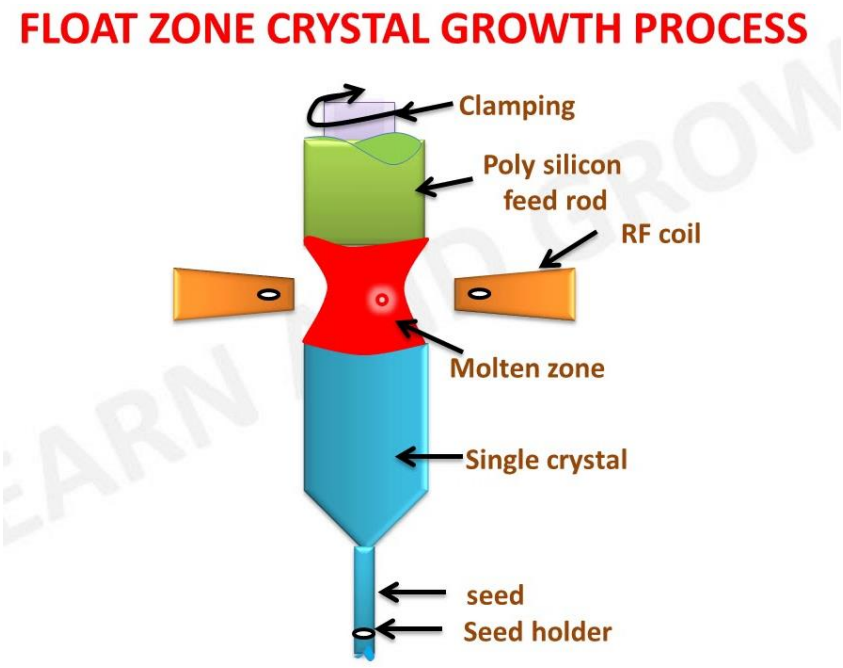
➢ Selecting the appropriate container can produce crystal of pure assigned diameter.

Disadvantage

➢ The compression of the solid by the contracting during cooling can lead to the devolpment of stresses high enough to nucleate dislocations in the material.

(d) Floating Zone Technique

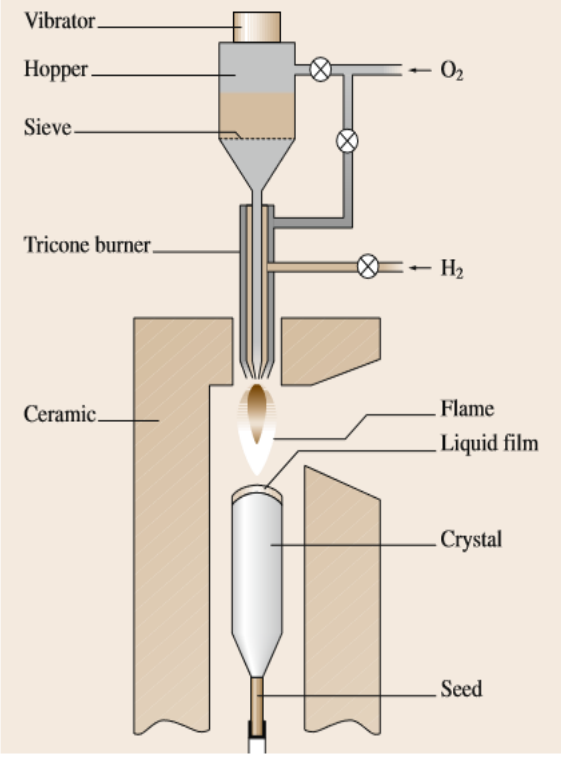
In this method of growing crystal from melt is the floating zone technique in which a section of starting material held vertically in the form of rod is melted by suitable heating. As the molten zone is moved along the rod, progressive melting of sample at one end, the whole rod can be converted into a single crystal. This method has an advantage that there is no contamination from crucible. It is similar to purification by zone refining and has been used routinely to grow single crystal of silicon.



(e) Verneuil method (flame fusion method)

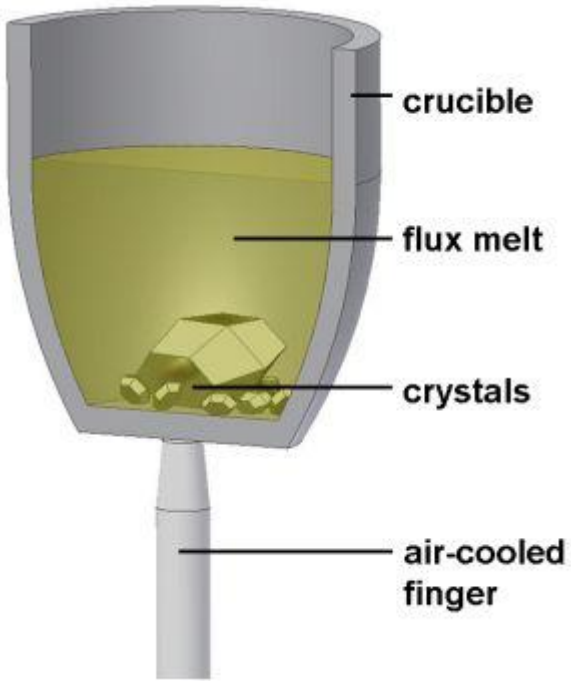
In the flame fusion method the power sample is directly fed in to an oxy hydrogen flame and the melt allowed dripping on a seed crystal. As the crystallization occurs on the top, the

growing seed is lowered slowly, facilitating the growth of large crystal. This process is considered to be founding step of modern industrial crystal growth technology



f) Vapour growth method

The advantage of vapour growth technique is that crystal tends to have a low dislocation density compared to crystal grown from the melt, the reason being, the temperature employed are usually lower than the melting temperature moreover, if the material melt incongruently, vapour growth can be the only choice for the growth of single crystal. Growth technique from vapour primarily involves three stages – vaporization, transport and deposition. The vapour is formed by heating solid or liquid to high temperature, transportation of vapour may occur through vacuum imparted by the K.E. from vaporization, deposition occurs by condensation or chemical reaction. Small size crystal of better quality can be grown like CdS and Al_2O_3 .



g) Solution Growth Method

Solution growth method is the simplest and oldest of all the methods and widely used. In growth from solution the solute usually forms complexes with solvent molecules and crystallizes at a temperature well below its melting point. The solute dissolves into a solvent which is most commonly water. The law of thermodynamics limits the amount of salt that can be dissolved in water. Once the solubility limit is reached, any more salt that can be added to the solution will not dissolve but it will simply sink to the bottom in solid form. The solubility limit is a function of temperature. Supposing a solution of salt and water has been prepared by adding as much salt as needed to reach the solubility limit and further. Since the solubility limit is lower at the lower temperature there is now an excess of salt dissolved in the solution. The excess salt must now precipitate from the solution. As precipitation occurs some of the dissolved salt begins to grow into small crystals and sink to the bottom. With some care using a very clean container and very pure material, it is possible to produce a single large crystal by this method. Materials which have high solubility and have variation in the solubility with temperature can be grown by solution growth technique. There are two methods in the solution

growth depending on the solvents and solubility of the solute. They are:

(i) High temperature solution growth

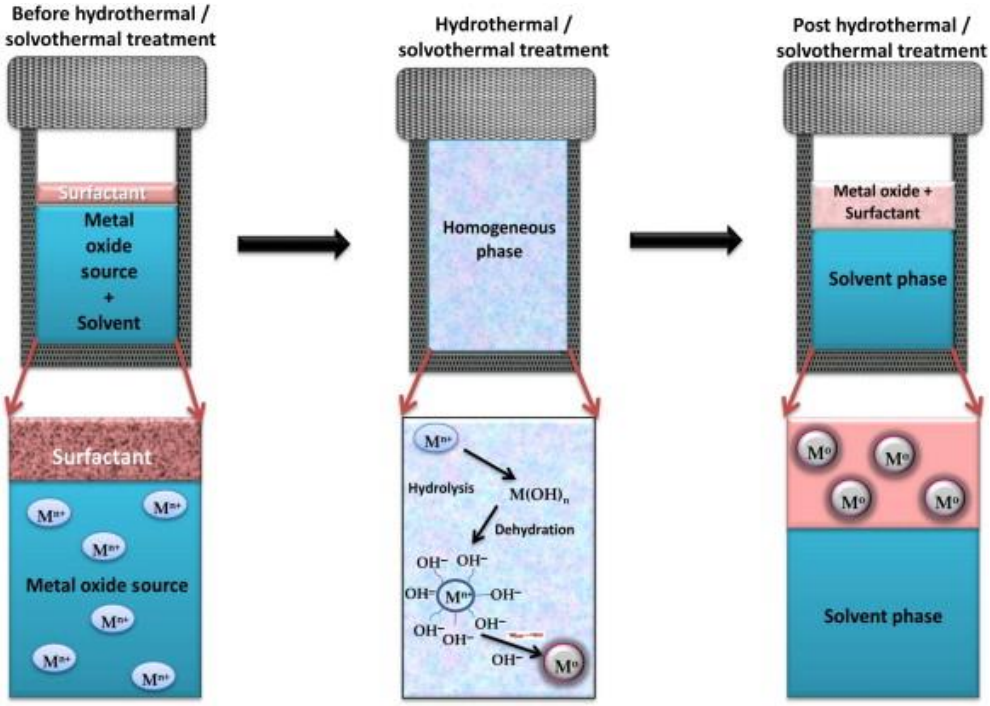
(ii) Low temperature solution growth

(i) High temperature solution growth

In high-temperature solution, the constituents of the material to be crystallized are dissolved in a suitable solvent and crystallization occurs as the solution becomes critically supersaturated. The supersaturated may be promoted by evaporation of the solvent, by cooling the solution or by a transport process in which the solute is made to flow from a hotter to a cooler region. This technique is widely used for the growth of oxide crystals.

(a) Hydrothermal growth

Hydrothermal growth implies the condition of high temperature. The substances like calcite, quartz are considered to be insoluble in water but at high temperature and pressure, these substances are soluble. This method of crystal growth at high temperature and pressure is known as hydrothermal method. The temperatures are typically in the range of 400˚C to 600˚C and the pressure involved is large (hundreds or thousands of atmospheres). Growth is usually carried out in steel autoclaves with gold or silver linings. Depending on the pressure the autoclaves are grouped into low, medium and high- pressure autoclaves. The concentration gradient required to produce growth is provided by a temperature difference between the nutrient and growth areas of high pressure point out some practical difficulties and there are only a few crystals of good quality and large dimensions are grown by this technique. The quartz is the outstanding example of industrial hydrothermal crystallization.



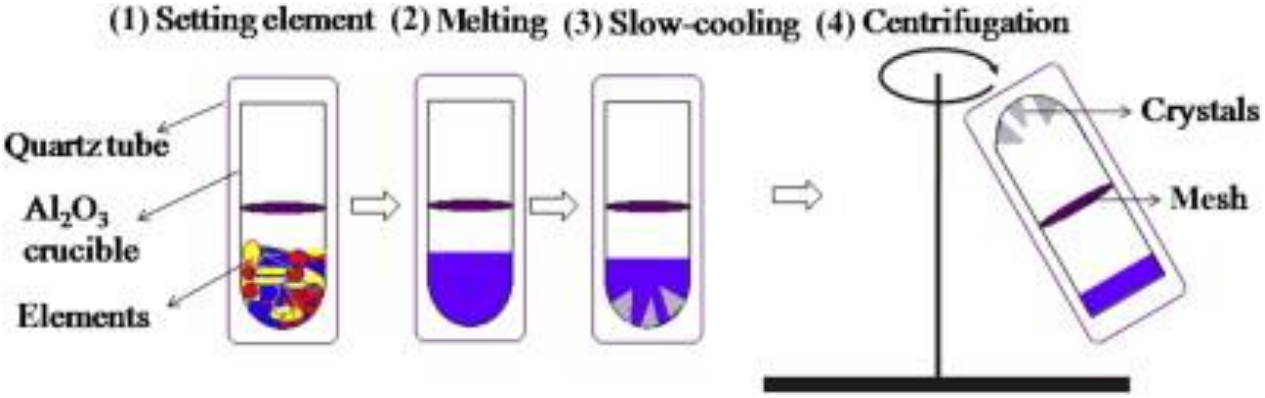
Disadvantage

➢ In this technique, there is a frequent incorporation of OH^- ions into the crystal, which makes them unsuitable for many applications.

(b) Flux growth

Molten salts are used as solvents in this method. For oxides and solid solutions of oxides, which have very high melting points and decompose prior to melting these are the only satisfactory solvents. The molten salts of such composition should be contained in platinum or iridium crucibles. The growth from molten salts is obtained by decreasing the temperature of a saturated solution to acquire supersaturation. Seeds are commonly used and these can be withdrawn before the solution is solidified. Flux growth is carried out at much lower temperatures than corresponding growth from melt. A distinct advantage of the method is that this is the only method for obtaining certain oxide solid solutions. However, the flux growth is

very slow and requires precise temperature control. The purity of the crystals grown by this method is often marginal. The flux growth is an expensive technique.



(ii) Low temperature solution growth

This method can be subdivided in the following

(a) Slow cooling method

(b) Slow evaporation method

(c) Temperature gradient method

(a) Slow cooling method

This is the best method to grow bulk single crystal from solution In this method, supersaturation is created by a change in temperature usually throughout the whole crystallizer. The crystallization is carried out in such a way that the point on the temperature dependence of the concentration moves into the metastable region along the saturation curve in the direction of the lower solubility. Since the volume of the crystallizer is finite and the amount of substance placed in it is limited. The supersaturation requires systematic cooling.

b) Slow Evaporation Method

In this method the solution loses particle which are weak bound to other components and therefore the volume of the solution decreases. An excess of a given solute is established by utilizing the difference between rates of evaporation of the solvent and the solute. Normally, the vapour pressure of the solvent above the solution is higher than the vapour pressure of the solute and therefore, the solvent evaporates more rapidly and the solution is higher than the

vapour pressure of the solute and therefore, the solvent evaporates more rapidly and the solution becomes supersaturated. It is sufficient to allow the vapour formed above the solution to escape freely into the atmosphere. Good control of evaporation rate can also be obtained by using some sort of condenser to allow the removal of condensed solvent at a controlled rate.

(c) Temperature Gradient Method

In this method, the materials are transported from a hot region containing the source material to be grown to a cooler region where the solution is supersaturated and the crystal grows. The main advantages of this method are that, this method is insensitive to changes in temperature provided, both the source and growing crystal undergo the same changes with the crystal growing at a fixed temperature and there is economy of the solvent and solute. On the other hand, the changes in the small temperature differences between the source and the crystal zones have a large effect on the growth rate.

Now a days, the Scientists are in search for new conversion materials for various device applications and the recent interest is focused on the development of the new semi organic materials with improved properties. Single crystals are important materials for electronic, optical devices and laser crystals. The efficient NLO materials has resulted in the development of a new class of materials called semi-organics. These materials have the potential for

combining the high optical nonlinearity and chemical flexibility of organic materials with the thermal stability of inorganic NLO materials. The impact of NLO on science is widely understood and has enabled, in one way or another, at least nine Nobel prizes in physics and chemistry. NLO phenomena have been observed at wavelengths from deep infrared to extreme UV, and even used to generate THz radiation. Optical nonlinearities are exhibited by crystals, amorphous materials, polymers, liquid crystals, semiconductors, organics, liquids, gases and plasmas. Early applications of NLO included second harmonic generation, Q-switching and modelocking, all of which extended the applications of lasers. Fiber optic communications early showed the deleterious impact of NLO in glass. With narrow-line lasers, ultra-fine resolution spectroscopy became possible. Today we see a plethora of promising future applications for NLO, including quantum optics, quantum computing, ultra-cold atoms, plasma physics and particle accelerators, to name a few, with many more to come.

Literature Survey

In 2019 Studies on α-nickel sulfate hexahydrate (NSH) crystals grown under different conditions were undertaken to investigate how changes in growth conditions affect crystal properties and whether or not there is any modification of the average crystal structure due to changes in crystallization conditions [1]. In 2018 Effect of nickel sulphate hexahydrate addition on crystal growth, structural, optical and biological applications of glycine single crystals was studied [2]. In 2015 Growth of Nickel Sulfate Hexahydrate (α-NiSO 4 · 6H 2 O) Single Crystals under Steady-State Conditions of Temperature Difference was studied [3]. In 2014 Nickel sulphate hexahydrate (NSH) and potassium magnesium nickel sulphate hexahydrate (KMNSH) single crystals were grown by slow evaporation method [4] . In 2002 high grade nickel sulphate hexahydrate crystals were grown from industrial de-copperized electrolyte using isopropanol [5].

OBJECTIVE

Now a days, the Scientists are in search for new conversion materials for various device applications and the recent interest is focused on the development of the new semi organic materials with improved properties. Single crystals are important materials for electronic, optical devices and laser crystals. The efficient NLO materials has resulted in the development of a new class of materials called semi-organics. These materials have the potential for combining the high optical nonlinearity and chemical flexibility of organic materials with the thermal stability of inorganic NLO materials.

The current era by which research and development of progress leads to the enormous scientific and technological advances and experiencing the technological revolution. Nonlinear optical (NLO) materials playan eminent role on information technology and industrial applications. Especially, NLO crystal materials exhibit finest applications such as laser frequency conversion, optical computing, optical information processing [6] optical storage, optical communication, photonics, electro-optic modulation, optical image processing etc. [7]. The organic NLO materials possess hyper NLO coefficients compared with inorganic materials [8]. Amino acids belong to a family of organic materials have wider NLO applications. The organic amino acid compounds like L-cysteine hydrochloride monohydrate [9], L-arginine [10.], LHistidine [11] and glycine [12] exhibits NLO applications.

Here an attempt is made to prepare L-Arginine and L-cysteine doped nickel sulphate crystals by slow evaporation method at room temperature. Single crystals obtained were then subjected to UV-Visible, FTIR and XRD studies.

EXPERIMENTAL METHOD

Chemicals Used :

Nickel Sulphate - 17.803 g (spectrum)

Arginine - 0.44 g (Himedia)

Cysteine- 1.2g (Himedia)

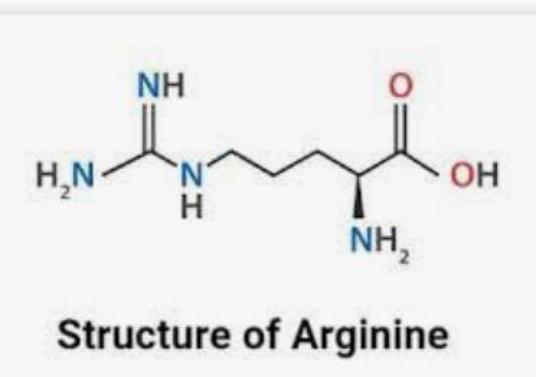
Water - 20 ml

Preparation of L-Arginine doped Nickel sulphate single crystals:

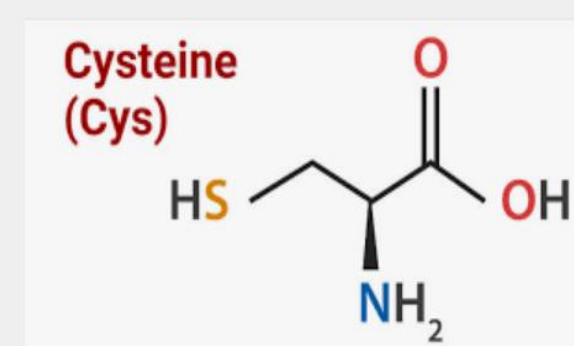
L-Arginine doped Nickel sulphate single crystals were grown by slow evaporation technique. Exactly 17.803g of nickel sulphate was dissolved in 20ml of water taken in a 100ml beaker. The beaker was then placed in a magnetic stirrer and was stirred uniformly for one hour to make a saturated solution . Then exactly 0.44g of the dopant L-arginine was added to increase the optical properties of a nickel sulphate crystal. The solution was again stirred for another 30 minutes . The beaker was placed at room temperature without any disturbance for the crystals to grow. Finally green coloured crystals were obtained.

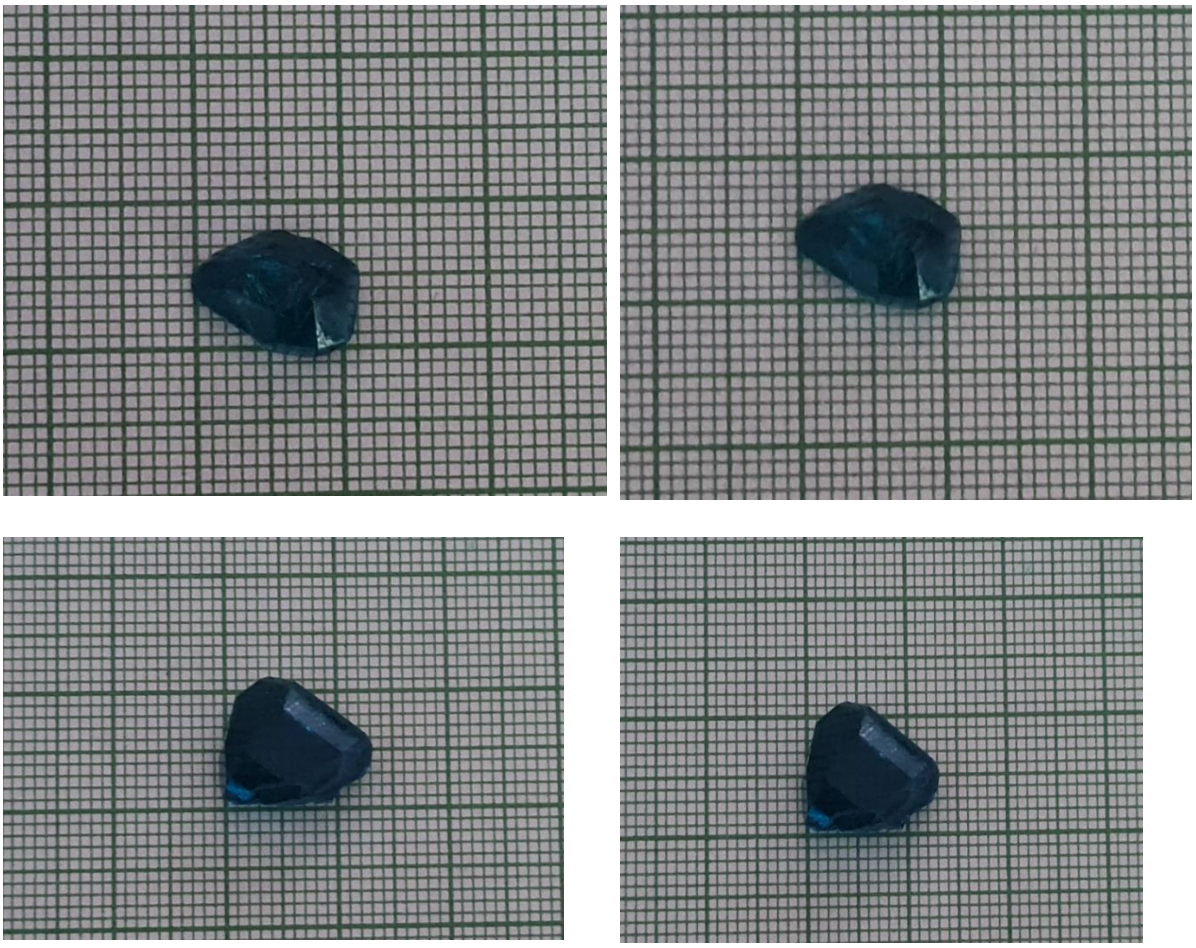
Preparation of L-Cysteine doped Nickel sulphate single crystals:

L-Cysteine doped Nickel sulphate single crystals were grown by slow evaporation technique. Exactly 17.803g of nickel sulphate was dissolved in 20ml of water taken in a 100ml beaker. The beaker was then placed in a magnetic stirrer and was stirred uniformly for one hour to make a saturated solution . Then exactly 1.2 g of the dopant L-cysteine was added to increase the optical properties of a nickel sulphate crystal. The solution was again stirred for another 30 minutes . The beaker was placed at room temperature without any disturbance for the crystals to grow. Finally green coloured crystals were obtained.

