Green Synthesis of Bimetallic Nanoparticles Using Aqueous Extract of *Deparia boryana,* a Himalyan Pteridophyte to Develop Novel Antimicrobial Agent.

A dissertation

Submitted in partial fulfillment of the requirement for the degree of

Masters of Science

in

Biochemistry

By

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June 2018

Certificate

This is to certify that the thesis entitled "**Green Synthesis of Bimetallic Nanoparticles Using Aqueous Extract of *Deparia boryana*, a Himalyan Pteridophyte to Develop Novel Antimicrobial Agent.**" submitted by Aastha Arora is an authentic record of work carried out as requirement for the award of the degree of **Masters of Biochemistry** at **Thapar Institute of Engineering and Technology**, Patiala, under the supervision of **Dr. Diptiman Choudhury**, Assistant Professor, School of Chemistry and Biochemistry, TIET, Patiala during the period of 6 months from **January 2018 to June 2018**. No part of the matter embodied in this report has been submitted to any other university or institute for the award of any degree.

It is certified that the above statement made by the student is correct to the best of my knowledge and belief.

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Candidate's Declaration

I hereby declare that the work being presented in the dissertation entitled "**Green Synthesis of Bimetallic Nanoparticles Using Aqueous Extract of *Deparia boryana*, a Himalyan Pteridophyte to Develop Novel Antimicrobial Agent.**" in the partial fulfillment of the requirements for the degree of Masters in Biochemistry and being submitted to School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala is my own work during the period of six months from January 2018 to June 2018, under the supervision of Dr. Diptiman Choudhury, Assistant Professor, School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala. No part of the matter embodied in this thesis has been submitted to any other University or Institute for the award of any degree.

Aastha Arora

Date: 14-08-2018

This is to certify the above statement made by the candidate is correct and true to the best of our knowledge.

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Abstract:

Increasing trend in the popularity of green synthesis owing to its cost effective and eco-friendly attributes, the plant *Deparia boryana*, a Himalyan pteridophyte is evaluated for its phytochemical makeup and its antibacterial and antihelmintic activities of its various extracts and biosynthesized nanoparticles. The chloroform, petroleum ether and silver nanoparticles were proved to be effective antihelmintic and antibacterial agents (against *E.coli* and *Bacillus subtilis*) while other nanoparticles like TiO_2 nanoparticles, TiO_2 coated Ag nanoparticles and Ag coated TiO_2 nanoparticles were proved to be antibacterial agents only. The MIC was found to be 1mg/ml. All the nanoparticles were synthesized by green synthesis method that is under sunlight without any chemically added reducing or capping agent. The synthesized nanoparticles were characterized by UV-Visible spectroscopy, FTIR, SEM-EDS and DLS. The UV-Visible spectra for AgNP, TiO_2 nanoparticles, TiO_2 coated AgNP, Ag coated TiO_2 nanoparticles were observed around 400- 412 nm, 280-320 nm, 405-427 and 284-328 nm, respectively. The DLS studies show that the average size of AgNP and TiO_2 coated AgNP were found to be around 30-90 nm i.e. within nano range, whereas the average size of the TiO_2 nanoparticles and Ag coated TiO_2 nanoparticles were found to be 90-140 nm, i.e. within micro range.

List of Figures

List of schemes

List of tables

List of abbreviations and symbols

- **ºC** Degree Celsius
- **%** Percent
- **gm** Gram
- **mg** Milligram
- **µg** Microgram
- **M** Molar
- **L** Litre
- **ml** Milliliter
- **min** Minute
- **nm** Nanometer
- **SEM** Scanning Electron Microscope
- **FTIR** Fourier Transform Infrared Spectroscopy
- **UV** Ultra Violet
- **EDS** Energy Dispersive Spectroscopy
- **DLS** Dynamic Light Scattering
- **CFU** Colony Forming Units
- **AgNP** Silver Nano Particles
- **Ag** Silver

List of Contents

1. Introduction:

The use of nanoscale materials and structures, as a rule reaching out from one to one hundred nanometers (nm), is an advancing region of nanoscience & nanotechnology. Nanomaterials can offer courses of action to creative and biological provocation in the region of sun-based energy change, catalysis, pharmaceutical, and waste water processing. This extending call must be joined by "green" blend strategies. In the overall undertakings to decrease precarious waste, "green" chemistry and manufactured strategies are progressively organizing with present day advancements in science and industry. [1]

Nanobiotechnology is a standout amongst the most encouraging areas in present day nanoscience & development. This advancing zone of investigation joins diverse orders of science, for instance, physics, chemistry, biological science and material science. [2]

Utilization of plants for composition of nanoparticles could be worthwhile over other nature friendly natural methods as this wipes out the expounded methodology of keeping up cell societies. If nanoparticles were conveyed extracelularly using plants or their concentrates and controled way as demonstrated by their size, scattering and structure, Biosynthetic systems for nanoparticles could be more vital [3]