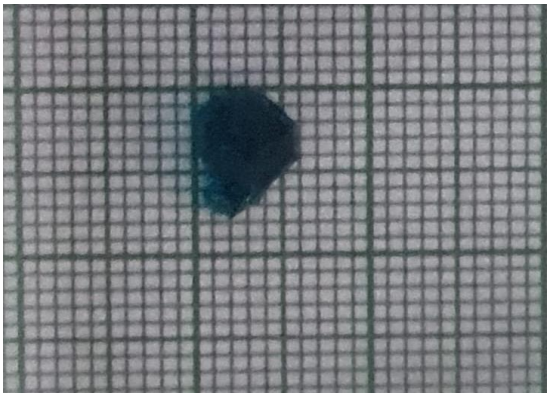


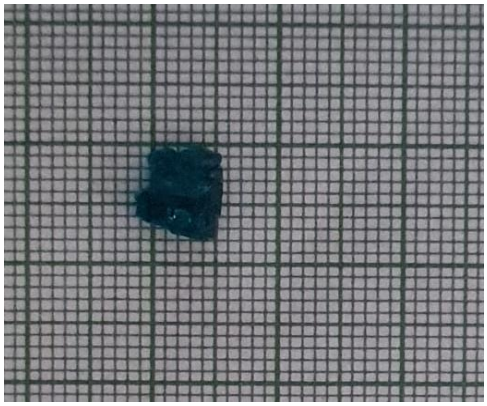
Structure of Arginine





L-Arginine Doped Nickel Sulphate Crystals





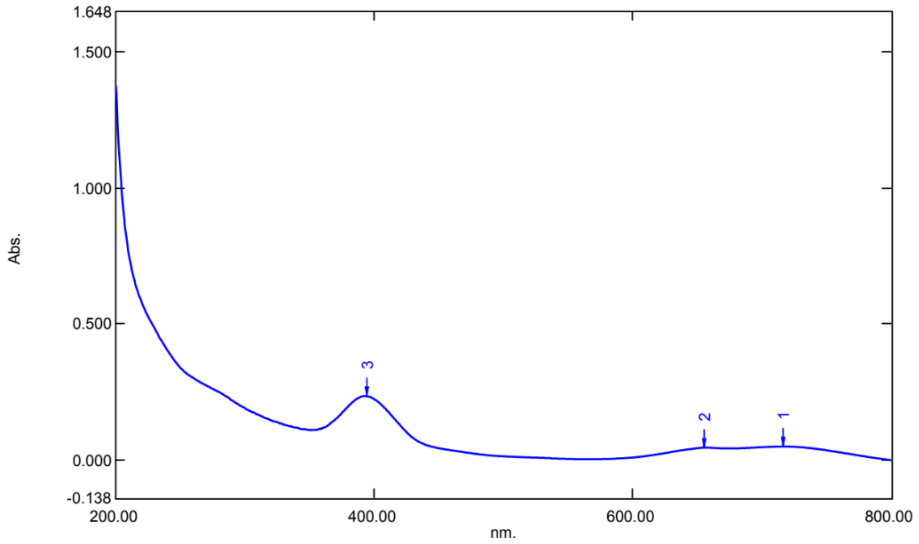
L-Cysteine Doped Nickel Sulphate Crystals

RESULTS AND DISCUSSION

UV-Visible spectra of L-Arginine and L-Cysteine doped Nickel sulphate Single Crystal:

The UV-Visible spectrum was recorded using Double Beam spectrophotometer in the wavelength region 200 – 800nm. UV-Vis spectrum for nickel sulphate hexahydrate single crystal earlier reported [13] has three transmittance peaks approximately centered at 300 nm, 490 nm and 850 nm and at the remaining wavelengths the crystal shows strong absorption. Thus the discontinuity in the optical transmittance at specific selective spectral wavelengths is the required property of any crystal to use it as band pass filter. This discontinuous spectral characteristics mainly arise from the absorption of hydrated transition metal ions $[Ni(H2O)_6]^{2+}$. The $(SO4)^{2-}$ units assume regular tetrahedral geometry which are linked with the $Ni(H_2O)6$ 2+ units through O-H…O hydrogen bond. Also it can be seen that NSH possesses a bandwidth in the range of 220-330 nm which is centered at 300 nm thereby making it a potential UV filter. Efficient non-linear optical crystals have an optical transparency lower cut-off wavelengths between 200-400nm as reported in literature. [14]. UV spectra for L-arginine shows one peak at 276nm and L-cysteine at 260nm as reported in literature.

In the case of the prepared L-Arginine and L-Cysteine doped Nickel Sulphate crystals The lower cut off region lies in the range 394 nm and 393nm respectively as shown below in Fig.1 and Fig.2 respectively. The low absorption in the visible and NIR regions along with low cut off wavelengths confirm the suitability of the grown crystals for NLO applications. The grown crystals has good transmission in UV as well as in visible regions. The single crystals can be mainly used for optical applications.



[Measurement Properties]

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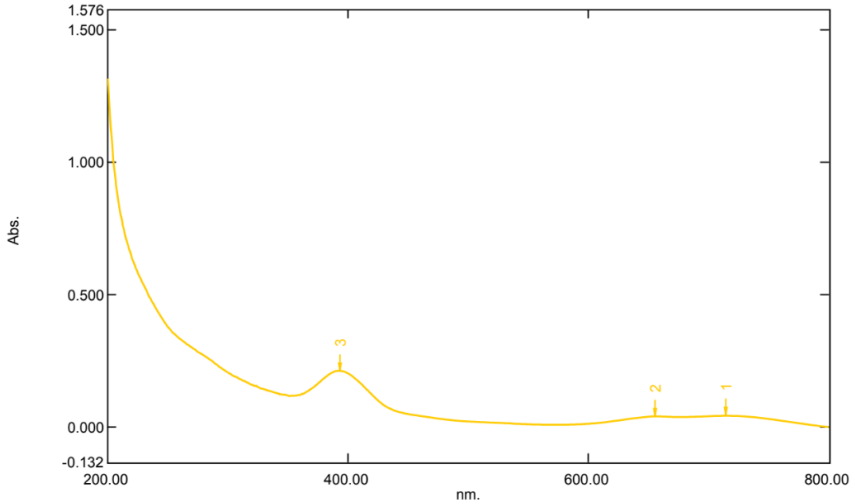
Auto Sampling Interval:	Enabled
Scan Mode:	Auto

No.	P/V	Wavelength	Abs.	Description
1	↑	717.00	0.050	
2	↑	656.00	0.046	
3	↑	394.00	0.235	

Fig. 1 UV-Visible Spectra of L-Arginine doped doped Nickel Sulphate crystals



[Measurement Properties]

Wavelength Range (nm.):	200.00 to 800.00
Scan Speed:	Fast
Sampling Interval:	0.5
Auto Sampling Interval:	Enabled
Scan Mode:	Auto

No.	P/V	Wavelength	Abs.	Description
1	↑	714.50	0.043	
2	↑	655.50	0.041	
3	↑	393.00	0.213	

Fig. 2 UV-Visible Spectra of L-Cysteine doped doped Nickel Sulphate crystals

IR spectra of L-Arginine and L-Cysteine doped Nickel sulphate Single Crystal:

The Fourier transform infrared (FTIR) spectrum was recorded in the spectral range 400-4000 cm^{-1} following KBr pellet method using Shimadzu FTIR spectrometer and is shown in Fig. 3 and Fig.4. The observed wavenumbers and tentative vibrational frequency assignment for reported nickel sulphate hexahydrate is as follows. The broad peak at 3641 and 3010 cm^{-1} is due to asymmetric and symmetric stretching modes of water molecules respectively. The vibration band observed at 1667 cm_1 is assigned to δ (H2O) bending vibration of water molecules. The strong band observed at 416 cm_1 is due to the Ni-O stretching vibration. Free $SO4^{-}$ ion has Td symmetry and has four fundamental vibrations namely a non degenerate mode at (ν_1) 1096 cm_1 and a doubly degenerate mode (ν_2) 465 cm_1 and triply degenerate modes (ν_3 and ν_4) at 1161 and 632 cm_1 respectively.

The reported IR spectra of l-arginine [15] N-H exhibits stretching frequencies at 3151cm^{-1}, stretching of C-C bond at 848cm^{-1}, bending vibration of NH_2 group at 896cm^{-1}, stretching of CNH group band at 794cm^{-1}, bending of CO group at 522cm^{-1}, rocking motion of NH_2 at 449cm^{-1}.

Doped nickel sulphate crystal of arginine shows symmetric and asymmetric O-H stretching due to H_2O in $NiSO_4$ at 3181cm^{-1} and 3472cm^{-1} and also N-H in arginine appears at 3899 cm^{-1}, thereby confirming the incorporation of the dopant L-Arginine in the nickel sulphate crystal lattice as shown in Fig.3 below.

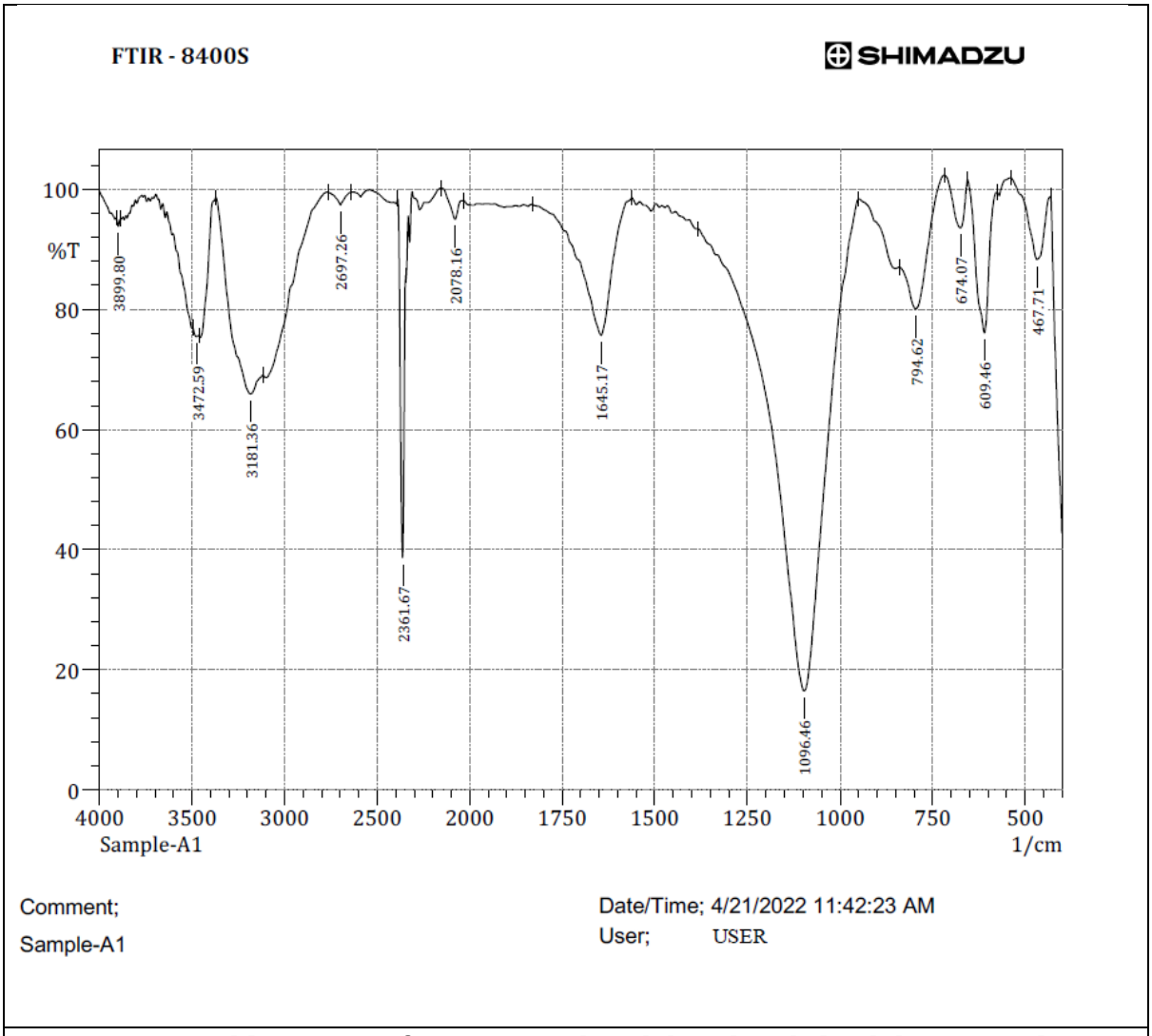


Fig. 3 FTIR-Visible Spectra of L-Arginine doped doped Nickel Sulphate crystals

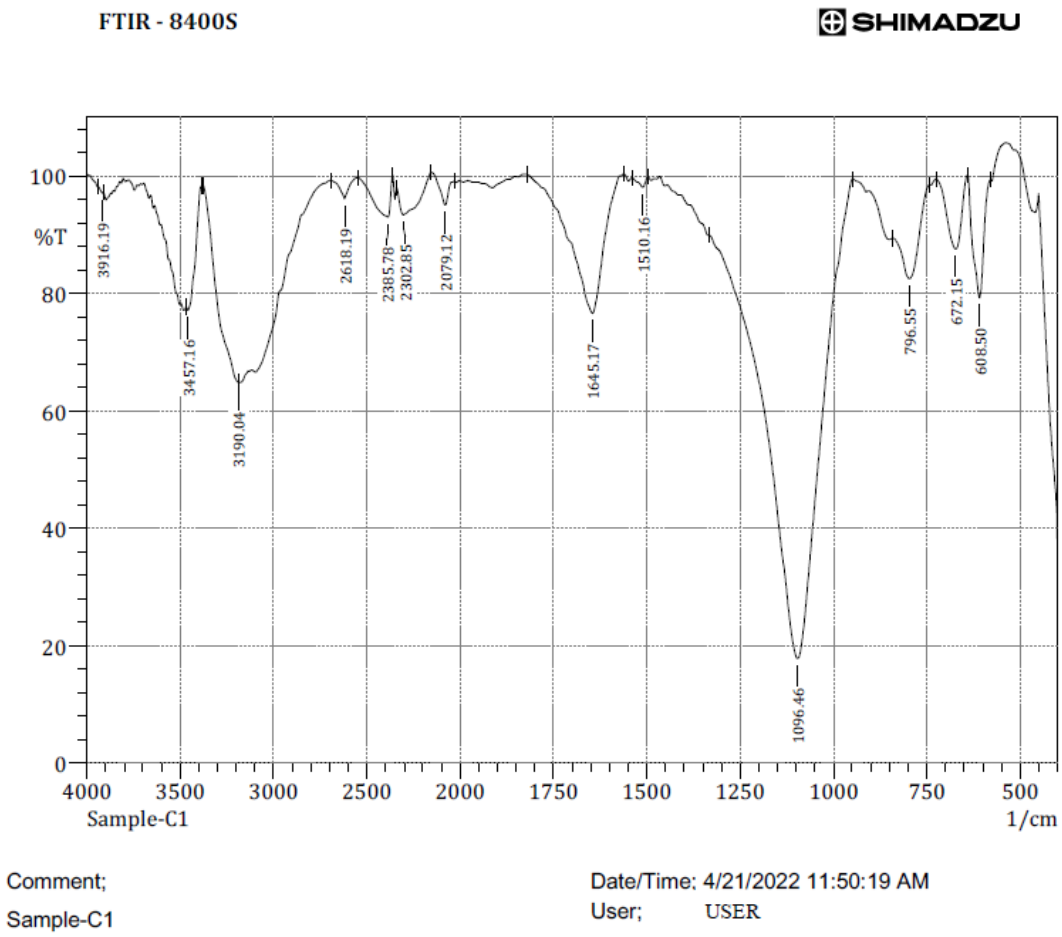


Fig. 4 FTIR-Visible Spectra of L-Cysteine doped doped Nickel Sulphate crystals

Doped nickel sulphate crystal of L-Cysteine shows symmetric and asymmetric O-H stretching due to H_2O in $NiSO_4$ at 3190cm^{-1} and 3916cm^{-1} and also N-H in cysteine appears at 3457 cm^{-1}, thereby confirming the incorporation of the dopant L-cysteine in the nickel sulphate crystal lattice as shown above in Fig.4.

XRD STUDIES

The grown crystals have been crushed into uniform fine powder and subjected to powder X-ray diffraction to identify the reflection planes. Advanced PAN analytical XPERT PRO diffractrometer was used to record the diffraction pattern of the grown sample with CuKα (λ=1.54056Å). The sample was scanned over the required 2θ range of 10˚- 90˚. X-ray diffraction data gives the angle of scattering (2θ) and the corresponding intensities of diffracted

beam for each reflection. All the reflections of powder XRD pattern of the grown crystals were indexed using INDEXING software package following of the procedure of Lipson and Steeple(1970). Indexing of the powder pattern consists of assignment of the numbers h,k,l (miller indices) to each reflection. The indexed powder X-ray diffraction pattern of the L-Arginine and L-Cystein doped nickel sulphate crystals is shown figure 5 and 6 respectively.. The occurrence of sharp peaks at specific Bragg's angle gives surety of high crystallinity of L-Arginine and L-Cysteine doped Nickel sulphate single crystals. XRD pattern of L-Arginine Doped Nickel Sulphate Single Crystal and L-Cysteine Doped Nickel Sulphate Single Crystal is as shown in Fig.6 and Fig.7 respectively. The presence of prominent Bragg's peak 2Ɵ angle confirms the perfect crystal line structure. The diffraction pattern shows sharp deflection corresponding to tetragonal structure for both L-Arginine Doped Nickel Sulphate Single Crystal and L-Cysteine Doped Nickel Sulphate Single Crystal .

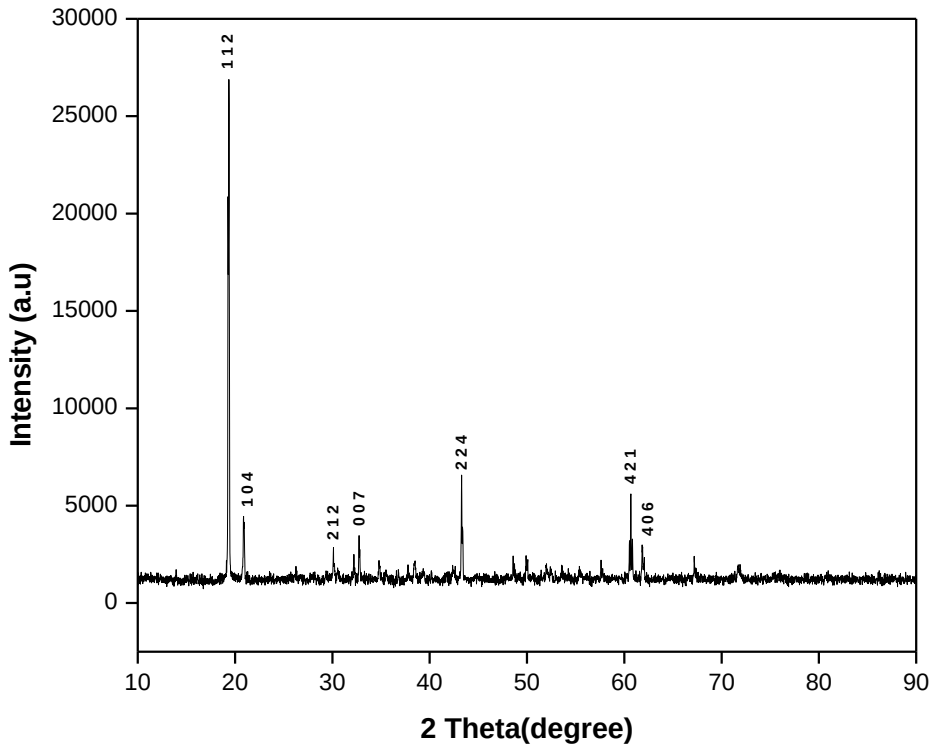


Fig. 5 XRD of L-Arginine Doped Nickel Sulphate Single Crystal

The Powder XRD pattern from the grown crystals with high intensity peaks were observed for L-Arginine Doped Nickel Sulphate Single Crystal at

$2\theta = 19.3025^{\circ}$ along the 112 h, k,l plane

$2\theta = 20.9684^{\circ}$ along the 104 h,k,l plane

$2\theta = 30.3198^{\circ}$ along the 212 h,k,l plane

$2\theta = 32.8298^{\circ}$ along the 007 h,k,l plane

$2\theta = 43.2030^{\circ}$ along the 224 h,k,l plane

$2\theta = 60.9062^{\circ}$ along the 421 h, k, l plane

$2\theta = 62.0168^{\circ}$ along the 406 h, k, l plane

From the above observation it is understood that the doped crystal exhibit tetragonal structure as confirmed from the JCPDS file: 75-0673. The absence of impurities peaks level that the

grown crystals exhibit high crystalline quality using β as Full Width at Half Maximum (FWHM) of a broad diffraction peak. Average grain size is estimated by applying the Scherrer's equation: D= K λ/β cos θ Where K= 0.9 (Scherrer's constant) λ is X-ray wavelength θ is Diffracted angle β is Full Width at Half Maximum(FWHM) The average particle size obtained from XRD data is found to be 66.82 nm

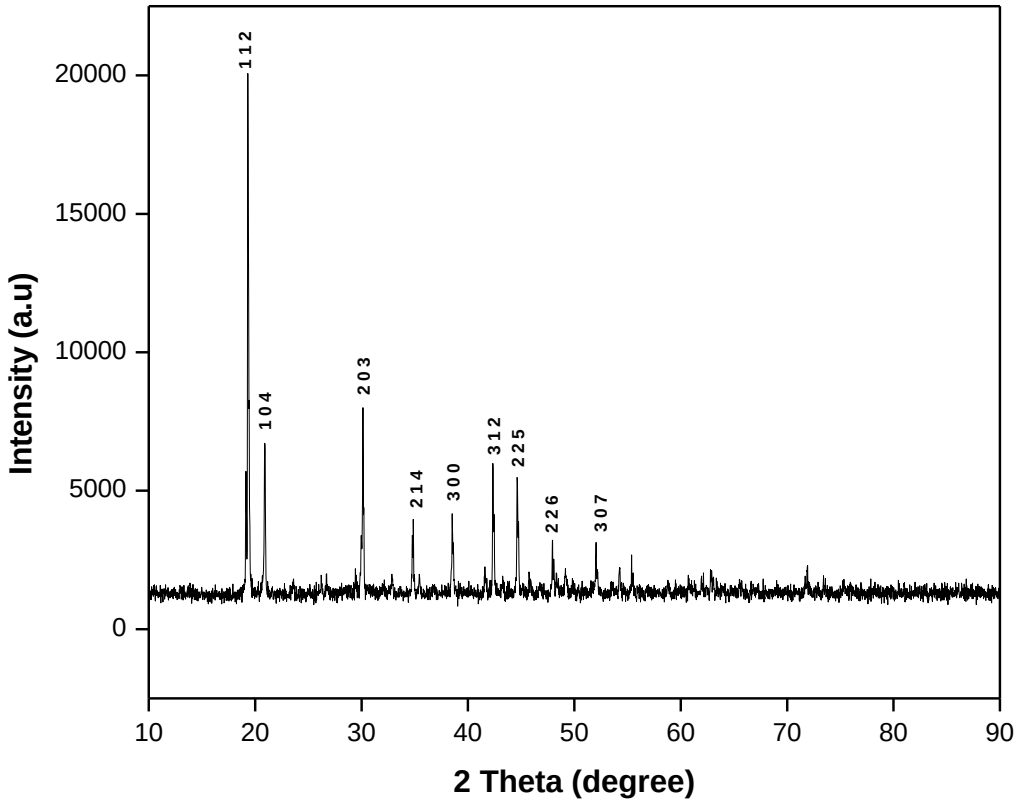


Fig. 6 XRD of L-Cysteine Doped Nickel Sulphate Single Crystal

The Powder XRD pattern from the grown crystals with high intensity peaks were observed for L-Cysteine Doped Nickel Sulphate Single Crystal at

2θ = 19.4802˚ along the 112 h, k, l plane

2θ = 21.1683 ˚along the 104 h, k, l plane

2θ = 30.1421 ˚along the 203 h, k, l plane

2θ = 35.1177 ˚along the 214 h, k, l plane

2θ = 38.6939 ˚along the 300 h, k, l plane

2θ = 42.2923˚ along the 312 h, k, l plane

2θ = 44.8689 ˚along the h, k, l plane

2θ = 48.06752˚along the h, k, l plane

2θ = 52.3989˚along the h, kl, plane

From the above observation it is understood that the doped crystal exhibit tetragonal structure as confirmed from the JCPDS file: 75-0673. The absence of impurities peaks level that the grown crystals exhibit high crystalline quality using β as Full Width at Half Maximum (FWHM) of a broad diffraction peak. Average grain size is estimated by applying the Scherrer's equation: D= K λ/β cos θ Where K= 0.9 (Scherrer's constant) λ is X-ray wavelength θ is Diffracted angle β is Full Width at Half Maximum(FWHM) The average particle size obtained from XRD data is found to be 61.34 nm.

CONCLUSION

L-Arginine and L-Cysteine doped single crystals were prepared by slow evaporation method at room temperature. Gren coloured single crystals obtained were then subjected to UV-Visible, FTIR and XRD studies. The following observations were made

- UV-Visible studies clearly indicate that in the case of the prepared L-Arginine and L-Cysteine doped doped Nickel Sulphate crystals the lower cut off region lies in the range 394 nm and 393nm respectively. The low absorption in the visible and NIR regions along with low cut off wavelengths confirm the suitability of the grown crystals for NLO applications. The grown crystals has good transmission in UV as well as in visible regions. The grown crystals has good transmission in UV as well as in visible regions.
- FTIR studies of doped nickel sulphate crystal of arginine shows symmetric and asymmetric O-H stretching due to H_2O in NiSO4 at 3181cm^{-1} and 3472cm^{-1} and also N-H in arginine appears at 3899cm^{-1}, thereby confirming the incorporation of the dopant L-Arginine in the nickel sulphate crystal lattice. Similarly Doped nickel sulphate crystal of L-Cysteine shows symmetric and asymmetric O-H stretching due to H_2O in NiSO4 at 3190cm^{-1} and 3916cm^{-1} and also N-H in cysteine appears at 3457cm-1cm^{-1}, thereby confirming the incorporation of the dopant L-cysteine in the nickel sulphate crystal lattice.
- XRD studies indicate clearly the presence of prominent Bragg's peak 2Ɵ angle which confirms the perfect crystal line structure in bothe the doped crystals. From the above observations it is understood that both the doped crystals exhibit tetragonal structure as confirmed from the JCPDS file: 75-0673. The average particle size obtained from XRD data is found to be 66.82nm in the case of L-Arginine doped nickel sulphate crystal and 61.34 nm in the case of L-Cysteine doped nickel sulphate crystal.

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BIOSYNTHESIS OF NANOPARTICLES USING *MORINGA OLEIFERA*

PROJECT IN CHEMISTRY

Submitted to St.Mary's College(Autonomous) in partial fulfillment for the award of the

Degree of Bachelor of Science in Chemistry

PROJECT DONE BY

B. ANUSH KAMALAM

A. FRANCIS VINISTA

S. JACKULIN

J. KAVITHA PRIYA

A. VINNARASI

St. Mary's College (Autonomous)

Re-accredited with A^+ Grade by NAAC

Thoothukudi – 628001

2021- 2022

DECLARATION

We hereby declare that the project entitled "BIOSYNTHESIS OF NANOPARTICLES USING *MORINGA OLEIFERA*" submitted to St. Mary's College (Autonomous), Thoothukudi, affiliated to Manonmaniam Sundaranar University, for the Degree of Bachelor of Science is our original work and that, it has not previously formed the basis for the award of any Degree, Diploma or similar title.

B. ANUSH KAMALAM

A. FRANCIS VINISTA

S. JACKULIN

J. KAVITHA PRIYA

A. VINNARASI

May 2022

Thoothukudi

CERTIFICATE

This is to certify that the report of the project in Chemistry entitled "BIOSYNTHESIS OF NANOPARTICLES USING *MORINGA OLEIFERA*" is submitted to St. Mary's College (Autonomous) Thoothukudi in partial fulfillment for the award of the degree of Bachelors of Science in Chemistry and is a record of the work done by the following students during the year 2021– 2022.

Names	**Reg .No**
B. ANUSH KAMALAM	19AUCH04
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Guide **Head of The Department**

Principal

Examiners

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2.

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CONTENTS

ABSTRACT

Silver, Platinum, Palladium nanoparticles are prepared using the extract of *Moringa Oleifera*. The effect of various process parameters like the reactant's concentration, mixing ratio of the reactants and the concentration of silver nitrate, Platinum, Palladium solution were studied. In the present study, Silver, Platinum, Palladium nanoparticles were rapidly synthesized at room temperature by treating Silver, Platinum, Palladium ions with *Moringa Oleifera* extract. About 10^{-3}M solution of silver nitrate, Platinum, Palladium solutions were prepared as a stock solution. *Moringa Oleifera* seeds are collected from the moringa trees in the garden. The seeds were dried under the shade properly and powdered and then it is grinded. The powder which we obtained from the grinding process must be sieved. About 20gm of prepared powder was weighed and heated to boiling. The extract was centrifuged at 10,000rpm to get a clear solution and to remove and undesired impurities from it. At first step, to fix the concentration of the *Moringa Oleifera* extracts, the extract is measured in the ratio of 1ml, 1.5ml, 2ml, 2.5ml, 3ml taken in a clean and a dry test tube. About 1ml of silver nitrate, Platinum, Palladium solutions were added in all the respective test tube individually and mixed to get a uniform distribution. Time has been noted from 0 minutes and observation has been done then and there. The exact concentration has been fixed by the appearance of colour change. After fixing the concentration the time taken for the formation of silver, platinum, palladium, nanoparticles are determined using potentiometric method, from this exact time at which the nanoparticle synthesized is determined for all the samples. Using silver, platinum, palladium, nanoparticles of *Moringa Oleifera* the physical process of settling the fine particles has been carried out. Hence, we conclude that nanoparticles synthesized using *Moringa Oleifera* extract can be used for filtration process.

INTRODUCTION

INTRODUCTION

Moringa Oleifera belongs to the family Moringaceae one of the well- known and most widely distributed species of a monogenetic and is popularly known as 'Sahajan' in Hindi and 'Miracle tree' in English [1]. It is reported to be medicinally important and almost all parts of Moringa tree are considered to possess medicinal properties [2]. Moringa oleifera tree is one of the world's most useful trees; in the tropics, it is used as the forage for the livestock; and in many countries, it is used as micronutrient powder and to treat various ailments [3]. The plant is highly valued since almost every part of the tree (leaves, roots, bark, fruits, flowers, immature pods, and seeds) is used as food with high nutritional value. In addition, the plant has been reported to possess antimicrobial properties, and this explains the reason for its wide use in the treatment of human diseases [4]. Recently, an *in vitro* antimicrobial activity of *Moringa Oleifera* L. seed extracts prepared in aqueous and organic solvents against *Staphylococcus aureus, Bacillus subtilis, E. coli, Aspergillus niger and Candida albicans* were reported [5]. These properties of *Moringa Oleifera* make it, as an effective alternative in many skin infections [6, 7].

Incredible Health Benefits of Moringa Seed:

Improves Sleep: Moringa seeds help you slip into slumber at night, and in turn will leave you energised to tackle the day. **High in Fiber:** "Moringa seeds are high in fibre, and help in moving food along your digestive system". **Regulate Blood Sugar Levels:** "Moringa seeds are a great source of zinc and can regulate blood sugar levels which can help manage or even prevent diabetes. "Moringa seeds are a great source of zinc.**Great Source of Iron**:This is especially important for vegetarians/vegans or those who suffer from low iron issues, as the body needs iron to enrich the blood and carry oxygen to our muscles, organs and tissues".**Reduces Joint Pain:** "Moringa seeds make for a great supplement of calcium and help those suffering from joint pain. They help in reducing inflammation and severe bone disorders like arthritis." Moringa seeds make for a great supplement of calcium and help those suffering from joint pain. **Lowers Cholesterol:**"Some plants have been known to reverse bad cholesterol and according to research, moringa is among them."**Induces Death of Cancer Cells:**"Moringa seeds are well known for their anti-carcinogenic effects. They can stop the growth and development of cancer cells by accelerating their death count. "Moringa seeds are well known for their anti-carcinogenic effects.**Promotes Heart**

Health:"Scientists have proved that moringa seeds can reduce the amount of oxidised lipids in our body and take care of our cardiac health by safeguarding the heart tissues from constructional damages,"**Powerhouse of Antioxidants:** "The oil extracted from moringa seeds contains almost 30 antioxidants. It contains vitamins A, B-complex, C and other free radical busters that save our body from severe oxidative damage. In other words, these antioxidant properties of moringa seeds can take care of our overall health". The antioxidant properties of moringa seeds can take care of our overall health.**Promotes Healthy Skin:** "Moringa seeds are packed with antioxidant, anti -inflammatory and antiseptic properties and are thus, very beneficial for skincare. The oil obtained from moringa seeds can be used as a moisturizer or used to treat skin problems like skin rashes and sunburn,". Moringa seeds are packed with antioxidant, anti-inflammatory and antiseptic properties.

Nanotechnology can play significant role in improved drug therapy. Nanoparticles synthesized by green method have applications in bactericidal, wound healing, medicine and electronics [8][9]. The antimicrobial potential of silver nano particle with *Moringa Oleifera* extract by green technology exhibits antimicrobial activity. Nanoparticles have a surprisingly long history. Their preparation is neither an exclusive result of modern research nor restricted to man-made materials. Naturally occurring nanoparticles include organic (proteins, polysaccharides, viruses, among others) as well as inorganic compounds (iron oxyhydroxides, aluminosilicates, metals, among others) [10], [11]. Nanoparticles are not necessarily produced by modern synthesis laboratories, but have obviously existed in nature for a long time, and therefore their use can be traced back to ancient times. While the application of clay minerals as natural nanomaterials does not seem to be very sophisticated, the controlled reinforcement of a ceramic matrix with natural asbestos nanofibers more than 4500 years ago is more intriguing [12]. However, the most spectacular effects were obtained with metal nanoparticles as color pigments in luster and glass technology [13], [14]. Metallic luster decorations of glazed ceramics

appeared in Mesopotamia during the 9th century [15]. These decorations showed amazing optical properties due to the presence of separate silver and/or copper nanoparticles dispersed within the outermost layers of the glaze [15]. Metal nanoparticles are able to color glass in an extraordinary way. Gold has been used for a long time to introduce a striking red color to glass. One of the finest examples of such ruby glass is the Lycurgus Cup in the British Musem manufactured by Romans in the fourth century it appears with a green color in daylight (but changes to red when illuminated from the inside (16), [17]. An interesting fact about the use of gold nanoparticles in ruby glass is that after the Romans the technology was forgotten, and was only rediscovered in Europe in the seventeenth century. Although the birth of gold-based glass and enamel colors is ascribed to Andreas Cassius, who subsequently received the name Purple of Cassius, the preparation of colloidal gold with a tin compound had been described several years earlier by Johann Rudolph Glauber [18]. However, there is no evidence that Glauber ever applied his knowledge to the coloring of glass. It was Johann Kunckel, who ran a glass factory in Potsdam between 1679 and 1689, that successfully used the purple precipitate to produce ruby glass [18]. From a scientific point of view, the next big step forward in nanoparticle research was made by Michael Faraday approximately 150 years ago. As a matter of fact, his systematic studies on the interaction of light with metal nanoparticles can be regarded as the beginning of modern colloid chemistry and the emergence of Nanoscience and Nanotechnology [19]. In 1857 he presented his work on ‘Experimental Relations of Gold (and other Metals) to Light’ to the Royal Society of London [20]. Faraday prepared his colloidal gold dispersions in a two-phase system consisting of an aqueous solution of a gold salt and a solution of phosphorus in carbon disulfide. After a short reaction time the bright yellow color of the Na [AuCl4] solution turned into a ruby color characteristic of gold nanoparticles The principal motivation to perform research on nanoparticles is founded in the so-called quantum size effect. Metal and semiconducting nanoparticles just a

few nanometers in diameter, and thus with sizes somewhere between single atoms/molecules and bulk materials, show pronounced size- (and also shape-) depending on the observation of such size effects raised expectations for the superior performance of nanomaterials compared to their bulk counterparts in many applications, if the size and the shape of the particles can be optimized in a rational way. Systematic work on the photocatalytic properties of colloidal CdS [15], [16] resulted in the description of the quantum size effect at the beginning of the 1980s. Brus et al. found that CdS crystallites in the size range of a few nanometers did not have the electronic spectra of the bulk material, even though they exhibited the same unit cell and bond length as the bulk material [17].These findings opened up a new and exciting possibility to tailor the chemical and physical properties of a material: new applications and properties as a result of controlling crystallite size and shape on a nanometer scale rather than by altering the composition [14]. Consequently, the development of advanced synthesis routes not only offering control over the composition, as typically required for traditional bulk synthesis, but also over particle size, size distribution, shape and surface properties became essential on the way to study and apply the size-dependent properties of nanomaterial sent electronic and optical properties [21], [22], [23]. The observation of such size effects raised expectations for the superior performance of nanomaterials compared to their bulk counterparts in many applications, if the size and the shape of the particles can be optimized in a rational way.

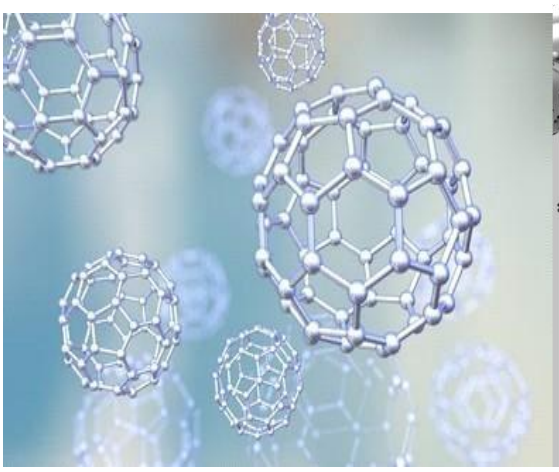
Importance of nanoparticles

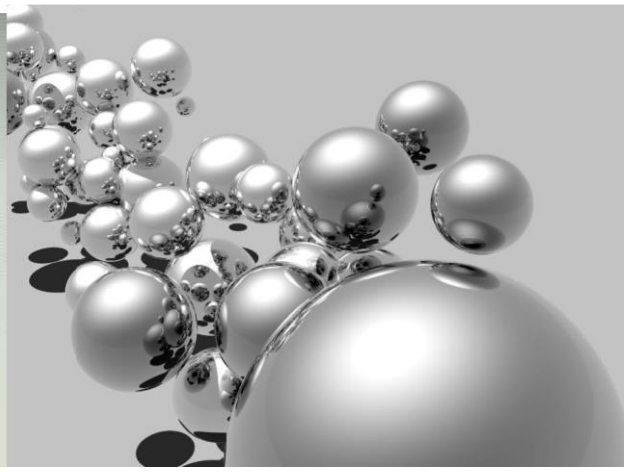
Nanoscale additives or surface treatments of fabrics can provide lightweight ballistic energy deflection in personal body armour, or can help the resist wrinkling, staining and bacterial growth. Clear nanoscale films on eyeglasses computer and camera displays, windows, and other surfaces can make them water and residue-repellent, anti-reflective, self-cleaning, resistant to ultraviolet or infrared light, anti-fog, antimicrobial, starch-

resistant, or electrically conductive. Nanoscale materials are beginning to enable washable durable "smart fabrics" equipped with flexible nanoscale sensors and electronics with capabilities for health monitoring, solar energy capture, and energy harvesting through movement. Light weighting of cars, trucks, airplanes, boats could lead to significant fuel savings. Nanoscale additives in polymer composite materials are being used in baseball boats, tennis, rockets, bicycles, motorcycle helmets, automobile parts, luggage, and power tool housing, making them lightweight, stiff, durable and resilient. Carbon nanotube sheets are now being produced for use in next-generation air vehicles.

Environmental importance:

Nanotechnology could help to meet the need for affordable, clean drinking water through rapid, low-cost detection and treatment of impurities in water. Engineers have developed a thin film membrane with nanopores for energy-efficient desalination. The molybdenum di sulphide (MoS:) membrane filtered two to five times more water than current conventional filters. Nanoparticles are being developed to clean industrial water pollutants in ground water through chemical reactions that render the pollutants harmless. This process would cost less than methods that required pumping the water out of the ground

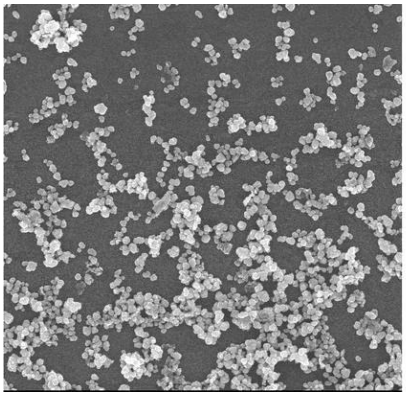


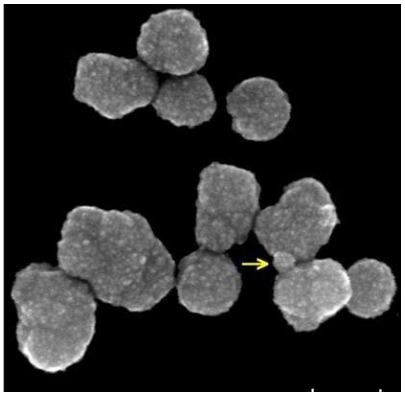


Nanotechnology is the study and application of tiny objects that may be used across different fields such as chemistry, biology, physics, and engineering. Nanoparticle is a major particle which performs as a whole unit in terms of transport and property. Nano means a billionth or 10–9 units. Its size ranges from 1 to 100 nm because it is too small in size and it occupies a position in various fields of nanoscience and nanotechnology [24]. Silver particles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnostics, antimicrobials and therapeutics; catalysis and microelectronics. Nowadays, there are several methods for the production of nanoparticles such as chemical and physical methods [25]. Silver nanoparticles (AgNO3) can be used to treat bacterial diseases [33]. Biosynthesized $AgNO_3$ had several applications in pharmaceutical and large-scale commercial production [34]. The present study focused on synthesizing $AgNO_3$ using *Moringa Oleifera* seeds and G. glabra stems. Synthesized $AgNO_3$ were used to evaluate the antibacterial efficacy of MRSA.

Science and technology of nanomaterials have rapidly grown in recent years and, as a consequence, great progress in the synthesis and characterization of materials in the nanometer regime has been achieved. The applications of these nanomaterials are diverse in the chemical industry, in electronics, and even in medicine [26,27]. One of the main applications of nanomaterials, however, is in catalysis. This is due to the positive impact that high superficial area of nanoparticles exerts on reaction time, costs, and process efficiency [27, 28-30]. However, in order to prevent sintering and have an easy recovery of the catalyst from the reaction medium, supports are employed. Carbon, titania, metal-organic frameworks, graphene, mesoporous silica, or various polymers have been successfully applied as supports by several research groups [31–41]. The conventional nanoparticle synthesis is complex and relatively expensive and is conducted at high temperatures. These undesirable features have motivated the search of a greener synthesis. To achieve so, the general approach that can be found in the literature is by

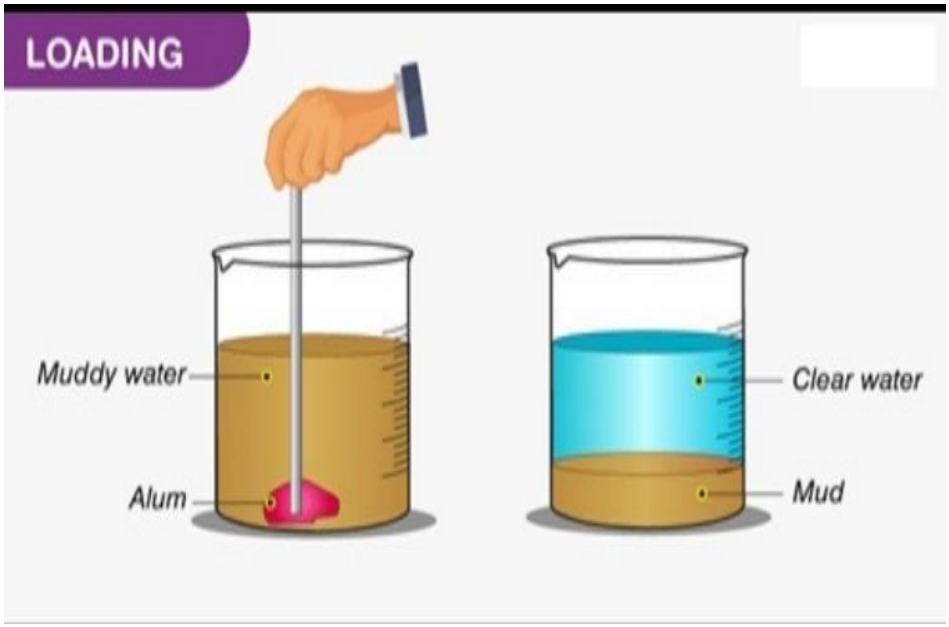
modifying the reaction medium to produce the nanoparticles, mainly in the liquid phase [42–45]. In this context, Lin et al. have reported the one step synthesis of platinum nanoparticles supported on wood, using hydroxyl groups (OH−) as reducing agent, to obtain a nanomaterial with high and relatively stable activities in the catalytic reduction of -nitrophenol [46]. Devia and Mandal reported the self-assembly of Ag nanoparticles using hydroxypropyl cyclodextrin for the catalytic reduction of p-nitrophenol [47]. Actually, cellulose has been studied as template since it is a natural carbohydrate rich in oxygen containing anhydro glucose units, linked by hydrogen bonds to form nonlinear molecular chain [48]. Bones, nonetheless, are constituted mainly by hydroxyapatite, which exhibits donor OH− and groups to anchor metallic ions. Therefore, the purpose of this study was to synthesize at relatively low-cost monodispersed Pt nanoparticles (NPs) onto a bovine bone, which is a renewable and novel bio support. In addition, the catalytic activity of the as-prepared material was assessed in the hydrogenation catalytic conversion of 2-butyne-1,4-diol to 2-butene-1,4-diol.





Palladium-nanoparticles (PdNPs) biohybrids have shown an important role in a widespread variety of chemical reactions [49-52]. The intrinsic properties of nanoparticles with an extremely large surface-to-volume ratio—provided advantages as catalysts compared to bulk materials. However, typical strategies for the transition metal nanoparticle syntheses require severe conditions, such as high T, pressure, flammable solvents, etc. [53]. Therefore, recently more simple, efficient, and sustainable synthetic strategies have been developed [54-58]. In this way,

the use of biological agents as plants, microorganisms, or fungi have allowed synthesizing Pd nanoparticles by a green way. However, important parameters such as the localization or morphology of the nanoparticles are subject to the microorganism species, since mixtures of enzymes and proteins can be involved in the process, producing nanoparticles of dissimilar sizes [59]. Therefore, the most suitable scheme would be the use of proteins or enzymes directly [60–62]. Protein scaffolds could offer splendid metal coordination environments that encourage biomineralization procedures, allowing the control of dimension, particle growing, and avoiding nanoparticles aggregation [63– 65]. Furthermore, using an enzyme, it could be possible to conjugate the activity of the metal nanoparticles with the enzymatic activity in a one-pot catalyst, being an additional advantage compared with other methods. Here, we describe the synthesis of high stable heterogeneous Pd nano catalysts (enzyme/PdNPs biohybrids), where NPs were created *in situ* from an aqueous solution containing an enzyme and a noble metal salt [66]. Different enzymes, such as scaffolds, were tested evaluating their role in the metal reduction, stabilization of nanoparticles, and remaining biocatalytic activity. The enzyme operated at once as a reducing, stabilizing, and supporting mediator a well as a bio-catalyst. These novel biohybrids were effectively used as catalysts in several chemical reactions, C-C bonding reaction, reductions, cascade processes in an aqueous or organic solvent as the reaction media, demonstrating their versatility as heterogeneous catalysts. During sedimentation, heavier particles settle down quickly but fine particles of clay settle down very slowly. The finer particles can be made to settle faster by dissolving a small quantity of "ALUM" in muddy water. This method is called loading. When alum is added to muddy water, the finer particles get loaded (stick) with alum particles and make them heavier and thus settle down rapidly (faster).


LOADING
Muddy water
Alum
Clear water
Mud

OBJECTIVES

Objectives

The present investigation was carried out with the following objectives.

- To study the synthesis of silver, platinum, palladium nanoparticles using extracts of "*Moringa Oleifera*" by various method like Visual, Potentiometric and Calorimetric methods.

- To study the application of the synthesized nanoparticles.

EXPERIMENTAL PART

EXPERIMENTAL PART

***Moringa oleifera*:**

Kingdom: Plantae

Clade: Tracheophytes, Angiosperms, Eudicots.

Order: Brassicales.

Family: Moringaceae.

Genus: Moring.

Species: M. oleifera.

Oleifera Binomial name: "*Moringa oleifera*"

The moringa tree *Moringa oleifera*, has probably been the most popular tree native to India but has been planted around the world and is naturalized in many locales Moringa goes by many names in the Philippines where the leaves of the moringa are cooked and fed to babies, it is called "mother's best friend" and "malunggay" Other names for it include the benzo live tree (Habi), horseradish tree (Florida), nobody (Senegal) and drumstick tree (India). There are about 13 species of moringa trees in the family *Moringaceae*. They are native to India, the Red Sea area and parts of Africa including Madagascar of these species, *Moringa Oleifera* is the most widely known referred to by their Latin name.

Preparation of *Moringa Oleifera* extract for synthesis of Silver Nanoparticles:

About 17ml of silver nitrate was dissolved in 100ml of distilled water($1x10^{-3}$M). The stock solution of the extract was prepared. The silver nanoparticles are synthesized by adding about 1ml,1.5ml,2ml,2.5ml,3ml of the *Moringa Oleifera* extract to each of 5 ml of silver nitrate solution.

By visual method by formation of brown colour solution shows the formation of silver nanoparticles. This is due to the reduction of Ag^{+} ions present in the solution. The time taken for synthesizing the silver Nanoparticles was studied by potentiometric method.

Preparation of *Moringa Oleifera* extract for synthesis of Platinum Nanoparticles:

About 20mg of the Platinum was dissolved in 100ml of distilled water ($1x10^{-3}$M). The stock solution of the extract was prepared. The Platinum Nanoparticles are synthesized by adding about 1ml,1.5ml,2ml, 2.5ml,3ml of the *Moringa Oleifera* extract to each 5ml of Platinum solution.

By visual method by formation of orange to brown colour shows the formation of Platinum nanoparticles. This is due to the reduction of Pt^{2+} present in the solution. The time taken for synthesizing the Platinum Nanoparticles was studied by potentiometric method.

Preparation of Moringa Oleifera for synthesis of Palladium Nanoparticles:

About 10mg of the palladium was dissolved in 100ml of dissolved water($1x10^{-3}$M). The stock solution of the extract was prepared. The Palladium Nanoparticles are synthesized by adding about 1ml,1.5ml,2ml,2.5ml,3ml of *Moringa Oleifera* extract to each 5ml of palladium solution.

By visual method by formation of Yellow to brown colour shows the formation of palladium nanoparticles. This is due to the reduction of Pd^{2+} ions present in the solution. The time taken for synthesizing the Palladium Nanoparticles was studied by potentiometric method.

POTENTIOMETRIC METHOD:

About 20ml of *Moringa Oleifera* extract is taken in a beaker and it is diluted with water to 40ml. The calomel electrode and platinum electrode are connected to the respective terminals of the potentiometer and are dipped in the extract taken in the beaker and titrated against silver nitrate solution taken in the burette. $AgNO_3$ solution taken in the burette is added in terms of 0.5ml to the extract and stirred well. The emf values are noted respectively and the timing is also noted for each reading. The reading is noted until a constant value of emf is obtained. From this method we can fix the concentration which the silver nanoparticles are synthesized and we can know the time taken for the synthesis of silver nanoparticles. A graph is plotted by taking the emf value and time taken for synthesis in the x-axis and the volume of $AgNO_3$ in the y-axis.