The use of plants for the composition of metal nanoparticles got the consideration of scientists for having a speedy, affordable, eco-accommodating and one-step proficiency for the biosynthesis strategy. [4] Ag is unquestionably the most utilized in nanotechnology; the alluring antimicrobial activity of Ag is certainly extended at the Nanoscale. Appropriately, AgNPs have been consolidated into many particular and proficient things extending from careful & sustenance managing the gadget, water purifiers, and textiles, beautifying objects, lesion dressing, child's toy & most parts constructed stages expected for tissue recuperation. A standout amongst the most beneficial work of Ag has been very unusual anti-bacterial specialist which is precarious to parasites, diseases, & green algae. A nanoparticle is portrayed as little objects that act overall unit as far as its transport and properties, in nanotechnology. Nanotechnology misuses the truth that when a solid material ends up being

little the surface zone expands, which prompts an extension in the surface reactivity what's more, quantum-related effects. [5][6]

For a long time, herbal medicines have been utilized and are still utilized as a part of developing nations as the essential origin of restorative treatment. Human relies upon drugs secondary to food. In poor nations because of constrained healthcare services, staggering expense and in availability, to the allopathic pharmaceuticals fused with poverty a large portion of the universal population rely upon standard enlistment of therapeutic plants. Plants have been utilized as a part of cure for their common germ-free properties. Consequently, investigation has advanced into exploring the probable properties and employments of telluric plants extracts as novel antimicrobial agents. Also, due to the global warming, the microbes that were earlier frozen in icebergs are exposing to the climate with high rate due to increase in global temperature and are posing greater threat to mankind. Thus, it is the dire need to develop new novel antimicrobial agents preferably from herbal plants because of their cost potency and easy approachability.

Titanium dioxide (TiO2) is a photo catalyst and its usage is done as a self-cleaning and self-sterilizing substance for exterior covering in varied functions and utilizations. It has an all the more pleasing part in our typical sanitization because of its non-toxicity, photo prompted super-hydrophobicity and antifogging control. They are used from time to time in beautifiers, medication, enamel, & paper industry. In any case, the little particle size of nanoparticles may have distinctive chemical, magnetic optical and auxiliary highlights, so they might have more differential lethal quality profiles than typically evaluated TiO2. [7]

The current investigation includes the biosynthesis of AgNP; TiO2 covered AgNP, TiO2 nanoparticles, Ag-covered TiO2 nanoparticles. The incorporated nanoparticles were described through different procedures, which incorporate UV-Visible spectroscopy, FT-IR (Fourier Transform Infrared Spectroscopy), SEM (Scanning Electron Microscopy), DLS (Dynamic Light Scattering), and EDS (Energy Dispersive Spectroscopy). The antibacterial exercises of the biosynthesized nanoparticles were checked against *E.coli* and *Bacillus Subtilis* by estimating the optical thickness (0D) at 600nm. 0ptical density decrease was seen with accession in the consolidation of nanoparticles. Additionally, the antihelmintic

movement of the nanoparticles and different concentrates were checked which was observed to be adequate. [8]

The lethal effects of silver on bacteria have been explored for over 60 years. The antibacterial movement of silver nanoparticles may have happened because of the release of the Ag+ particles from the silver nanoparticles and they wedge itself to the DNA thereby uncoiling it and deteriorating the genomic structure. Likewise, because of inactivation of the inhaling chain which might lead to the formation of hydroxyl radicals and consequently killing of the microorganisms. The silver nanoparticles also discharge reactive oxygen species, stop the bacterial growth by S phase or growth phase arrest and by chromosomal DNA degradation. [9]

They are known to have oligodynamic properties and can be lethal to antibiotic-resistant microbes while applying limited cytotoxicity against animal body cells. They are known to break microbial cell walls, dividers, denature cell proteins, clock respiration, and inevitably prompt cell death. The method of synthesis plays an essential part in choosing the plasmonic properties of silver nanoparticles that further emphasize their antimicrobial activity. [10]

Smaller nanoparticles apply greater toxicity. The surface properties of silver nanoparticles give a fairly crucial impact on their intensity. [11]

2. Literature review

2.1 About the plant

Deparia boryana, a Himalyan pteridophyte is a plant which is located in Himalyan range. It is the species of ferns belonging to *Athyriaceae* family. It is native from tropical and temperate Asia to NE Himalayas and Malaysia. It is mainly cultivated to the areas to the altitude of 1350–2700 m. *Deparia boryana* has circular sori 4-13 per pinnule-lobe, which are approximately 0.6 mm in diameter. [12] A fern is any of a group of around 20,000 types of plants arranged in the phylum or division Pteridophyta. The term pteridophytes have customarily been utilized to depict all seedless vascular plants so is synonymous with "ferns and fern allies" A fern is a vascular plant that contrasts from the more primitive lycophytes in having genuine leaves (megaphylls), and from the further developed seed plants (gymnosperms and angiosperms) in lacking seeds. Most plants replicate sexually, and that includes meiosis and fertilization. When you are thinking about the common big plant, what it does is, by meiosis, produces spores, and spores have the number chromosomes of the huge parent plant. [13]