Similarly, about 20ml of *Moringa Oleifera* extract is taken in a beaker and is diluted with water to 40ml. The calomel electrode and platinum electrode are connected to the respective terminals of the potentiometer and are dipped in the extract taken in the beaker and titrated against Platinum solution taken in the burette. Pt solution taken in the burette is added interms of 0.5ml to the extract and stirred well. The emf values are noted respectively and the timing is also noted for each reading. The reading is noted until a constant value of emf is obtained. From this method we can fix the concentration at which the Platinum nanoparticles is synthesized and we can know the time taken for the synthesis of silver nanoparticles. A graph is plotted by taking the emf value and time taken for synthesis in the x - axis and the volume of Platinum solution in the y- axis.

Similarly, about 20ml of *Moringa Oleifera* extract is taken in a beaker and is diluted with water to 40ml. The calomel electrode and platinum electrode are connected to the respective terminals of the potentiometer and are dipped in the extract taken in the beaker and titrated against Palladium solution taken in the burette. Pd solution taken in the burette is added interms of 0.5ml to the extract and stirred well. The emf values are noted respectively and the timing is also noted for each reading. The reading is noted until a constant value of emf is obtained. From this method we can fix the concentration at which the Palladium nanoparticles is synthesized and we can know the time taken for the synthesis of silver nanoparticles. A graph is plotted by taking the emf value and time taken for synthesis in the x - axis and the volume of Palladium solution in the y- axis.

COLORIMETRIC METHOD:

The samples for the *Moringa Oleifera* extract of about 5ml is pipette out in a provided test tube and about 0.5ml,1ml,2ml,2.5ml of AgNO3, platinum and palladium solution are added to it individually. The solution is allowed to stand for a few minutes for uniform distribution. The optical density is measured in the colorimeter by keeping the nm range at 420. The absorbance values are noted. A graph is drawn between the concentration along x-axis and the optical density along y-axis.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION:

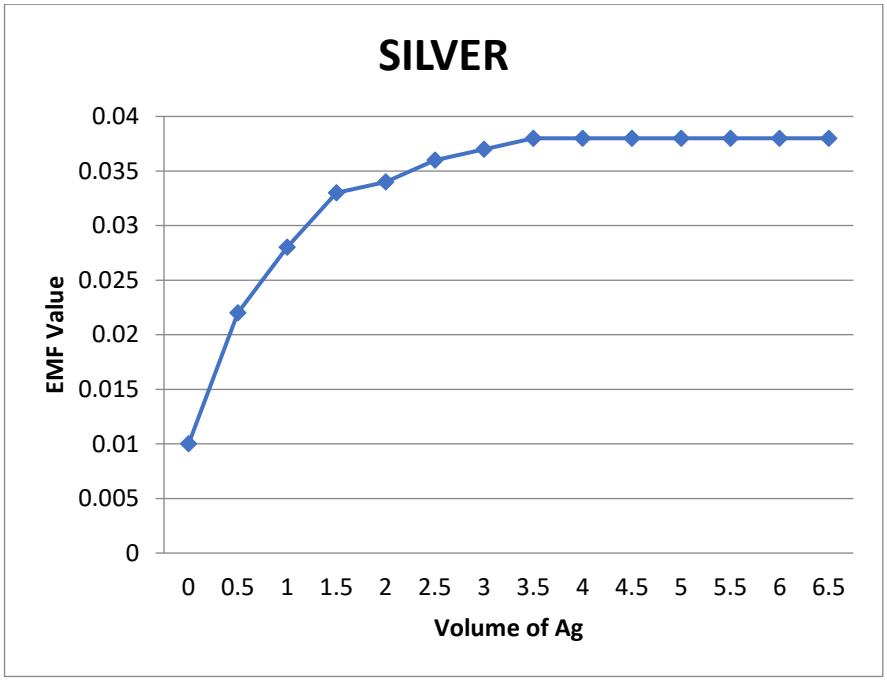
Visual Method

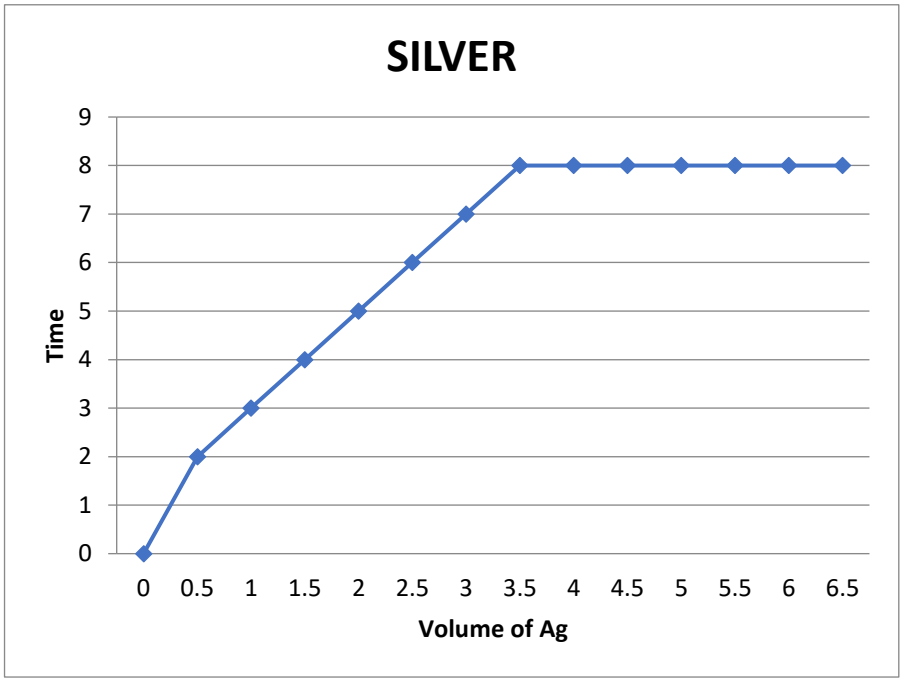
The above figure shows the formation of Silver, Platinum and Palladium Nanoparticles.

POTENTIOMETRIC METHOD:

SILVER:

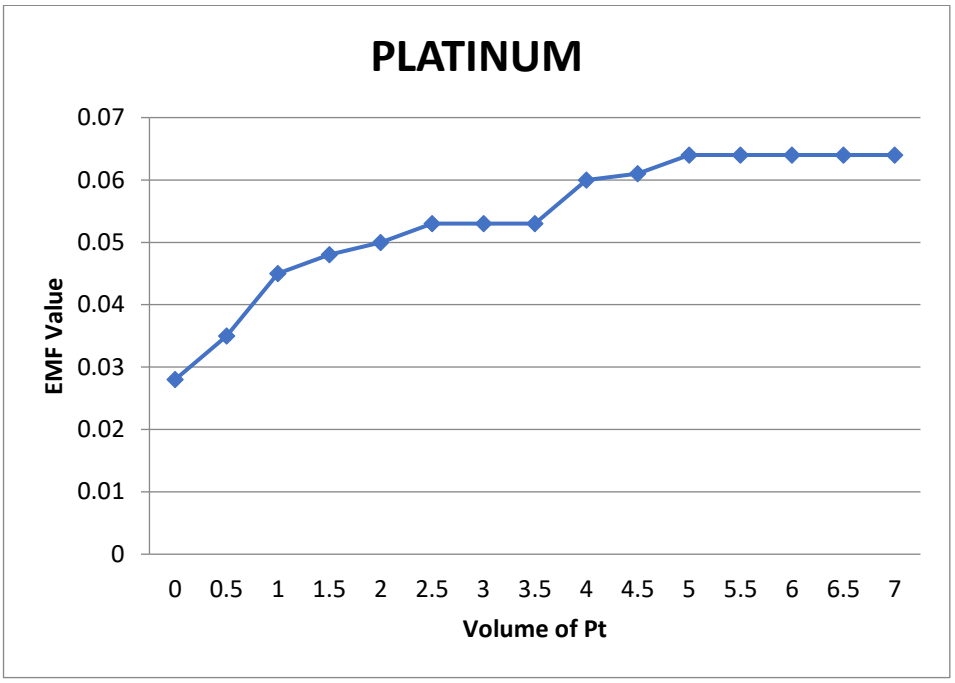
S.NO	Volume of Ag	EMF Value	Time
1.	0	0.010	0
2.	0.5	0.022	2
3.	1	0.028	3
4.	1.5	0.033	4
5.	2	0.034	5
6.	2.5	0.036	6
7.	3	0.037	7
8.	3.5	0.038	8
9.	4	0.038	8
10.	4.5	0.038	8
11.	5	0.038	8
12.	5.5	0.038	8
13.	6	0.038	8
14.	6.5	0.038	8

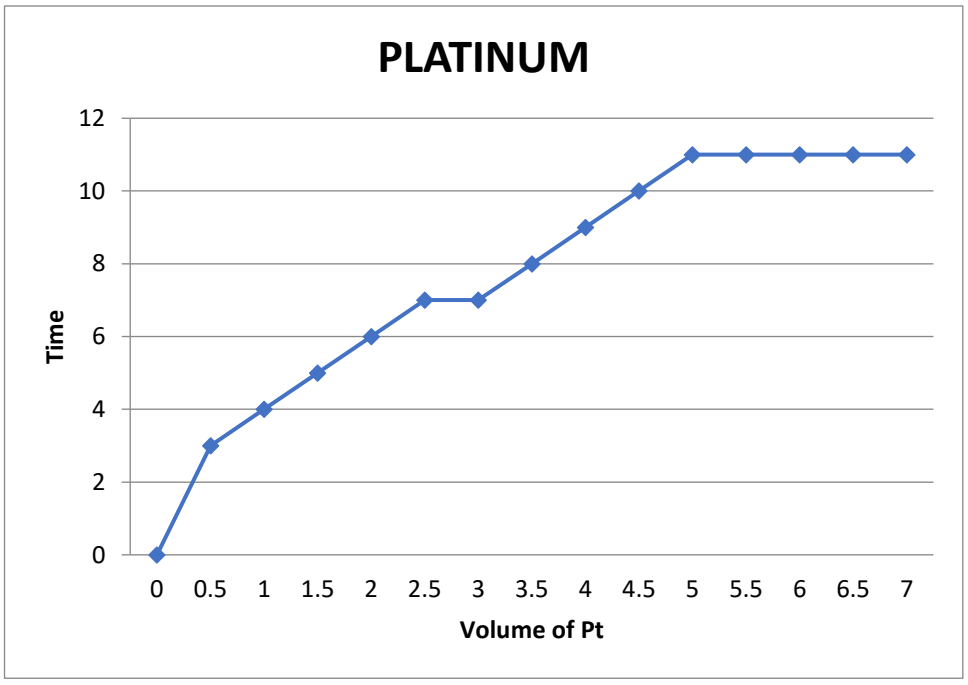

SILVER
EMF Value
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5
Volume of Ag


SILVER
Time
9
8
7
6
5
4
3
2
1
0
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5
Volume of Ag

PLATINUM:

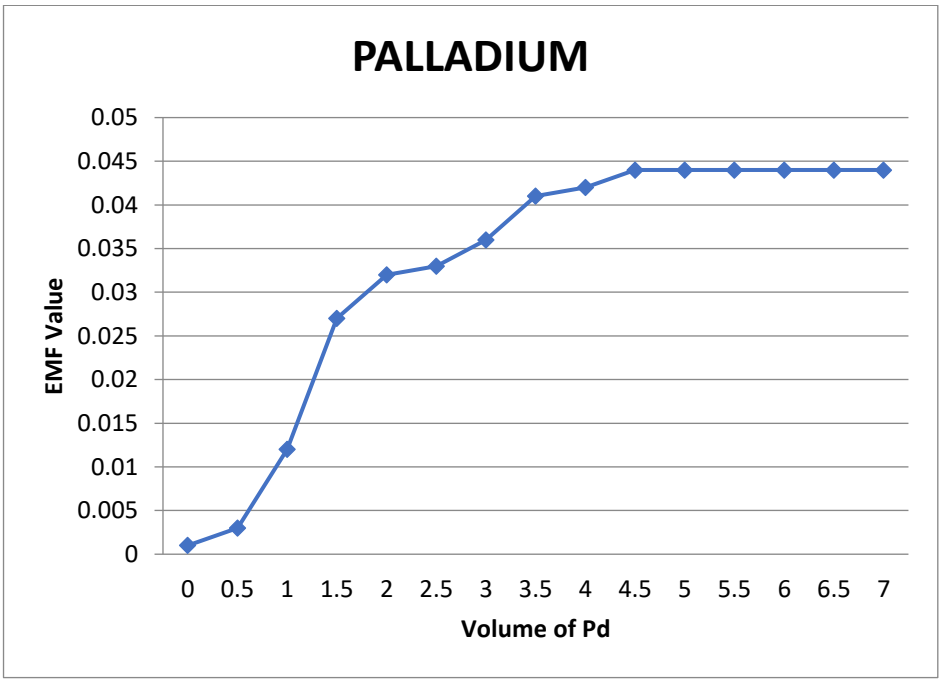
S.NO	Volume of Pt	EMF Value	Time
1.	0	0.028	0
2.	0.5	0.032	3
3.	1	0.045	4
4.	1.5	0.048	5
5.	2	0.050	6
6.	2.5	0.053	7
7.	3	0.053	7
8.	3.5	0.053	8
9.	4	0.060	9
10.	4.5	0.061	10
11.	5	0.063	11
12.	5.5	0.064	11
13.	6	0.064	11
14.	6.5	0.064	11
15.	7	0.064	11

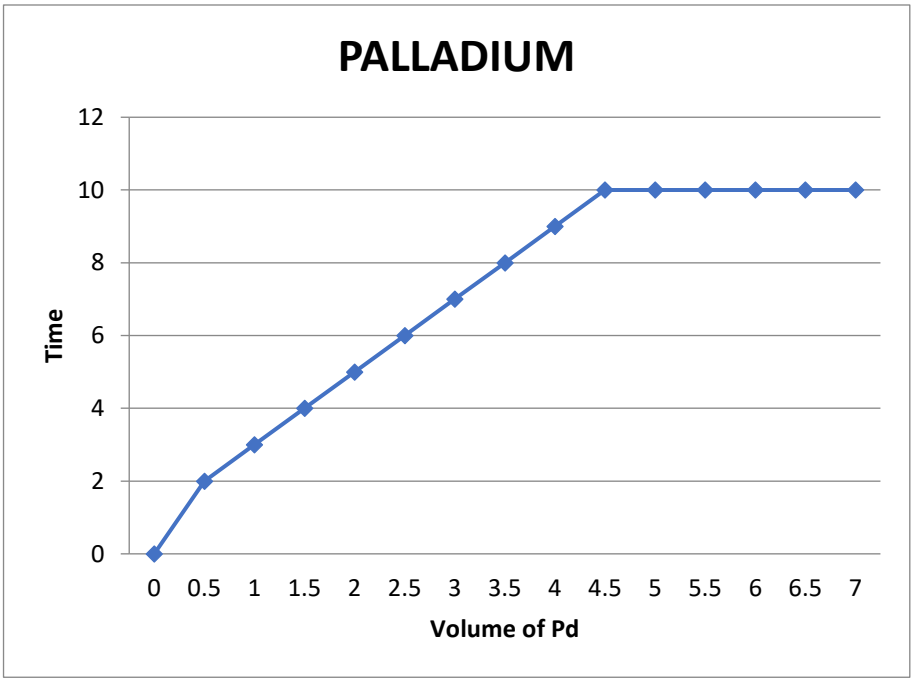

PLATINUM
EMF Value
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7
Volume of Pt


PLATINUM
Time
12
10
8
6
4
2
0
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7
Volume of Pt

PALLADIUM:

S.NO	Volume of Pd	EMF Value	TIME
1.	0	0.001	0
2.	0.5	0.003	2
3.	1	0.012	3
4.	1.5	0.027	4
5.	2	0.032	5
6.	2.5	0.033	6
7.	3	0.036	7
8.	3.5	0.041	8
9.	4	0.042	9
10.	4.5	0.044	10
11.	5	0.044	10
12.	5.5	0.044	10
13.	6	0.044	10
14.	6.5	0.044	10
15.	7	0.044	10


PALLADIUM
EMF Value
0.05
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7
Volume of Pd

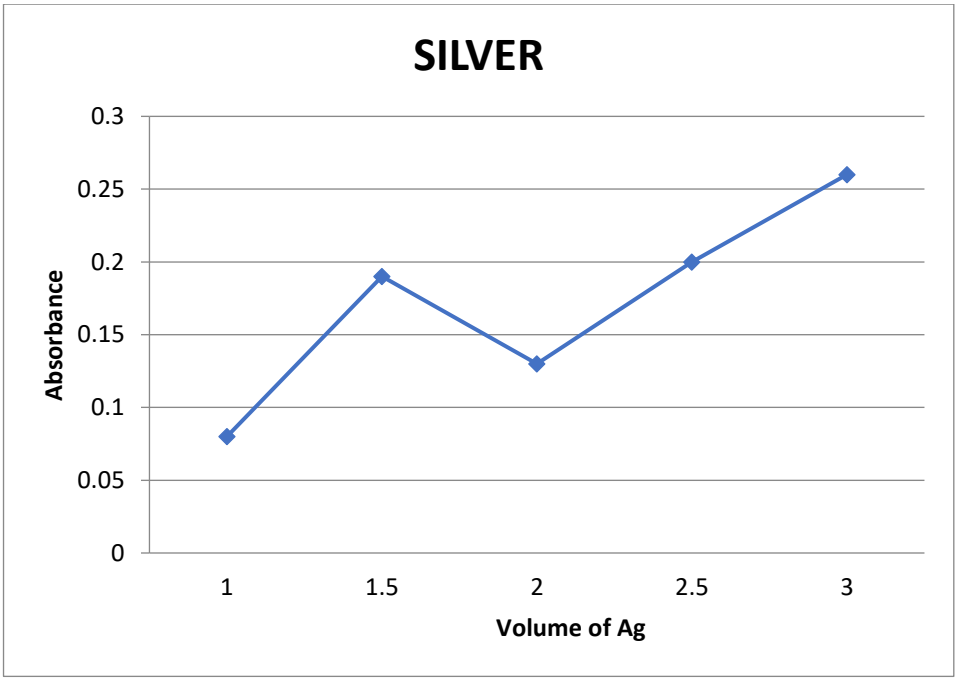

PALLADIUM
Time
12
10
8
6
4
2
0
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7
Volume of Pd

From the above reading the formation of the Silver ,Platinum , Palladium nanoparticles is noted by the constant emf value and the time is recorded for the formation of the nanoparticles and in addition the extract volume of Silver, platinum , Palladium nanoparticles needed for the formation is also noted. From the above reading the formation of the Silver nanoparticles is noted by the constant emf value and the time is recorded for the 3.5 ml and is formed around 8 minutes with the constant emf value of 0.038. Platinum nanoparticles is noted by the constant emf value and time is recorded for the 5 ml and is formed around 11 minutes with constant emf value of 0.064. Palladium nanoparticles is noted by the constant emf value and time is recorded for the 4.5 ml and is formed around 10 minutes with constant emf value of 0.044.

CALORIMETRIC METHOD:

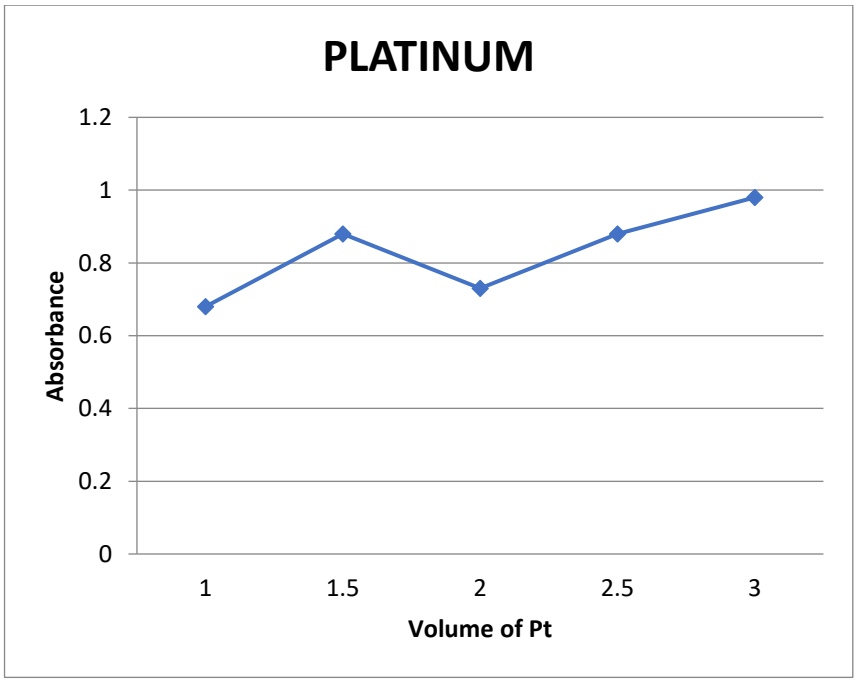
SILVER:

S.No	Volume of Ag	Calorimeter reading [Abs]
1.	1	0.08
2.	1.5	1.19
3.	2	1.13
4.	2.5	1.20
5.	3	1.26



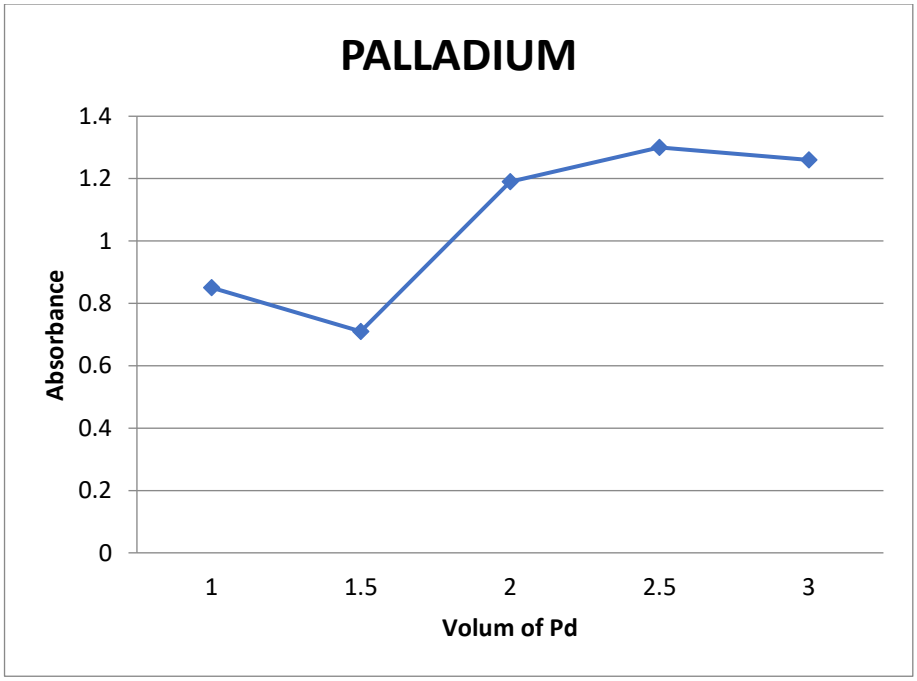
PLATINUM:

S.No	Volume of Pt	Calorimeter reading [Abs]
1.	1	0.68
2.	1.5	0.88
3.	2	0.73
4.	2.5	0.88
5.	3	0.98



PALLADIUM:

S.No	Volume of Pd	Calorimeter reading [Abs]
1.	1	0.85
2.	1.5	0.71
3.	2	1.19
4.	2.5	1.30
5.	3	1.26



From the above reading it is observed that as the concentration increases the absorbance increases. From the graph it was clear that as the concentration of nanoparticles increases the absorbance value also increases.

Application of *Moringa Olifera*

During sedimentation, heavier particles settle down quickly but fine particles of clay settle down very slowly. The finer particles can be made to settle faster by dissolving a small quantity of "ALUM" in muddy water. This method is called loading. When alum is added to muddy water, the finer particles get loaded (stick) with alum particles and make them heavier and thus settle down rapidly (faster). Using *Moringa Oleifera* extract the solutions are used for loading and hence we took two beakers of the same size with 200 ml of water. 5g of moringa leaf powder is added in first beaker as shown in figure. In another beaker moringa leaf and moringa seed powder are added. When allowed to stand for few minutes much more leaf powder has settled in a rapid manner.

Moringa Oleifera leaf powder alone

Moringa Oleifera leaf powder along with *Moringa Oleifera* seed

Using *Moringa Oleifera* extract the solutions are used for loading and hence we took two beakers of the same size with 200 ml of water. 5g of cocoa powder is added in first beaker as shown in figure. In another beaker cocoa powder and moringa seed powder are added. When allowed to stand for few minutes much more Cocoa powder has settled in a rapid manner.

	
Cocoa powder alone	Cocoa powder along with *Moringa Oleifera* seed

Using *Moringa Oleifera* extract the solutions are used for loading and hence we took two beakers of the same size with 200 ml of water. 5g of Tomato sauce is added in first beaker as shown in figure. In another beaker Tomato sauce and moringa seed powder are added. When allowed to stand for few minutes much more Tomato sauce has settled in a rapid manner.

Tomato sauce alone	Tomato sauce along with *Moringa Oleifera* seed

Using *Moringa Oleifera* extract the solutions are used for loading and hence we took two beakers of the same size with 200 ml of water. 5g of garden soil is added in first beaker as shown in figure. In another beaker garden soil and moringa seed powder are added. When allowed to stand for few minutes much more garden soil has settled in a rapid manner.

garden soil alone	garden soil along with *Moringa Oleifera* seed

Plants are rich in secondary metabolites and are being used for the treatment of various ailments in the indigenous system of medicine. Many developing countries are facing illnesses, and deaths among children are caused by germs, which get into the mouth via water and food.

In addition, it has been estimated that up to 80% of all disease and sickness in the world is caused by inadequate sanitation, polluted water or unavailability of water. Thus, this study investigates that *Moringa oleifera* seed powder can be used for water purifying property ie.,helps to settle the fine dust particles that are present in the water and also determines the role of seed extracts against a few bacterial growths.

CONCLUSION

CONCLUSION

Silver, Platinum, Palladium nanoparticles are prepared using the extract of *Moringa Oleifera*. extract. The exact concentration has been fixed by the appearance of colour change. After fixing the concentration the time taken for the formation of silver, platinum, palladium, nanoparticles are determined using potentiometric method and calorimetric method, from this exact time at which the nanoparticle synthesized is determined for all the samples. Using silver, platinum, palladium, nanoparticles of *Moringa Oleifera* the physical process of settling the fine particles has been carried out. Hence, we conclude that nanoparticles synthesized using *Moringa Oleifera* extract can be used for filtration process.

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A STUDY ON SYNTHESIS OF DIFFERENT NANOPARTICLES USING EXTRACTS OF *TERMINALIA BELLIRICA*

PROJECT IN CHEMISTRY

Submitted to St.Mary's College(Autonomous) in partial fulfillment for the award of the

Degree of Bachelor of Science in Chemistry

PROJECT DONE BY

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2021- 2022

DECLARATION

We hereby declare that the project entitled **"A STUDY ON SYNTHESIS OF DIFFERENT NANOPARTICLES USING EXTRACTS OF *TERMINALIA BELLIRICA*"** submitted to St. Mary's College (Autonomous), Thoothukudi, affiliated to Manonmaniam Sundaranar University, for the Degree of Bachelor of Science is our original work and that, it has not previously formed the basis for the award of any Degree, Diploma or similar title.

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May 2022

Thoothukudi

CERTIFICATE

This is to certify that the report of the project in Chemistry entitled **"A STUDY ON SYNTHESIS OF DIFFERENT NANOPARTICLES USING EXTRACTS OF *TERMINALIA BELLIRICA*"** is submitted to St. Mary's College (Autonomous) Thoothukudi in partial fulfillment for the award of the degree of Bachelors of Science in Chemistry and is a record of the work done by the following students during the year 2021– 2022.

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CONTENTS

ABSTRACT

ABSTRACT

In the present study, Silver, Platinum, Palladium nanoparticles were rapidly synthesized at room temperature by treating Silver, Platinum, Palladium ions with *Terminalia Bellirica* extract. The effect of various process parameters like the reactant concentration, mixing ratio of the reactants and the concentration silver nitrate, Platinum, Palladium were studied. Silver, Platinum, Palladium nanoparticles are prepared using the extract of Terminalia Bellerica. About 10^{-3}M, solution of Silver nitrate, Platinum, Palladium are prepared as a stock solution individually. *Terminalia Bellirica* fruits were collected from the fields. Cleaning of fruits are done and are spread for 10 to 15 days for drying. Separation of hard portion from the seeds is done only when the fruits are properly dried. After separation of hard portion from the seeds, the separated hard cover or pulp are kept for further drying for 4 - 5 days. Then it is grinded. The powder which we obtained from the grinding process must be sieved. About 20gm of prepared powder was taken in a 200ml beaker diluted with water and heated to boiling.The extract was centrifuged for 2 minutes at 10,000rpm to get a clear solution and to remove the undesired impurities from it. At first step, to fix the concentration of the *Terminalia Bellirica* extracts, the extract are measured in the ratio of 1ml, 1.5ml, 2ml, 2.5ml, 3ml taken in a clean and a dry test tube. About 1ml of Silver nitrate, Platinum, Palladium solution were added in all the respective test tube individually and mixed to get an uniform distribution. Time has been noted from 0 minutes and observation has been done then and there. The exact concentration has been fixed by

the appearance of yellowish brown colour for silver, Light yellow to brown colour for Platinum, Orange to dark brown colour for Palladium. After fixing the concentration, the time taken for the formation of Silver, Platinum, Palladium nanoparticles are determined using potentiometric method, from this the exact time at which the nanoparticle synthesized is determined for all the samples. Hence, we conclude that Silver, Platinum, Palladium nanoparticles synthesized using *Terminalia Bellirica* extract can be used to enhance the long shelf life period of vegetables like tomato.

INTRODUCTION

INTRODUCTION:

Plants produce a wide range of bioactive compounds and constitute a rich source of medicines. The ancient Ayurvedic, Unani and Siddha systems of medicine is based on the healing ability of plants. In different regions of the world, plant derived medicinal systems remain important in the treatment of various diseases. Ayurvedic remedy is quite commonly practiced in India with an estimated 85 % of Indians still using crude plant preparations for the treatment of a wide variety of diseases and ailments (Kamboj, 2000)[1]. Many of the prescription drugs currently promoted for a wide variety of ailments were originally isolated from plants and are semi-synthetic analogues of plant derived chemicals. It has been estimated that approximately 25 % of all prescription drugs currently in use are of plant origin (Walsh, 2003; Newman and Cragg, 2007)[2][3]. Furthermore, approximately 75% of new anticancer drugs used between 1981 and 2006 were derived from plant compounds (Newman and Cragg, 2007)[3]. *Terminalia bellirica Roxb*. (Family Combretaceae) is a large deciduous tree with broadly elliptic leaves clustered at the ends of branches (Meena et al.,2010)[4]. It is widely distributed throughout the world especially in Indian subcontinent, Srilanka, Pakistan, Nepal and South East Asia. *Terminalia bellirica* is used in traditional medicine due to the wide spectrum of pharmacological activities associated with the biologically active secondary metabolites present in this plant. Variety of phytochemicals are isolated from various parts of the plant which include alkaloid, coumarin, flavones, steroids, lignans, tannins, glycosides, terpenoid, saponin etc.(Abraham et al., 2014)[5]. Nature serves as primary source for the cure of ailments. It is estimated that, in many developing countries, two third of the population is dependent on medicinal plants to meet primary healthcare needs. The use of herbal medicine is increasing due to its safety, efficacy and therapeutic potential as compared to synthetic pharmaceutical products. However, the potential of higher plants as a source of herbal medicine is unexplored.Therefore,

there is a need for thorough literature search on some species to update the current state of knowledge and one such plant is *Terminalia bellirica*. *Terminalia bellirica* commonly known as bibhitaki belongs to the family Combretaceae. It is called vibheetaki in Sanskrit which means "fearless", the fruit that takes away the fear of disease. In Indian history, it is said that, *Terminalia bellirica* is inhabited by demons and those who sat under its shade were vulnerable to an attack by the same. Due to its medicinal properties tree has Sanskrit synonym of Anila-ghnaka, or "wind-killing". The generic name '*Terminalia*' is derived from Latin word, 'terminus' or 'terminalis' (ending), which means habit of the leaves being crowded or borne on the tips of shoots. It is a compound of rasayana preparation, made up of three myrobalan fruits, known as Triphala, which is important in Indian as well as Tibetan traditional medicines. *Terminalia bellirica*. (Family Combretaceae) is a large deciduous tree with broadly elliptic leaves clustered at the ends of branches (Meena et al.,2010)[4].

Traditional Uses :

Terminalia bellirica fruits exhibit medicinal activity. These are used as laxative, astringent, anthelmintic and antipyretic. Fruits are useful in treatment of hepatitis, bronchitis, asthma, dyspepsia, piles, diarrhoea, coughs, hoarseness of voice, eye diseases, scorpion-sting and also used as a hair tonic (Singh et al., 2011; Rastogi and Mehrotra, 2004)[6,7]. Decoction of the green fruit is used for cough. Pulp of the fruit is useful in dysenteric-diarrhoea, dropsy, piles and leprosy. Half ripe fruit is used as purgative. Kernel of the fruit is narcotic. Inhabitants of Khagrachari in Bangladesh use fruits in menstrual disorder. Seed oil is used in rheumatism.

Gum of the bark is demulcent and purgative. The triterpenoid present in the fruits possess significant antimicrobial activity. Kernel oil has purgative action and its prolonged use is well tolerated in mice (Ghani, 2003)[8].

Antimicrobial activity :

Infectious diseases have become the major concern for public health issues because of the emergence of drug resistant strains with less susceptibility to antibiotics. *Terminalia bellirica* has shown potent action against infectious agents *in vitro*. Fruit extract contains phenol, tannins, alkaloid and flavonoids. Alkaloid could be responsible for inhibiting the microorganism by impairing the enzymes involved in energy production, interfering with the integrity of cell membrane and structural component synthesis. Tannins in the fruit extract of *Terminalia bellirica* could be implicated in preventing the development of microorganisms by precipitating the microbial protein and making nutritional proteins unavailable for them (Hung and Chung, 2003)[9]. Tannins have been found to form irreversible complexes with proline rich proteins resulting in the inhibition of cell protein synthesis (Hagerman and Butler, 1981)[10]. Coagulase is major virulence factor of *S. aureus* which converts the host plasma fibrinogen to fibrin, forming blood clots. *Terminalia bellirica* extract inhibit the activity of this enzyme when *S. aureus* grown in the presence of *Terminalia bellirica* extract. The lower MIC values of crude and methanol extracts against *S. aureus* suggested the efficacy of *Terminalia bellirica* phytoconstituents (Elizabeth, 2005)[11]. *Terminalia bellirica* aqueous fruit extract has shown activity against numerous pathogenic bacteria viz., *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Shigella flexneri, and Salmonella typhi* (Devi et al., 2014)[12]. In a study conducted by Saraphanchotiwitthaya et al. (2008[13]), stem and leaf extracts of *Terminalia bellirica* also showed antibacterial activity against many Gram positive and Gram negative bacteria including *Corynebacterium rubrum, Staphylococcus epidermidis, K. pneumonie, E. coli and S. typhimurium*. None of these studies identified the antibacterial components. However,

studies have demonstrated that antibacterial activity was associated with the polar extracts (Aneja and Joshi, 2009; Aneja et al., 2012)[14].

Nanoparticles :

A nanoparticle or ultrafine particle is usually defined as a particle of matter that is between 1 and 100 nanometres (nm) in diameter.[15][16]. The term is sometimes used for larger particles, upto 500 nm, or fibers and tubes that are less than 100 nm in only two directions.[17] At the lowest range, metal particles smaller than 1 nm are usually called atom clusters instead. Nanoparticles are usually distinguished from microparticles (1-1000 μm), "fine particles" (sized between 100 and 2500 nm), and "coarse particles" (ranging from 2500 to 10,000 nm) because their smaller size drives very different physical or chemical properties, like colloidal properties and ultrafast optical effects[18] or electric properties. Being more subject to the brownian motion, they usually do not sediment, like colloidal particles that conversely are usually understood to range from 1 to 1000 nm. Being much smaller than the wavelengths of visible light (400-700 nm), nanoparticles cannot be seen with ordinary optical microscopes, requiring the use of electron microscopes or microscopes with laser. For the same reason, dispersions of nanoparticles in transparent media can be transparent,[19] whereas suspensions of larger particles usually scatter some or all visible light incident on them. Nanoparticles also easily pass through common filters, such as common ceramic candles,[20] so that separation from liquids requires special nanofiltration techniques. The properties of nanoparticles often differ markedly from those of larger particles of the same substance. Since the typical diameter of an atom is between 0.15 and 0.6 nm, a large fraction of the nanoparticle's material lies within a few atomic diameters of its surface. Therefore, the properties of that surface layer may dominate over those of the bulk material. This effect is particularly strong for nanoparticles dispersed in a medium of different

composition since the interactions between the two materials at their interface also becomes significant.[21] Nanoparticles occur widely in nature and are objects of study in many sciences such as chemistry, physics, geology and biology. Being at the transition between bulk materials and atomic or molecular structures, they often exhibit phenomena that are not observed at either scale. They are an important component of atmospheric pollution, and key ingredients in many industrialized products such as paints, plastics, metals, ceramics, and magnetic products. The production of nanoparticles with specific properties is a branch of nanotechnology. In general, the small size of nanoparticles leads to a lower concentration of point defects compared to their bulk counterparts,[22] but they do support a variety of dislocations that can be visualized using high-resolution electron microscopes.[23] However, nanoparticles exhibit different dislocation mechanics, which, together with their unique surface structures, results in mechanical properties that are different from the bulk material.[24][25][26] Non-spherical nanonparticles (e.g., prisms, cubes, rods etc.) exhibit shape-dependent and size-dependent (both chemical and physical) properties (anisotropy).[27][28] Non-spherical nanoparticles of gold (Au), silver (Ag), and platinum (Pt) due to their fascinating optical properties are finding diverse applications. Non-spherical geometries of nanoprisms give rise to high effective cross-sections and deeper colors of the colloidal solutions.[29] The possibility of shifting the resonance wavelengths by tuning the particle geometry allows using them in the fields of molecular labeling, biomolecular assays, trace metal detection, or nano technical applications. Anisotropic nanoparticles display a specific absorption behavior and stochastic particle orientation under unpolarized light, showing a distinct resonance mode for each excitable axis. This property can be explained by the fact that on a daily basis there are new developments being made in the field of synthesis of these nanoparticles for preparing them in high yield [29].

Silver Nanoparticles :

Silver nanoparticles (AgNPs) are increasingly used in various fields, including medical, food, health care, consumer, and industrial purposes, due to their unique physical and chemical properties. These include optical, electrical, and thermal, high electrical conductivity, and biological properties [30, 31, 32]. Due to their peculiar properties, they have been used for several applications, including as antibacterial agents, in industrial, household, and healthcare-related products, in consumer products, medical device coatings, optical sensors, and cosmetics, in the pharmaceutical industry, the food industry, in diagnostics, orthopedics, drug delivery, as anticancer agents, and have ultimately enhanced the tumor-killing effects of anticancer drugs [33]. Recently, AgNPs have been frequently used in many textiles, keyboards, wound dressings, and biomedical devices [33,34,35]. Nanosized metallic particles are unique and can considerably change physical, chemical, and biological properties due to their surface-to-volume ratio; therefore, these nanoparticles have been exploited for various purposes [36,37]. In order to fulfill the requirement of AgNPs, various methods have been adopted for synthesis. Generally, conventional physical and chemical methods seem to be very expensive and hazardous [30,38]. Interestingly, biologically-prepared AgNPs show high yield, solubility, and high stability [30]. Among several synthetic methods for AgNPs, biological methods seem to be simple, rapid, non-toxic, dependable, and green approaches that can produce well-defined size and morphology under optimized conditions for translational research.[39]

Platinum Nanoparticles :

Platinum nanoparticles are usually in the form of a suspension or colloid of nanoparticles of platinum in a fluid, usually water. A colloid is technically defined as a stable dispersion of particles in a fluid medium (liquid or gas). Spherical platinum nanoparticles can be made with

sizes between about 2 and 100 nanometres (nm), depending on reaction conditions.[40][41] Platinum nanoparticles are suspended in the colloidal solution of brownish-red or black color. Nanoparticles come in wide variety of shapes including spheres, rods, cubes,[42] and tetrahedral.[43] Platinum nanoparticles are the subject of substantial research,[44][45][46] with potential applications in a wide variety of areas. These include catalysis,[46] medicine,[44] and the synthesis of novel materials with unique properties.[41][45][46]

Palladium nanoparticles :

Modern science has experienced one of its major breakthroughs with nanotechnology in the last decades. This enabled nano-dimensional materials (in the 1–100 nm size domain) to be produced and applied in a number of technological and consumer fields due to their peculiar physico-chemical properties [47]. Metallic based nanomaterials have attracted great scientific attention in the nanotechnology field. Particularly, given that noble metals are characterized by remarkable catalytic, electronic, magnetic, optical, and mechanical properties [48,49,50], the study of the characteristics and possible uses of nano-sized noble metal materials has emerged as hugely important and valuable for the benefits potentially offered in a number of different applications.

In this regard, palladium (Pd) is a rare and precious metal that belongs to the Platinum group elements. It is largely employed as an active catalyst material in automotive catalytic converters, but finds also application in the electronic, engineering, biomedical, and jewelry sectors [51,52,53,54]. Palladium-NPs offer the opportunity for being even more effective catalyst materials due to their high surface area to volume ratio and high surface energy [55]. Therefore, the emerging applications of these NPs, due to the release of such materials in living and occupational environments, raise public health and scientific concerns over possible environmental and human health implications [56].

OBJECTIVES

Objectives

The present investigation was carried out with the following objectives.

* To study the synthesis of Silver, platinum, palladium nanoparticles using extract of *"Terminalia Bellirica"* by various method like Potentiometric, Calorimetric methods.

* To study the application of the synthesized nanoparticles.

EXPERIMENTAL METHOD

EXPERIMENTAL METHODS

Terminalia Bellirica

Family Name: Combretaceae

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Myrtales

Family: Combretaceae

Genus: Terminalia

Species:belerica

Terminalia bellirica is also referred to as *Beleric Myrobalan* in English, *Bibhitaki* in Sanskrit, and locally known as Bahera in India. It has been used for centuries in the Ayurveda, a holistic system of medicine originating from India. It is a large (10-20 meter) deciduous tree with characteristic thick brownish gray bark and is found throughout the Indian forests and plains. The stems are straight, frequently buttressed when large. Leaves are alternate, broadly elliptic, clustered towards at the ends of branches when young but glabrous on maturity and the nerves are prominent on both surfaces. Flowers are greenish yellow, borne in axillary, spender spikes longer than the petioles and having offensive odor. Fruit a drupe about 2.5 cm long, globose or narrowed at the base, silky-brownish-velvety.

FORMATION OF POWDER FROM *TERMINALIA BELLERICA*

1) COLLECTION OF *TERMINALIA BELLIRICA* FRUITS :

Fruits of *Terminalia bellerica* are collected from the farm/fields and transported to the mills for further processing. While collecting the fruits from the tree, it must be ensure that fruits are well matured and do not have any microbial growth over it.

2) CLEANING :

Cleaning of fruits should be done in proper manner so that every foreign particles and dust will be removed. While cleaning one should ensure that the water used for cleaning must be fresh and free from any contaminants. The utensils used for cleaning the fruits must be of food grade quality and non reactive.

3) SPREADING :

After cleaning, fruits are spread for 10 to 15 days for drying. Layer of thickness should be uniform while spreading. Spreading area must be free from hazardous substances or any other thing which may impact the quality of fruits.

4) SEPARATION OF HARD PORTION :

Separation of hard portion from the seeds is done only when the fruits are properly dried. It can be done either manually or mechanically. For smaller amount of fruits the operation is performed manually while for larger amount it is done mechanically to make the operation more cost effective.

5) DRYING :

After separation of hard portion from the seeds, the separated hard cover or pulp are kept for further drying for 4 - 5 days. Drying area must be free from hazardous substances or any other thing which may impact the quality of fruits.

6) GRINDING:

Grinding of pulp is mainly done with the help of grinding machine and it should be smoothly grind so that texture of powder will be uniform and of good quality.

7) SIEVING :

The powder which we obtained from the grinding process must be sieved with the help of proper size of sieve so that size of powder should be uniform. The process of sieving also helps in avoiding the unwanted large particles from the final products.

Preparation of *Terminalia Bellirica* Extract for synthesis of Ag, Pt, Pd Nanoparticles :

*About 17mg of the Silver Nitrate was dissolved in 100 ml of distilled water (1×10^{-3} M). The stock solution of the extract was prepared. The Silver nanoparticles are synthesized by adding about 1ml, 1.5 ml, 2 ml, 2.5 ml, 3 ml, of the *Terminalia bellirica* extract of each of 5 ml Silver nitrate solution.

By visual method the formation of brownish yellow colour shows the formation of silver nanoparticles. This is due to the reduction of Ag^{+} ions present in the solution. The time taken for synthesizing the silver nanoparticles was studied by potentiometric method.

* About 20mg of the Platinum was dissolved in 100 ml of distilled water (1×10^{-3} M). The stock solution of the extract was prepared. The Platinum nanoparticles are synthesized by adding about 1ml, 1.5 ml, 2 ml, 2.5 ml, 3 ml, of the *Terminalia bellirica* extract of each of 5 ml Platinum solution

By visual method by formation of orange to brown colour shows the formation of Platinum nanoparticles. This is due to the reduction of Pt^{2+} present in the solution. The time taken for synthesizing the Platinum nanoparticles was studied by potentiometric method.

* About 10mg of the Palladium was dissolved in 100 ml of distilled water (1×10^{-3}M). The stock solution of the extract was prepared. The Palladium nanoparticles are synthesized by adding about 1ml, 1.5 ml, 2 ml, 2.5 ml, 3 ml, of the *Terminalia bellirica* extract of each of 5 ml Palladium solution.

By visual method by formation of Yellow to brown colour shows the formation of Palladium nanoparticles. This is due to the reduction of Pd^{2+} present in the solution. The time taken for synthesizing the Palladium nanoparticles was studied by potentiometric method.

POTENTIOMETRIC METHOD :

About 20ml of *Terminalia bellirica* extract is taken in a beaker and is diluted with water to 40ml. The calomel electrode and platinum electrode are connected to the respective terminals of the potentiometer and are dipped in the extract taken in the beaker and titrated against silver nitrate solution taken in the burette. AgNO3 solution taken in the burette is added interms of 0.5ml to the extract and stirred well. The emf values are noted respectively and the timing is also noted for each reading. The reading is noted until a constant value of emf is obtained. From this method we can fix the concentration at which the silver nanoparticles is synthesized and we can know the time taken for the synthesis of silver nanoparticles. A graph is plotted by taking the emf value and time taken for synthesis in the x - axis and the volume of AgNO3 in the y- axis.

Similarly, about 20ml of *Terminalia bellirica* extract is taken in a beaker and is diluted with water to 40ml. The calomel electrode and platinum electrode are connected to the respective terminals of the potentiometer and are dipped in the extract taken in the beaker and titrated against Platinum solution taken in the burette. Pt solution taken in the burette is added interms of 0.5ml to the extract and stirred well. The emf values are noted respectively and the timing is also noted for each reading. The reading is noted until a constant value of emf is obtained. From this method we can fix the concentration at which the Platinum nanoparticles is synthesized and we can know the time taken for the synthesis of silver nanoparticles. A graph is plotted by taking the emf value and time taken for synthesis in the x - axis and the volume of Platinum solution in the y- axis.

Similarly, about 20ml of *Terminalia bellirica* extract is taken in a beaker and is diluted with water to 40ml. The calomel electrode and platinum electrode are connected to the respective terminals of the potentiometer and are dipped in the extract taken in the beaker and titrated against Palladium solution taken in the burette. Pd solution taken in the burette is added interms of 0.5ml to the extract and stirred well. The emf values are noted respectively and the timing is also noted for each reading. The reading is noted until a constant value of emf is obtained. From this method we can fix the concentration at which the Palladium nanoparticles is synthesized and we can know the time taken for the synthesis of silver nanoparticles. A graph is plotted by taking the emf value and time taken for synthesis in the x - axis and the volume of Palladium solution in the y- axis.

CALORIMETERIC METHOD :

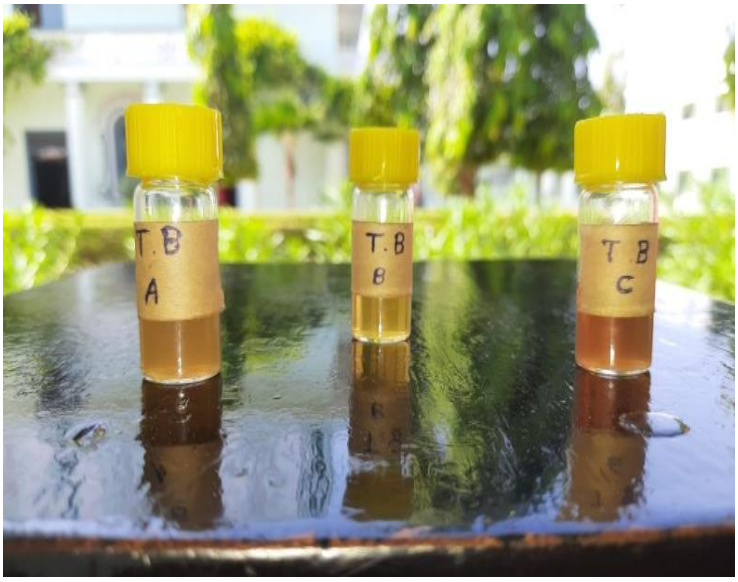
The samples of the *Terminalia bellirica* extract of about 5ml is pipette out in a provided test tubes and about 0.5ml,1ml,1.5ml,2ml,2.5ml of AgNO3,Platinum and Palladium solution is added to it individually.

The solution is allowed to stand for few minutes for uniform distribution. The optical density is measured in the colorimeter by keeping the nm range at 420. The absorbance values are noted. A graph is drawn between the concentration along x-axis and the optical density along y-axis.

RESULTS AND DISCUSSION

Results And Discussion :

Visual Method

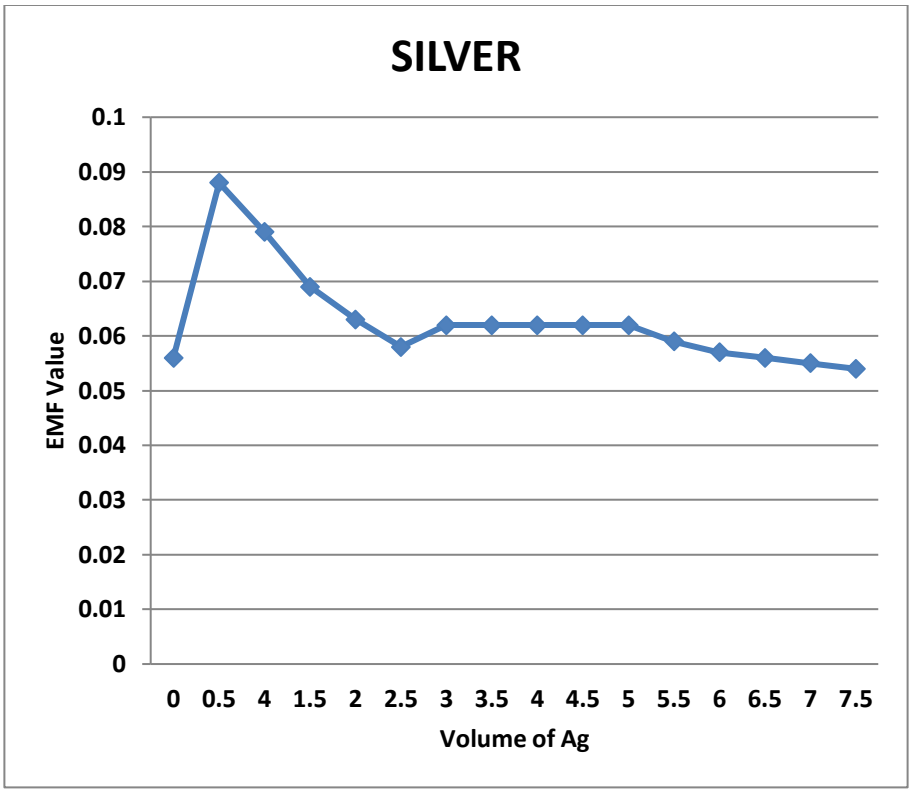


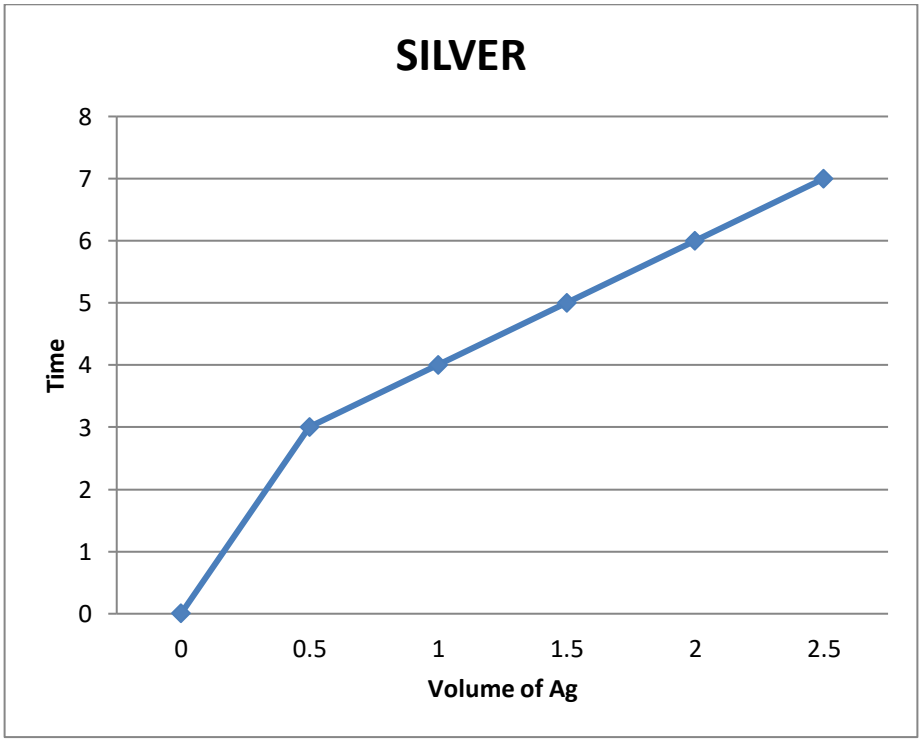
The above figure shows the formation of Silver, Platinum and Palladium Nanomaterials.

POTENTIOMETRIC METHOD

SILVER:

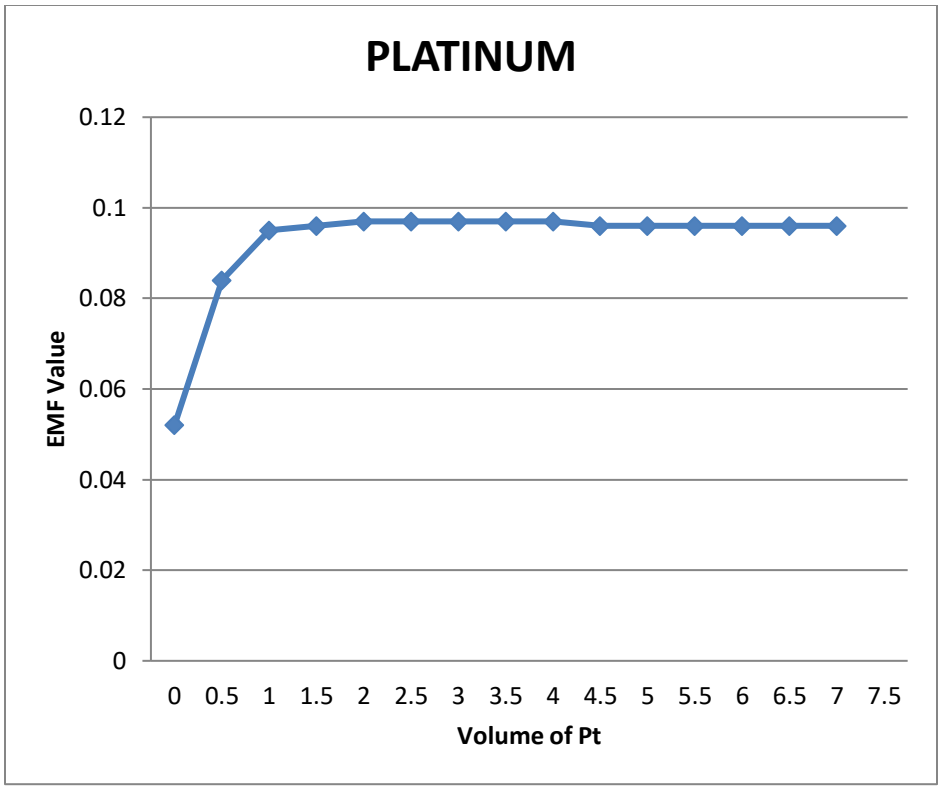
S.No	Volume of Ag	EMF value	TIME
1.	0	0.056	0
2.	0.5	0.088	3
3.	1	0.079	4
4.	1.5	0.069	5
5.	2	0.063	6
6.	2.5	0.058	7
7.	3	0.062	8
8.	3.5	0.062	8
9.	4	0.062	8
10.	4.5	0.062	8
11.	5	0.062	8
12.	5.5	0.059	9
13.	6	0.057	9
14.	6.5	0.056	10
15.	7	0.055	11
16.	7.5	0.054	12

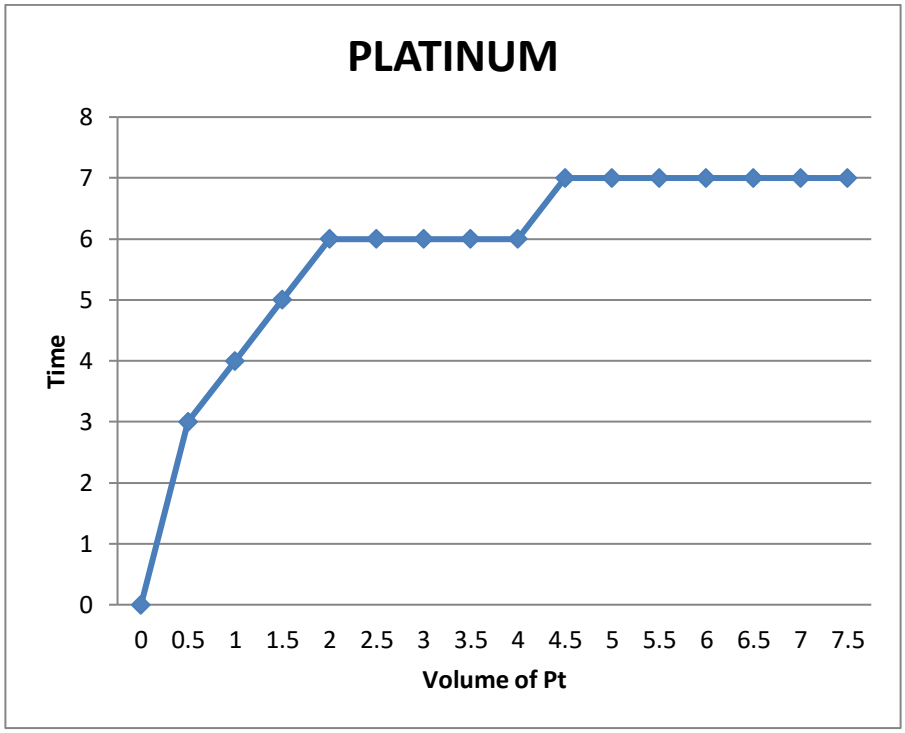

SILVER
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
EMF Value
0 0.5 4 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5
Volume of Ag


SILVER
8
7
6
5
4
3
2
1
0
Time
0 0.5 1 1.5 2 2.5
Volume of Ag

PLATINUM:

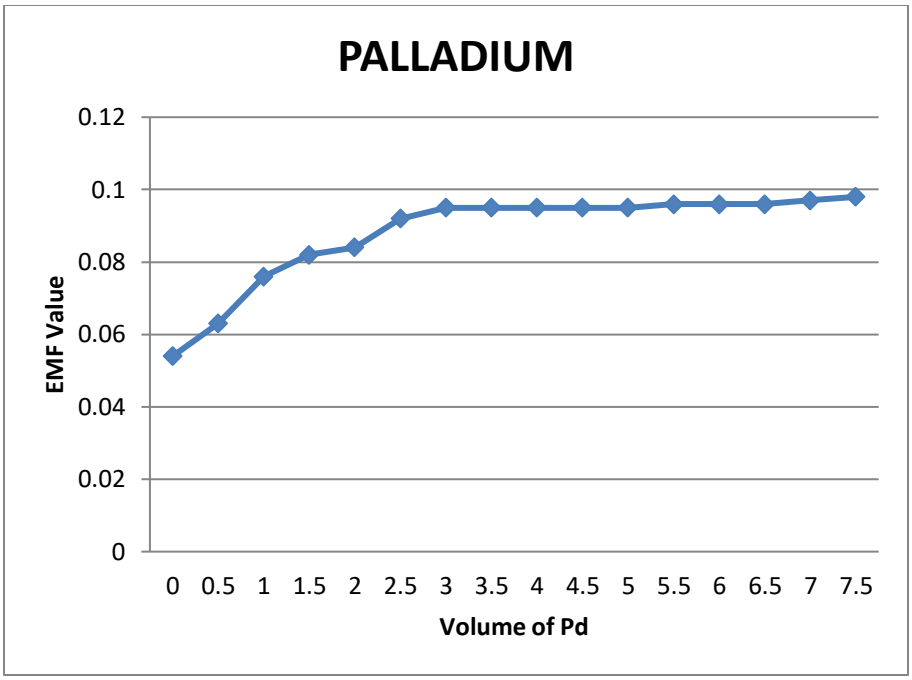
S.No	Volume of Pt	EMF Value	TIME
1.	0	0.052	0
2.	0.5	0.084	3
3.	1	0.095	4
4.	1.5	0.096	5
5.	2	0.097	6
6.	2.5	0.097	6
7.	3	0.097	6
8.	3.5	0.097	6
9.	4	0.097	6
10.	4.5	0.096	7
11.	5	0.096	7
12.	5.5	0.096	7
13.	6	0.096	7
14.	6.5	0.096	7
15.	7	0.096	7

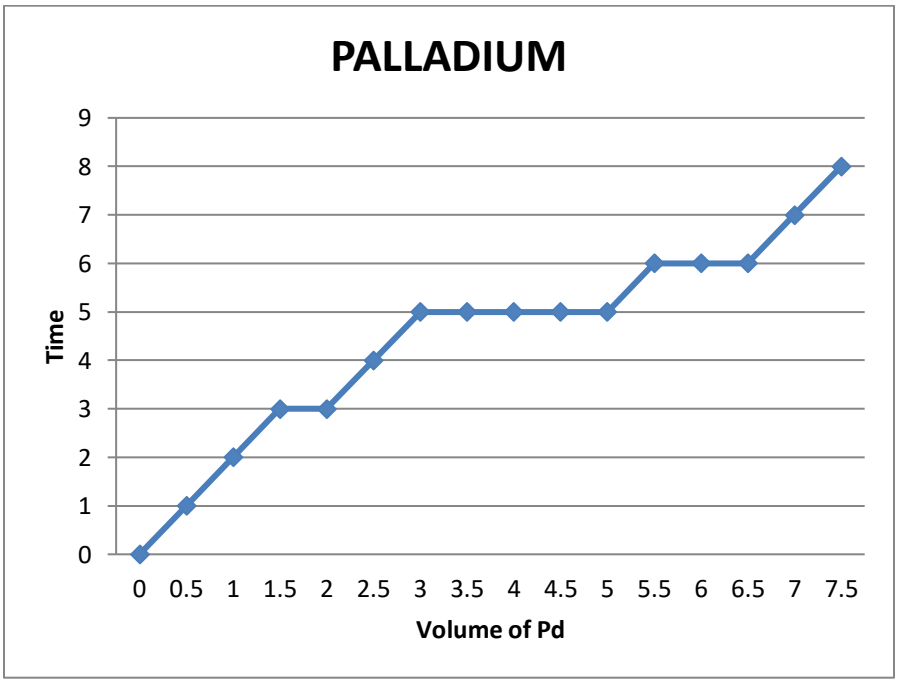

PLATINUM
0.12
0.1
0.08
0.06
0.04
0.02
0
EMF Value
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5
Volume of Pt


PLATINUM
8
7
6
5
4
3
2
1
0
Time
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5
Volume of Pt

PALLADIUM:

S.No	Volume of Pd	EMF Value	TIME
1.	0	0.054	0
2.	0.5	0.063	1
3.	1	0.076	2
4.	1.5	0.082	3
5.	2	0.084	3
6.	2.5	0.092	4
7.	3	0.095	5
8.	3.5	0.095	5
9.	4	0.095	5
10.	4.5	0.095	5
11.	5	0.095	5
12.	5.5	0.096	6
13.	6	0.096	6
14.	6.5	0.096	6
15.	7	0.097	7
16.	7.5	0.098	8


PALLADIUM
0.12
0.1
0.08
0.06
0.04
0.02
0
EMF Value
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5
Volume of Pd

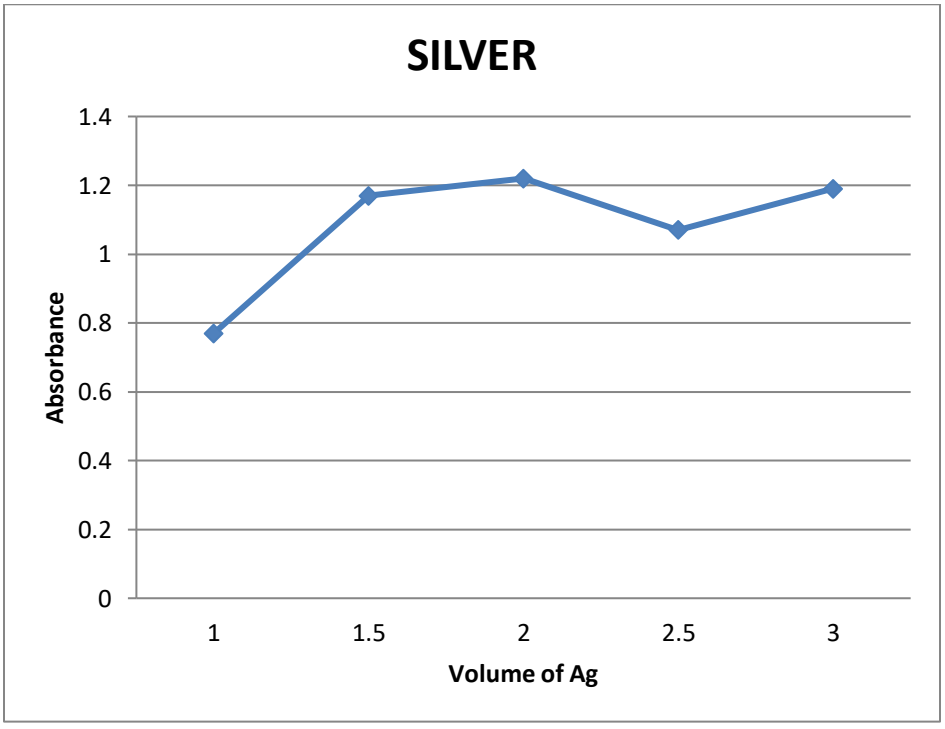

PALLADIUM
9
8
7
6
5
4
3
2
1
0
Time
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5
Volume of Pd

From the above reading the formation of the Silver ,Platinum , Palladium nanoparticles is noted by the constant emf value and the time is recorded for the formation of the nanoparticles and in addition the extract volume of Silver, platinum , Palladium nanoparticles needed for the formation is also noted. From the above reading the formation of the Silver nanoparticles is noted by the constant emf value and the time is recorded for the 3 ml and is formed around 8 minutes with the constant emf value of 0.062. Platinum nanoparticles is noted by the constant emf value and time is recorded for the 1.5ml and is formed around 6 minutes with constant emf value of 0.097. Palladium nanoparticles is noted by the constant emf value and time is recorded for the 1ml and is formed around 3 minutes with constant emf value of 0.095.

CALORIMETRIC METHOD:

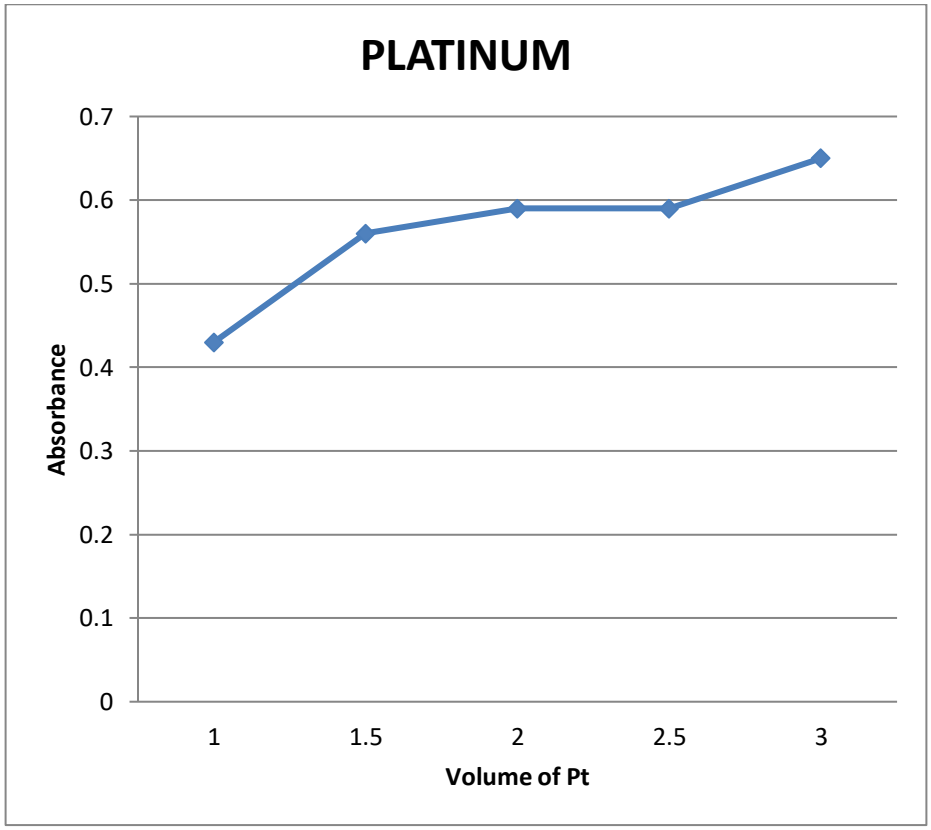
SILVER:

S.No	Volume of Ag	Calorimeter reading [Abs]
1.	1	0.77
2	1.5	1.17
3.	2	1.22
4.	2.5	1.07
5.	3	1.19



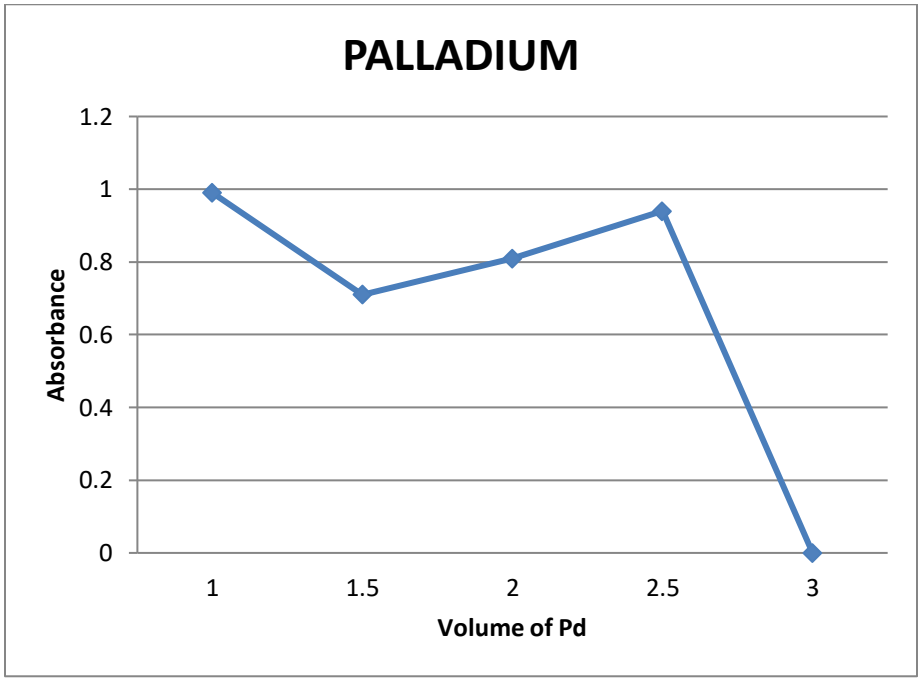
PLATINUM:

S.No	Volume of Pt	Calorimeter reading [Abs]
1.	1	0.43
2.	1.5	0.56
3.	2	0.59
4.	2.5	0.59
5.	3	0.65



PALLADIUM:

S.No	Volume of Pd	Calorimeter reading
1.	1	0.99
2.	1.5	0.71
3.	2	0.81
4.	2.5	0.94
5.	3	0.86



From the above reading it is observed that as the concentration increases the absorbance increases. From the graph it was clear that as the concentration of nanoparticles increases the absorbance value also increases.

Application of Silver, Platinum, Palladium Nanoparticles :

The prepared nanoparticles are used for preserving the vegetables for few days without decay. Usually the vegetables are stored in referigerators for preserving the vegetables. During travel like excursions, we carry fruits and vegetables along with us for usage. To avoid quick decay we can sprinkle the prepared extract over the vegetables and we can carry during travel and it remains fresh for few more days. Thus we conclude that the prepared nanoparticles enhances the shelf life of the fruits and vegetables. We observed this by taking a tomato and kept for five days under examination. The tomato without sprinkling the extract got shrinked and at the same time the tomato which is sprinkled with the extract remain fresh. Hence we conclude that the nanoparticles prepared using *Terminalia Bellirica* can be used to enhance the shelf life of the vegetables without the use of refrigeration which will be useful during travel.

CONCLUSION

Conclusion:

Silver, Platinum, Palladium nanoparticles were synthesized at room temperature by using *Terminalia Bellirica* extract. The effect of various process parameters like the reactant concentration, mixing ratio of the reactants and the concentration silver nitrate, Platinum, Palladium were studied. Silver, Platinum, Palladium nanoparticles are prepared using the extract of *Terminalia Bellirica*. About 10^{-3}M, solution of Silver nitrate, Platinum, Palladium are prepared as a stock solution individually. *Terminalia Bellirica* fruits were collected, cleaned, of are properly dried and it is grinded and the powder is sieved.. The extract is prepared by heated to boiling, centrifuged for 2 minutes at 10,000rpm to get a clear solution and to remove the undesired impurities from it. At first step through visual method, the concentration of the *Terminalia Bellirica* extracts is fixed. The exact concentration has been fixed by the appearance of yellowish brown colour for silver, Light yellow to brown colour for Platinum, Orange to dark brown colour for Palladium. After fixing the concentration, the time taken for the formation of Silver, Platinum, Palladium nanoparticles are determined using potentiometric method, from this the exact time at which the nanoparticle synthesized is determined for all the samples. The prepared nanoparticles are used to preserve the vegetables and fruits and to avoid rapid decay during transportation. Hence, we conclude that Silver, Platinum, Palladium nanoparticles synthesized using *Terminalia Bellirica* extract can be used to enhance the long shelf life period of vegetables like tomato.

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REFERENCES

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“IMPACT OF MIXTURES OF COPPER AND AMMONIA ON CHOSEN BIOCHEMICAL PARAMETERS IN A EICHHORNIA CRASSIPES”

Project in chemistry

Submitted to St. Mary’s college (Autonomous) in partial fulfilment for the award of the Degree of Bachelor of Science in Chemistry

PROJECT DONE BY

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THOOTHUKUDI- 628001

(2021-2022)

DECLARATION

We hereby declare that the project entitled **"IMPACT OF MIXTURES OF COPPER AND AMMONIA ON CHOSEN BIOCHEMICAL PARAMETERS IN A EICHHORNIA CRASSIPES"**submitted to St. Mary'sCollege (Autonomous) , Thoothukudi, affiliated to ManonmaniamSundaranar University, for the Degree of Bachelor of Science in our original work and that , it has not previously formed the basis for the award of any Degree, Diploma or similar title .

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May ,2022

Thoothukudi.

CERTIFICATE

This is to certify that the report of the project in chemistry entitled "**IMPACT OF MIXTURES OF COPPER AND AMMONIA ON CHOSEN BIOCHEMICAL PARAMETERS IN A EICHHORNIA CRASSIPES"** is submitted to St. Mary's College (Autonomous) Thoothukudi in partial fulfilment for the award of the Degree of Bachelor of Science in Chemistry and is a record of the work done by the following student's during the year 2021-2022.

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All praise and glory to the almighty God

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CONTENTS

1. **INTRODUCTION**

Water hyacinth was introduced into the U.S. in 1884 at the Cotton States Exposition in New Orleans as an aquatic ornamental plant. It can now be found in freshwater systems throughout the southeast, as well as in California and Washington state. In Florida, where for 100 years water hyacinth had the upper-hand in plant management, it is under maintenance control and not nearly as abundant as it once was.

Aquatic plant managers work to keep water hyacinth at their lowest possible levels, in exchange for the rivers and lakes remaining usable.Eichhornia crassipes forms dense, floating mats. As a free-floating plant, all its nutrients come from the water column. Leaves are thick, waxy, rounded, andglossy and rise well above the surface on water stalks

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Species Characteristics :

Family: Pontederiaceae

- Habit: perennial free-floating, aquatic plant with long dark roots.
- Leaves: formed in rosettes; petioles to 30 cm (12 in) or more, spongy, usually inflated or bulbous, especially near base; leaf blades roundish or broadly elliptic, glossy green, to 15 cm (6 in) wide.

- Flowers: showy spike above rosette, to 30 cm (12 in) long; lavender-blue with a yellow blotch, to 5 cm (2 in) wide, somewhat 2-lipped; 6 petals, 6 stamens.
- 2 Fruit: a 3-celled capsule
- Seeds: ovoid, ribbed capsule with as many as 50 Seeds.
- Distribution in Florida: statewide Water hyacinth grows in all types of freshwaters environments.
- This plant varies in size from a few inchesto over three feet tall. They have showy lavender flower

and the leaves are rounded and leathery, attached to spongy and sometimes inflated stalks. Water hyacinth has dark feathery roots and may be confused with frog's-bit (Limnobium spongia) which has a somewhat similar appearance.Indiscriminate discharge of raw and partially treated industrial effluents into aquatic system leads to deterioration of the environment.Various metals are present in industrial effluents in significant quantities and they usually occur as components of metal mixtures Some metallic elements playimportant roles inthe biochemical life processes ofplants and animals and their presence in the environment in trace amounts is essential. However, heavy metals are particularly toxic to organisms even in trace levels. Aquatic animals and other organisms in freshwater are severely affected by metal poisoning (Brown *et al.,* 1970). Ammonia iswidespread in the environment due to agricultural and industrial run off's and decomposition of biological waste.Ammonia is a major toxicant to fishes and other aquatic life. Ammonia is also the main

nitrogen waste material in fishes, and is generated as a product of protein catabolism (Randall and the relative proportion of each form is dependent on pH, temperature andsalinity ofwater bodies (Bower and Bidwell, 1978) .Mixtures of pollutants in the environment can influence the toxicity of each other; this influence could be different from the individual toxicological effects alone (Brown, 1980; James *et al.*, 1991; 1992). Copper salts are widely used as algicides in fisheries and fungicides in agriculture. The use of high concentrations of copper salts, however, can create pollution problems. Ammonia is the major nitrogenous end product of aquatic organisms (Colt and Tohobanoglosus, 1978) and in the presence of other chemicals toxicity of ammonia is enhanced . Ammonia combines with copper to form very stable complex cations of cuprammonium $[Cu(NH_3)_4]^{2+}$ (Herbert and VanDyke, 1964) and it enhances the copper toxicity. Toxic effects of individual pollutants on fish have been well documented (Sreedevi *et al.*, 1992; Mayer and Kramer, 1973).

1.1 Chlorophyll :

Leaf chlorophyll concentration is an important parameter that is regularly measured as an indicator of chloroplast content, photosynthetic mechanism and of plant metabolism. Chlorophyll is an antioxidant compounds which are present and stored in the chloroplast of green leaf plants and mainly it is present in the green area of leaves, stems, flowers and

roots . However the chlorophyll production is mainly depended on penetration of sun light and it is the main source of energy for plant . In the laboratory it is commonly determined by using pestle and mortar to extract the pigments using an organic solvent such as acetone. Chlorophyll

a and Chlorophyll b are essential pigments of the plant photosystems. Moreover the chlorophyll ais the primary photosynthetic pigment in plants which helps to produce energy in plant . However the chlorophyll a concentration is 2-3 times higher than that of secondary chlorophyll b in plants . To obtain ratio of chlorophyll a and b, the readings should be taken at the wavelength of 650 nm, which was in between the absorption maxima of both. Green plants have different characters because of the presence of various pigments like chlorophyll, carotenoid, other pigments and water content which together constitute the spectral characters of a plant body However the chlorophyll content has medicinal qualities. The chlorophyll is also plays important role in plant physiology and it can be act as nutrition in decline blood sugar conditions, detoxification, digestion, excretion and decreasing allergens . However in using modern technique like satellite remote sensing technology being used for analysis of leaf chlorophyll concentration can also be measured. Variation in leaf chlorophyll content can provide information about the physiological condition of a leaf or plant. Destructive methods of leaf chlorophyll content quantification include traditional method using extraction and Calorimetric or HPLC measurement, but they are considered time consuming and expensive. Biochemical components (green pigment and nutrient) of forest canopies are among essential parameters that control physiological processes. Present paper records the chlorophyll content of different plant leaves. Difference between chlorophyll content in young and adult leaves of same plant

species were studied. It is essential to do this kind of study to know the photosynthetic activity of physiological changes of young and adult leaves of plants. This work was an experimental study and objective of study was to analyze chlorophyll a and b content in young and adult leaf of selected plants. However the chlorophyll is very important macromolecule

which indicates performance of photosynthesis and energy utilization rate. Also it gives us energy in the form of food or plant material. Chlorophyll bears antioxidant properties which can be used in a medicinal drug discovery. In the article it has explicitly been explained the chlorophyll content in young and adult leaves and its interaction with other macromolecules.

1.2 Carbohydrate :

Carbohydrates are one of the most important components in many foods. Carbohydrates may be present as isolated molecules or they may be physically associated or chemically bound to other molecules. Individual molecules can be classified according to the number of monomers that they contain as monosaccharides, oligosaccharides or polysaccharides. Molecules in which the carbohydrates are covalently attached to proteins are known as glycoproteins, whereas those in which the carbohydrates are covalently attached to lipids are known as glycolipids. Some carbohydrates are digestible by humans and therefore provide an important source of energy, whereas others are indigestible and therefore do not provide energy. Indigestible carbohydrates form part of a group of substances known as dietary fiber, which also includes lignin. Consumption of

significant quantities of dietary fiber has been shown to be beneficial to human nutrition, helping

reduce the risk of certain types of cancer, coronary heart disease, diabetes and constipation. As well as being an important source of energy and dietary fiber, carbohydrates also contribute to the sweetness, appearance and textural characteristics of many foods. It is important to determine the type and concentration of carbohydrates in foods for a number of reasons.

Standards of Identity - foods must have compositions which conform to government regulations.

Nutritional Labeling - to inform consumers of the nutritional content of foods

Detection of Adulteration - each food type has a carbohydrate "fingerprint"

Food Quality - physicochemical properties of foods such as sweetness, appearance, stability and texture depend on the type and concentration of carbohydrates present.

Economic - industry doesn't want to give away expensive ingredients Food Processing - the efficiency of many food processing operations depends on the type and concentration of carbohydrates that are present

1.3 Lipids :

Lipids are one of the major constituents of foods, and are important in our diet for a number of reasons. They are a major source of energy and provide essential lipid nutrients. Nevertheless, over-consumption of certain lipid components can be detrimental to our health, e.g. cholesterol and saturated fats. In many foods the lipid component plays a major role in determining the overall

physical characteristics, such as flavor, texture, mouthfeel and appearance. For this reason, it is difficult to develop low-fat alternatives of many foods, because once the fat is removed some of the most important physical characteristics are lost. Finally, many fats are prone to lipid oxidation, which leads to the formation of off-flavors and potentially harmful products. Some of the most important properties of concern to the food analyst are:

- Total lipid concentration
- Type of lipids present
- Physicochemical properties of lipids, e.g., crystallization, melting point, smoke point, rheology, density and color
- Structural organization of lipids within a food.

1.4Toxicity of Ammonia :

The toxicity of ammonia was evaluated and an estimate is given of (mass) concentration for no adverse effect: 75 μg/m3 for a yearly average, 600μg/m3 for 24 h and 10 000 μg/m3 for 1 h. Ammoniacan cause various types of injury, including necrosis,growth reduction, growth stimulation and increasedfrost sensitivity. Several plant species have beenassessed for sensitivity to ammonia. Some coniferspecies were relatively sensitive to lowconcentrations in the long term; some cultivars ofcauliflower and tomato were relatively sensitive tosomewhat higher concentrations for a short term.

Plants were more sensitive in the dark than indaylight and better adapted to ammonia in high thanin low temperatures. Availability of carbohydratesprobably plays an important role: the plant can

detoxify ammonia as long as it can convert ammoniainto amino acids.Special attention has been paid to plant injuryaround intensively managed livestock. The emissionfrom these sources consists of a large number ofcomponents, ammonia proving to be the main toxiccomponent.

Ammonium toxicity damages plantroots and water-conducting (xylem) tissues. As thexylem collapses and roots are damaged, wateruptake is restricted and wilting and stunting occur.Some plants may die and the marketability ofsurviving plants is reduced. In addition, freeammonium can

reduce seed germination.In most surface waters, total ammoniaconcentrations greater than about 2

milligrams perlitre are toxic to aquatic animals.

An excess of ammonia will kill most of theaquarium plants in your planted tank. You shouldmonitor ammonium levels in your aquarium toprevent not only your plants from dying but alsoyour fish and other living things within theaquarium. it’s important to know that itcanharminplants (and eventually fish). Some plants can helpkeep it in check, and maintaining otherenvironmental factors can also mitigate some risk.

2. ABSTRACT

To study the impact of copper and ammonia on biochemical components and chlorophyll in chosen hydrophyte. To quantity the accumulation of copper in *Eichhornia crassipes* . To identify /determination of cuprammonium complex.

KEY WORD

Pond ,Aathur,Chlorophyll,Carbohydrate,protein,lipid,calorimeter

3.MATERIALS AND METHODS

3.1 Estimation of protein

The protein content of plant / animal sample was estimated calorimetrically following the method of Lowry *et al.* (1951).

Reagents

Biuret Reagent.

Procedure

A Standard solution of sample was prepared by dissolving 0.6g of Bovine Serum Albumin in 100 mL of distilled water. A working standard was prepared by diluting 10mL in a standard flask using distilled water. This working standard contains 6 mg of protein / mL. Pipette out into a series of tubes 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mL of the sample solution and make up the total volume to 5.0 mL with addition of distilled water. A blank tube(B) will contain only 5.0mL of water. Add 6.0mL of biuret reagent to each tube and mix well. Keep the test tube at room temperature for 10 minutes. Measure the optical density of each tube at 620 nm (green filter) using the reagent blank. Draw a standard graph using concentration along x-axis and optical density along y-axis.

The sample was made up to 5mL with water repeated the above same procedure with these test solutions also. Cut the standard graph using the optical density obtained for the test solutions. This gives the concentration of protein in the test solutions. From the calculate amount of protein present in the sample of serum given.

3.2 Estimation of carbohydrate

Total carbohydrate contents of the plant / animal samples were estimated by the Anthrone method.

Principle

Carbohydrates are dehydrated by sulphuric acid to form furfural. Furfural condenses with anthrone to form a blue coloured complex, which is measured Colorimetrically.

Reagents

Anthrone reagent

Procedure

100 mg of plant was homogenized. The homogenate was centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and measured and it was used for the analysis of carbohydrate. One ml of the supernatant was taken in a test tube. To this 5 ml of anthrone reagent was added and mixed well. The test tube containing the mixture was kept in boiling water bath for 10 minutes. The test tubes were then cooled at room temperature and optical density was measured in a calorimeter at 620 nm. With the use of the standard graph, the amount of carbohydrate present in the sample was converted for one gram.

3.3 Estimation of lipid

Total lipid content in plant / animal sample was analysed by Bradgon method (1951).

Reagents

1. Chloroform
2. 2% Potassium dichromate
3. Concentrated sulphuric acid

Procedure

A known weight of about 15 mg of the plant / animal sample was grounded well with a few ml of choloroform and the solution was centrifuged. The supernatant was evaporated to dryness. Then 3 ml of 2% potassium dichromate in concentrated sulphuric acid was added which was followed by 3 ml of distilled water. The developed colour was read in calorimeter using filter 620 nm.

3.4 Estimation of chlorophyll content

The chlorophyll content of *Eichhornia crassipes*was estimated following the method of Arnon (1949).

Procedure

1) Select only most green parts of leaf for chlorophyll estimation.
2) Take 1 (or) 2 gm of fresh plant weight and divide it into two equally weighted parts. Keep one part in the oven at 105^0C for the determination of dry weight and use another part for chlorophyll estimation.
3) Add little quantity of 90% of acetone to the plant material in a morter and pestle and add few ml of magnesium carbonate. Grind thoroughly. Keep the sample at 4^0C for 4 – 6 hours for pigments to elute.
4) Centrifuge at 2500 rpm for 15 minutes. Decant the extract to a volumetric flask and make up the volume of 25 to 50 ml using 90% acetone. If the solution is very dark green, make up to the volume to 100 ml.
5) Take absorbance at 620 nm.

4. RESULTS AND DISCUSSION

4.1.Ammonium Chloride in*EICHHORNIA CRASSIPES* :

The data presented in Table 1 showed the amount of protein , carbohydrate and lipid in water hyacinth , E. Crassipes exposed to various levels of Ammonium Chloride. Protein content of the plant E. Crassipes was 22 mg /g wet weight on day 0 and declined to 18 , 15 and 14 mg/g wet weight in plant exposed to 2,4 and 6 ppm of Ammonium chloride respectively. On day 6, similar result was obtained in carbohydrate and lipid contents also (Table 1; Fig.1)

4.2.Copper Sulphate in *EICHHORNIA CRASSIPES*:

On exposure of E. Crassipes to various levels of Copper Sulphate , the organic components of plant were altered. The protein content of plant were altered. The protein content of plant was high on day 0 and it highly decreased in plant exposed to different levels of Copper Sulphate (Table 2;2 Fig;2). Similar results were also obtained in Carbohydrate and Lipid contents like protein. However, the amount of tested organic contents were highly reduced in plant exposed to Copper Sulphate as compared to plant exposed to Ammonium Chloride (Table 1 and 2). For instance, the Carbohydrate content of plant exposed to 6 ppm of Ammonium Chloride was 8.26 mg/g wet weight and it decreased to 7.13mg/g wet weight in the same plant exposed to 3ppm of Copper Sulphate. Similar results were obtained in protein and Lipid contents also.

4. 3.Mixtures of Ammonium Chloride and Copper Sulphate:

Like Ammonium Chloride and Copper Sulphate individual exposures, the protein and other organic components were similar in plants exposed to mixture Ammonium Chloride and Copper Sulphate. However, the amount of tested organic components were highly reduced in

Copper Sulphate exposures as compared to Ammonium Chloride among the exposure, the decline of organic contents was high in chosen plant exposed to Copper Sulphate (3ppm) with different levels (1-3ppm) of Ammonium Chloride as compared to plant exposed to Ammonium Chloride with different levels of Copper Sulphate (Table 3 and 4; Fig 3 and 4). The decline of organic components was severely decreased in 3 ppm Copper Sulphate + 6ppm Ammonium Chloride followed by 6ppm Ammonium Chloride + 3ppm Copper Sulphate as compared to other exposures (see table 3 and 4).

4.4.Ammonium Chloride/ Copper Sulphate on Chlorophyll in E.Crassipes:

The Chlorophyll content of plant E.Crassipes was high in control plant. However, the amount of Chlorophyll content declined with increasing the concentrations of Ammonium chloride / Copper Sulphate and extension of exposure period from day 0-6(Tables 5-8; Fig 5-8).For instance, the chlorophyll content of 6ppm Ammonium Chloride exposed plant was 0.37,0.26,0.20 and 0.13 mg/g wet weight on days 0,2,4 and 6 respectively (Table 5).There was 3 times reduction of Chlorophyll content in 6 ppm Ammonium Chloride exposed plant on day 6 as compared to day 0 (Table 5). However, there was 4 times reduction of Chlorophyll content in plant exposed to 3ppm or high concentration of Copper (Table 6). It showed that Copper Sulphate affected the Chlorphyll content in E.Crassipes as compared to Ammonium Chloride (see tables 5 and 6).

4.5.Mixtures of Ammonium Chloride and Copper Sulphate in E.Crassipes:

The Chlorophyll content of E.Crassipes was drastically affected in plant exposed to Copper Sulphate with different levels of Ammonium Chloride (Table 8) as compared to Ammonium Chloride with different levels of Copper Sulphate (Table 7 and 8; Fig 7 and 8). For example, the Chlorophyll content of 3ppm Copper Sulphate + 4 and 6 ppm Ammonium Chloride was gradually decreased with extension of exposure period (0-6th days) and Nil on day 6.

The decrease in tested organic components (Protein,Carbohydrate and Lipid) and Chlorophyll in plant E.Crassipes showed the toxic effects of pollutants (Ammonium Chloride and Copper Sulphate). The reduction of organic components and Chlorophyll in the leaves might be due to the absorption of pollutants / toxicants through root of the plant to leaves which in turn reduced the tested components.

In the present investigation, the *EICHHORNIA CRASSIPES*exposed to various levels of Ammonium Chloride and Copper Sulphate showed that, decrease in total Chlorophyll content and colour change with Similar results were also made by Mhatre and Chaphekar (1984). This probably appears to be a result of inhibition of biosynthesis of Chlorophyll and Lipids, especially galactolipids as discovered in the fresh water algae (*Ankistro-desmus bravhii and Euglena gractilis)* due to Mecury Chloride and Methyl Mecury Chloride (Matson et al.,1982).

The present study revealed that, the individual effect of Copper was more toxic than Ammonia and two of the combination were more toxic than the individual toxic effect of Copper and ammonia. Biochemical parameters of plant showed that when Copper combines with Ammonia, the toxic effect of copper was enhanced several fold (see tables 3,4,7 and 8). It indicates synergistic (or additive) effects of Copper and Ammonia. Copper has High affinity for Ammonia , resulting in the formation of Cuprammonium ions and perhaps , these could have elevated the copper toxicity. Herbert and VanDyke (1964) studied the synergistic effects of Copper and Ammonia and observed the formation of Cuprammonium ions predominate during their interactions.

5.TABULATION AND GRAPHICAL REPRESENTATION

Table:5.1 Effect of ammonium chloride levels (ppm) on Protein, carbohydrate and lipid in a plant Eichhornia crassipes.

Components	Days	Concentration of NH4CL(ppm)		
		2	4	6
Protein	0	22.17	22.17	22.17
	6	17.68	15.08	14.11
Carbohydrate	0	14.16	14.16	14.16
	6	12.01	10.08	8.26
Lipid	0	6.86	6.86	6.86
	6	5.40	4.85	4.10

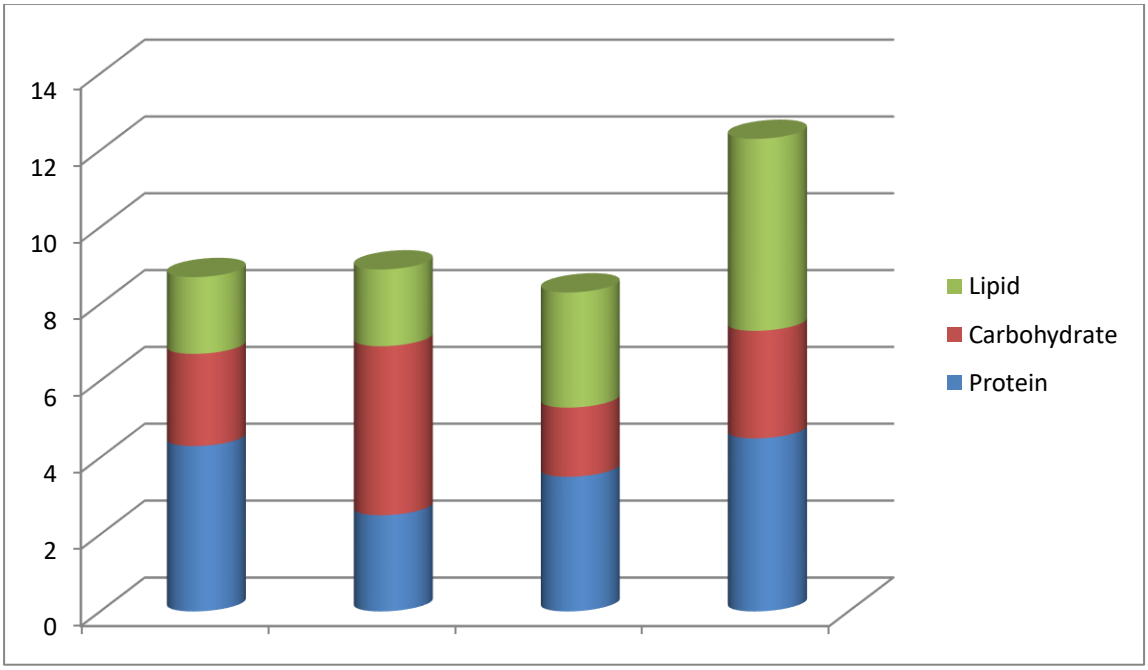


Table : 5.2 Effect of Copper Sulphate levels (ppm) on protein, carbohydrate and lipid in a plant Eichhornia Crassipes.

Components	Days	Concentration of CuSO4(ppm)		
		1	2	3
Protein	0	22.17	22.17	22.17
	6	16.20	14.00	12.96
Carbohydrate	0	14.16	14.16	14.16
	6	13.29	8.55	7.13
Lipid	0	6.86	6.86	6.86
	6	5.10	4.45	4.12

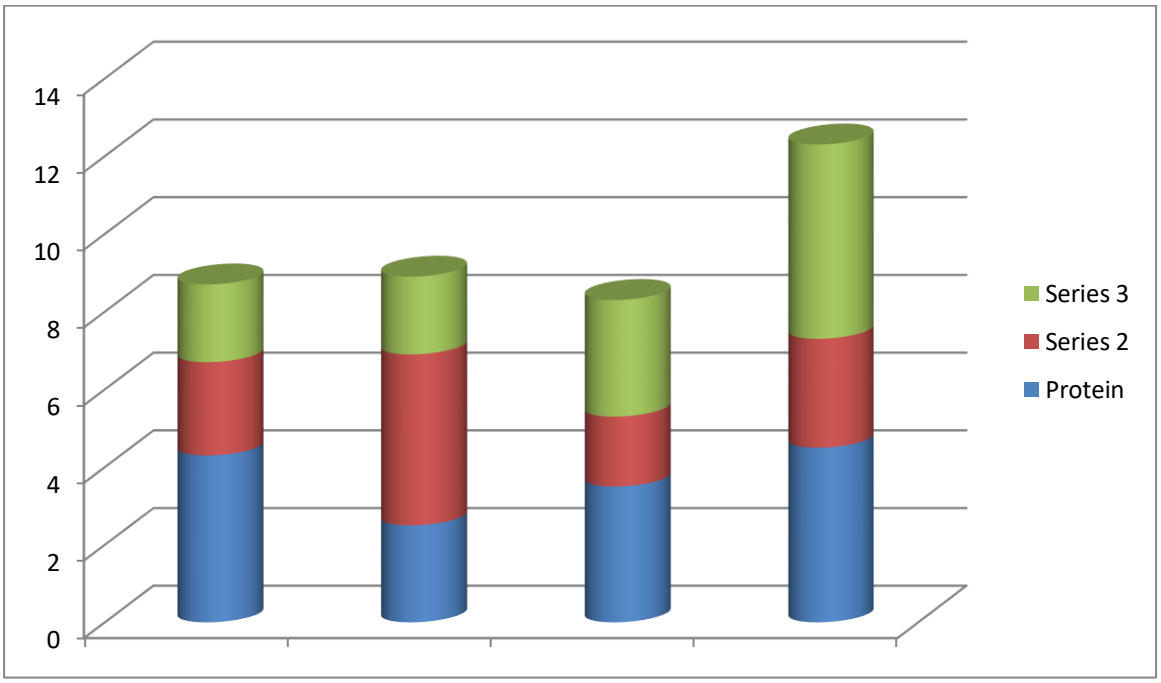


Table 5.3: Impact of ammonium chloride with different levels of copper sulphate on protein, carbohydrate and lipid in a plant Eichhornia Crassipes.

Components	Days	Concentrations (ppm)		
		6ppmNH4Cl+ 1ppm CuSO4	6ppm NH4Cl+ 2ppm CuSO4	6ppm NH4Cl + 3ppm CuSO4
Protein	0	22.17	22.17	22.17
	6	14.62	17.03	8.23
Carbohydrate	0	14.16	14.16	14.16
	6	11.40	7.05	4.25
Lipid	0	6.86	6.86	6.86
	6	4.95	4.28	3.60

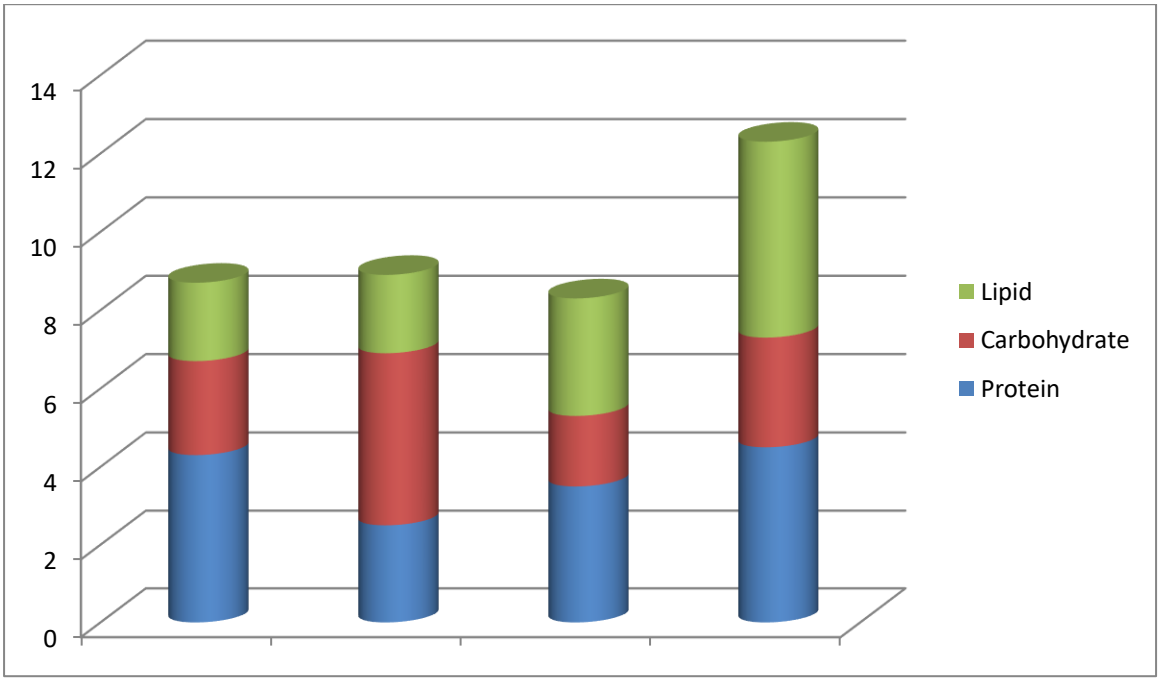


Table:5.4 Impact of copper sulphate with various levels(ppm) of Ammonium Chloride on protein, carbohydrate and lipid in a plant Eichhornia Crassipes.

Components	Days	Concentration(ppm)		
		3ppm CuSO4+2pP mNH4CL	3ppm CuSo4+4ppm NH4Cl	3ppm Cuso4+6ppm NH4Cl
Protein	0	22.17	22.17	22.17
	6	13.10	8.33	7.08
Carbohydrate	0	14.16	14.16	14.16
	6	7.20	5.10	3.00
Lipid	0	6.86	6.86	6.86
	6	4.60	4.00	3.13

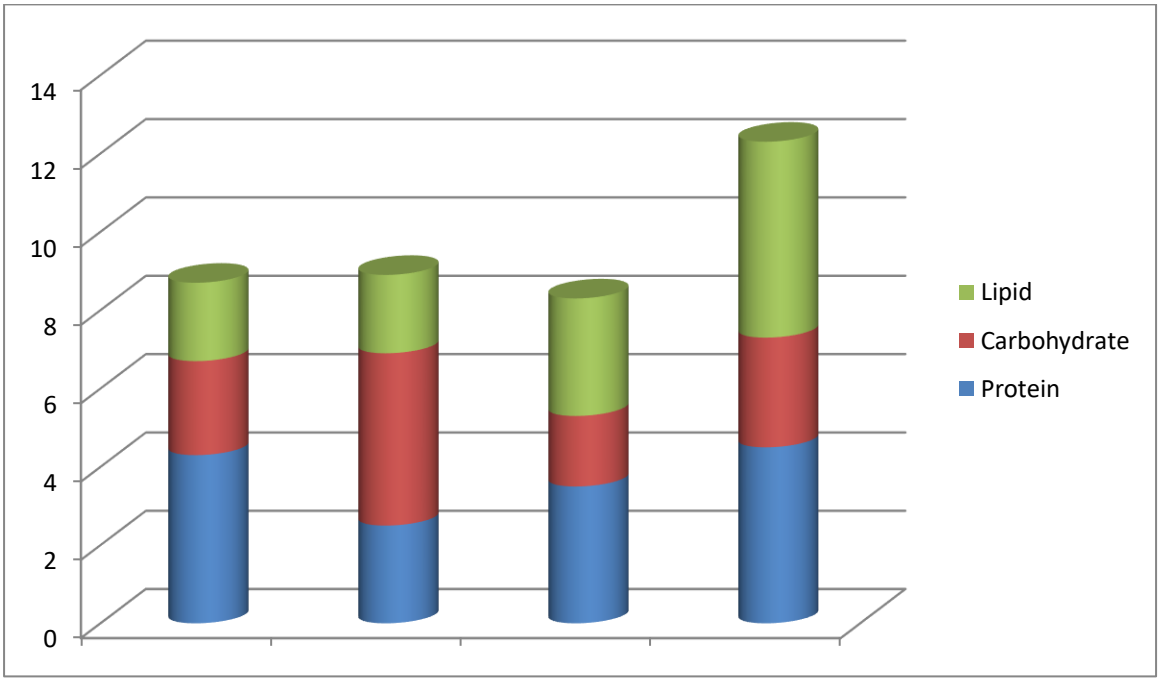


Table:5.5 Effect of ammonium chloride levels (ppm) on chlorophyll content (mg g^{-1}wet weight) in a plant Eichhorhia Crassipes.

Days	Concentrations (ppm)		
	2	4	6
0	0.37	0.37	0.37
2	0.30	0.28	0.26
4	0.22	0.21	0.20
6	0.16	0.15	0.13

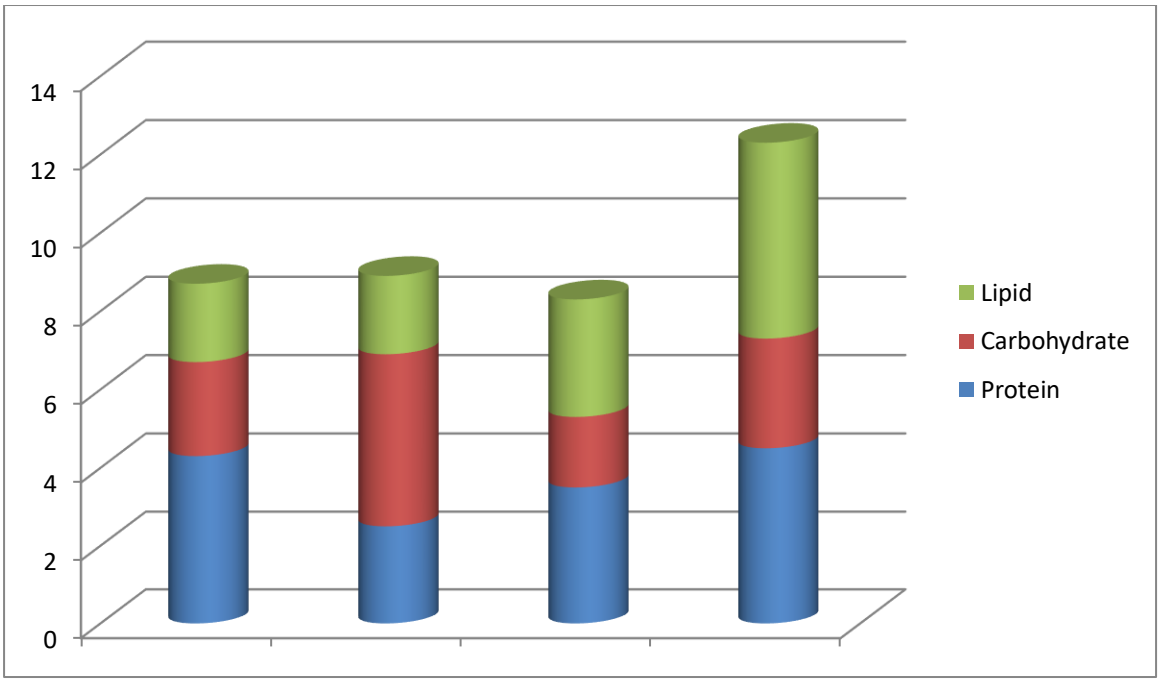


Table: 5.6 Effect of copper sulphate levels (ppm) on chlorophyll content (mg g^{-1} wet weight) in a plant Eichornia Crassipes.

Days	Concentrations (ppm)		
	1	2	3
0	0.37	0.37	0.37
2	0.28	0.28	0.24
4	0.20	0.18	0.16
6	0.14	0.12	0.09

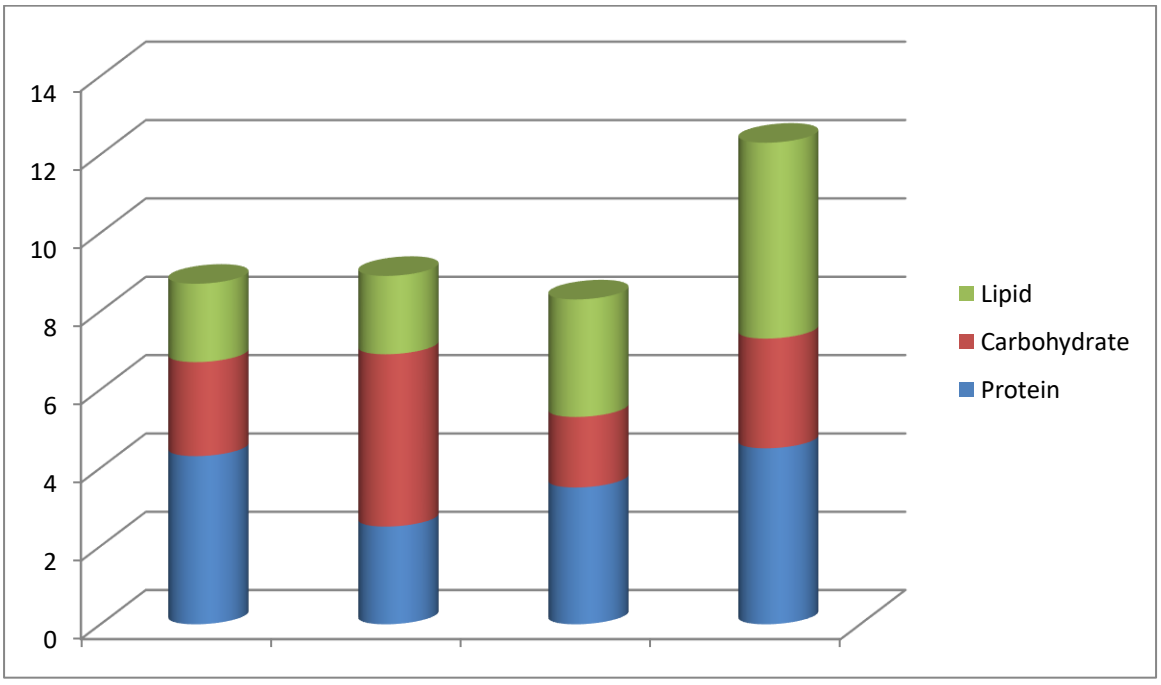


Table: 5.7 Effect of ammonium chloride with different levels (ppm) of copper sulphate on chlorophyll content (mg g^{-1} wet weight) in a plant Eichhornia Crassipes.

Days	Concentrations (ppm)		
	6ppm NH4Cl+ 1ppmCuSO4	6ppm NH4Cl+ 2 ppmCuSO4	6ppm NH4Cl+ 3 ppmCuSO4
0	0.37	0.37	0.37
2	0.25	0.25	0.23
4	0.16	0.14	0.11
6	0.09	0.07	0.05

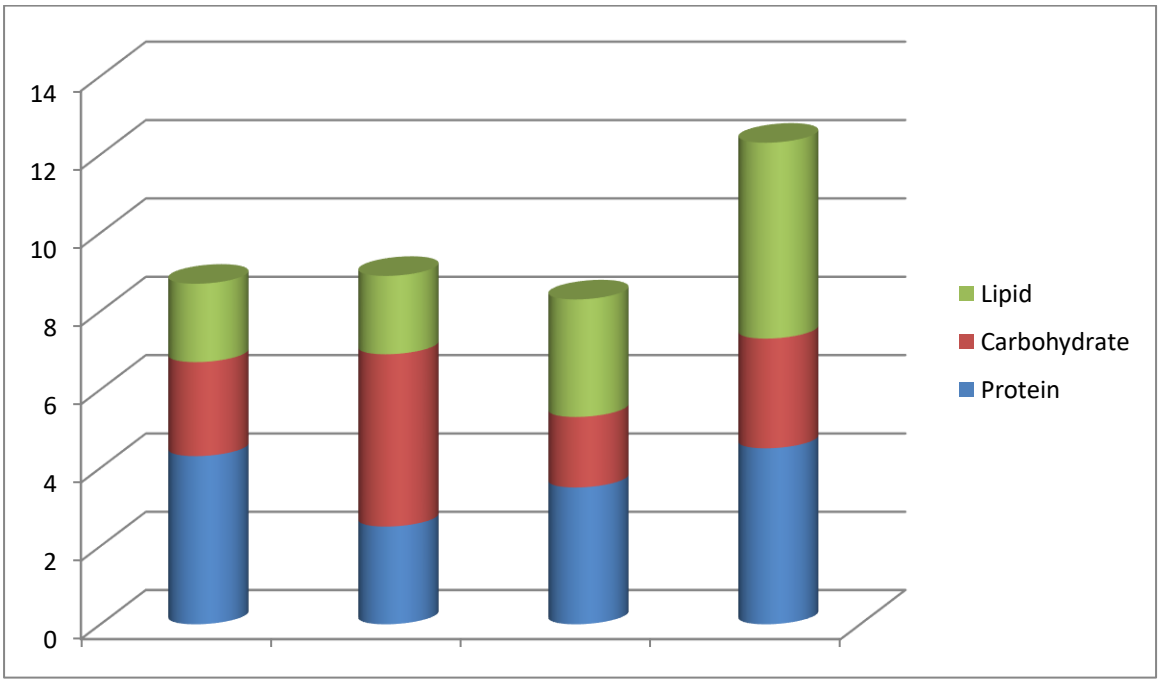
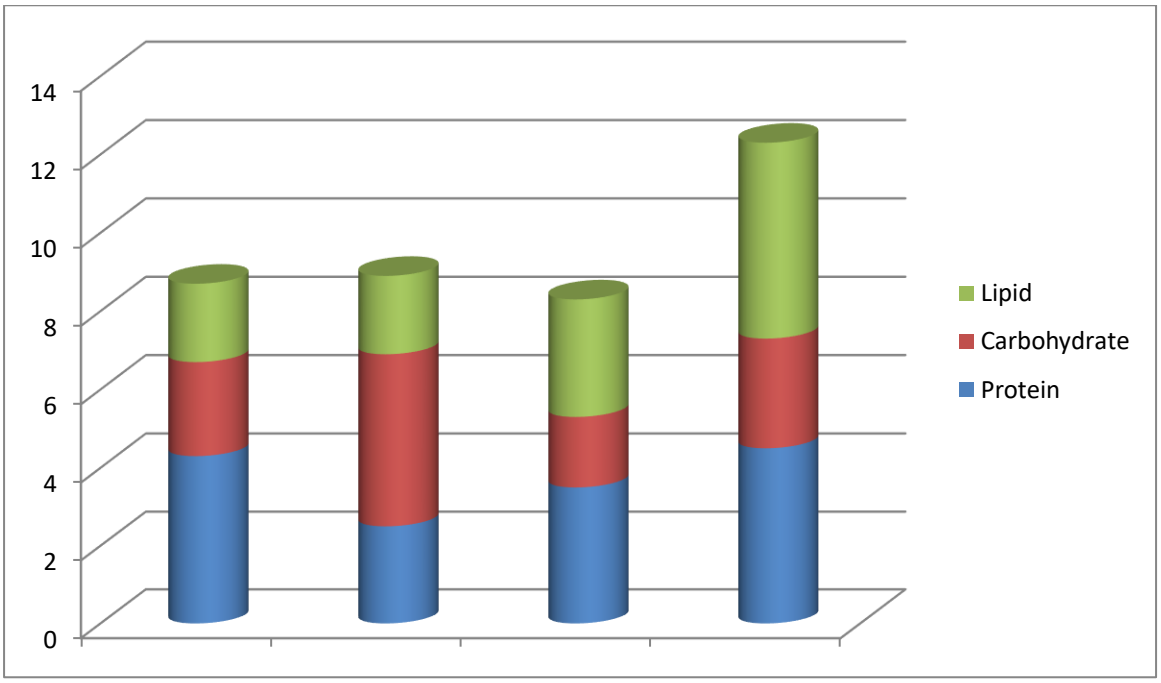


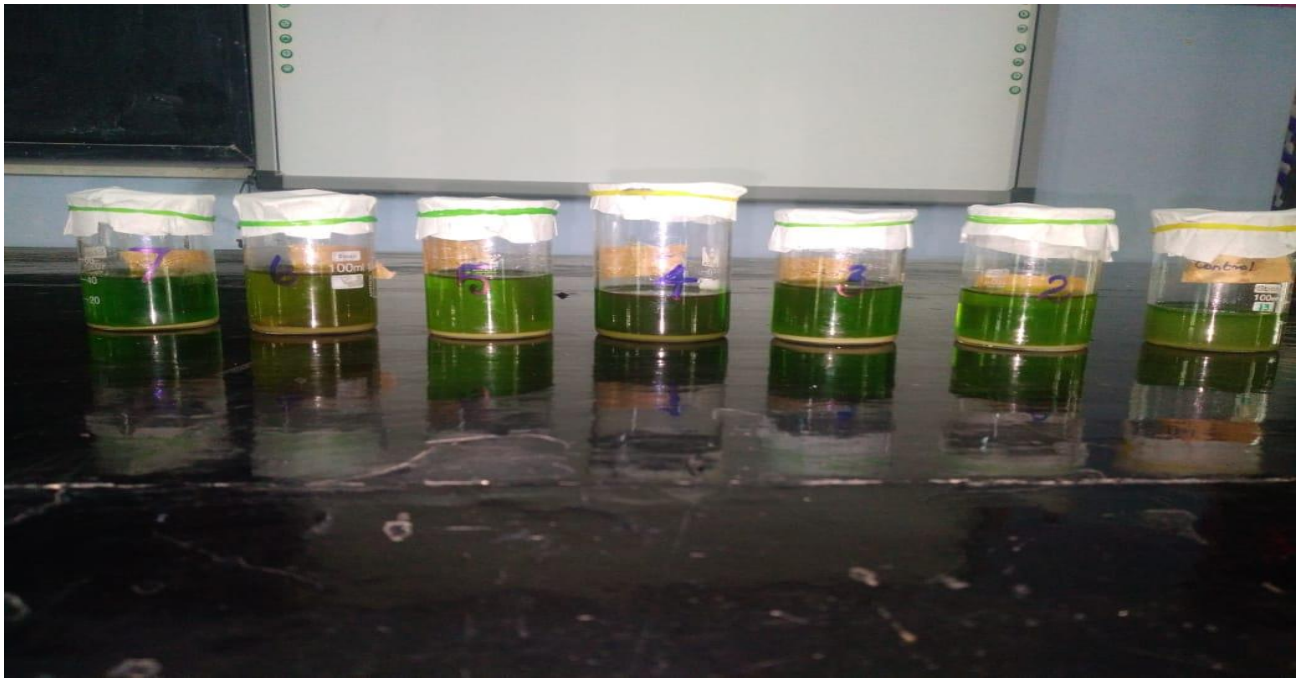
Table:5.8 Impact of copper sulphate with various levels (ppm) of ammonium chloride on chlorophyll content (mg g^{-1} wet weight) in a plant Eichhornia Crassipes.

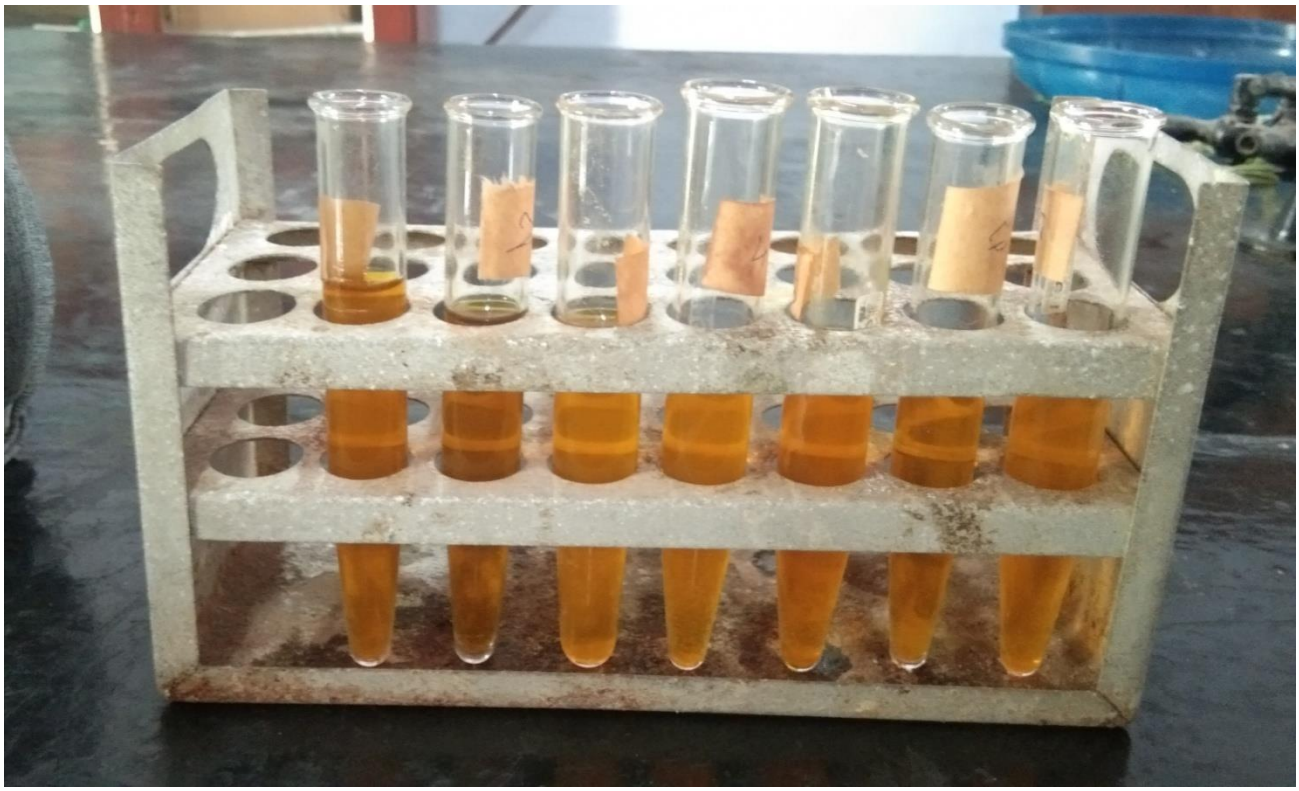
Days	Concentrations (ppm)		
	3ppm CuSO4+ 2ppm NH4Cl	3ppm CuSO4+ 4 ppm NH4Cl	3ppm CuSO4+ 6 ppm NH4Cl
0	0.37	0.37	0.37
2	0.25	0.20	0.18
4	0.15	0.07	0.05
6	0.07	Nil	Nil



FIGURES


3PPM CuSO4+6PPM NH4Cl
3PPM CuSO4+4PPM NH4Cl
6PPM NH4Cl+1PPM CuSO4
3PPM CuSO4 +2PPM NH4Cl
6PPM NH4Cl+2PPM CuSO4
2PPM NH4Cl+3PPM CuSO4


7
6
5
4
3
2
Control



25

7. CONCLUSION

By visual observation , the leaf green colour was gradually changed into brown colour in the test media during the experimental period it may be due to Copper sulphate and Ammonium chloride toxicity individually and in mixtures . It was more in plants exposed mixtures followed by Ammonium chloride and copper sulphate exposures. Eichhornia crassipes is used to clean up waste water in small scale sewage treatment plants. It has been shown to remove Nitrogen and Phosphorous,as well as Biological Oxygen Demands (BOD). It removes trace toxic metals as well. Its ability to remove to toxic heavy metals is the applied value of the weed, Water Hyacinth for treating the waste water. Water Hyacinth roots absorbs pollutants including toxic metals such as Mercury, Cadmium, Copper , Lead and Strontium 90 and other carcinogenic compounds. Further, Water Hyacinth has been found to remove bad odour producing compounds, bacteria, algae and etc.. from the polluted water bodies.

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