2.2 Phytochemistry

Phytochemistry is the branch of chemistry executing the chemical mechanism related with plant life and chemical compound created by plants. Different therapeutic properties have been ascribed to regular herbs. Therapeutic plants consist of the main origin of new therapeutical and healthcare items. The classical scenery of plants being utilized for the restorative reason is likely as decrepit as the ancient backdrop of humankind. The usage of restorative plants in the technical civil regulation has been followed to the extrication & enhancement of a few medicaments from such plants and also from traditionally utilized folk pharmaceutical. Extrication and characterization of a few dynamic phyto compounds from such green factories have brought some

very high activity profile drugs in light. The utilization of conventional medicine is widespread in India. A developing assortment of proof demonstrates that secondary plant metabolites assume a demanding role in human wellbeing & might be nutritiously essential. It is trusted that crude extract from restorative plants is more naturally active than segregated compounds because of their synergistic impacts. Phytochemical screening of plants has uncovered the presence of varied synthetic chemicals involving alkaloids, tannins, flavonoids, steroids, glycosides, and saponins etc. Secondary metabolites of plants serve as barrier systems against predation by numerous microorganisms, insects, and herbivores. Herbal medicines have turned out to be more prevalent in the treatment of numerous infections because of mainstream thinking that green medication is safe, adequately reachable and with fewer side-effects. Undoubtedly, the market and social trade have been great to the point that there is an exceptional jeopardy that numerous remedial plants today, confront either eradication or loss of genetic diversity. [14] Phytochemicals are arranged in various groupings according to their substance structures and to some degree based on their ability. Written beneath are the most widely recognized phytochemicals.

1. Alkaloids: the term ‘alkaloid’ (alkali-like) is commonly used to designate basic heterocyclic nitrogenous compounds of plant origin that are physiologically active. They are said to be as ‘True alkaloids’ if they have nitrogen in the heterocycle.

   For example: Morphine

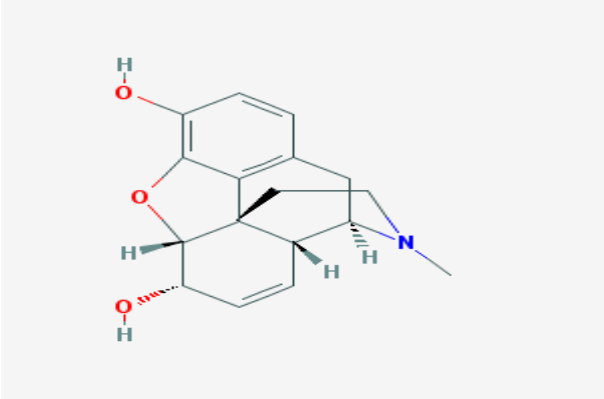


Fig 2.1 Structure of Morphine

Source: PubChem

2. Terpenoids: Terpenoids otherwise called isoprenoids are likely the most widespread group of natural products. They are the group of hydrocarbons that contain terpenes, got from five-carbon isoprene units and can be additionaly arranged into classes as indicated by the quantity of isoprene units framing their parent terpene platforms. They include various compounds like: saponins, limonene etc.

   For example: limonene

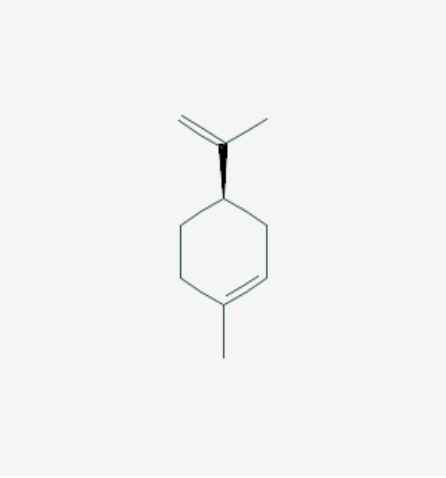


Fig. 2.2 Structure of Limonene

Source: PubChem

3. Steroids: Steroid, any of a class of normal or built natural compounds described by a sub-atomic structure of 17 carbon particles orchestrated in four rings. The steroid comprises of all sex hormones, adrenal cortical hormones, bile salts and so on.

   For example: dietary lipid- cholesterol, sex hormones- estrogen and testosterone.

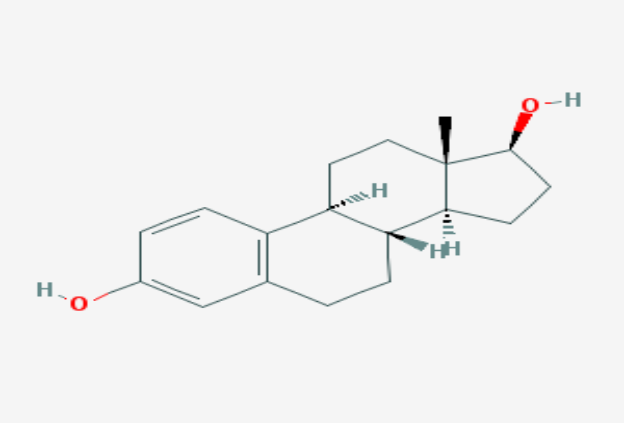


Fig. 2.3 Structure of Estradiol

Source: PubChem

4. Flavonoids: Flavonoids are the most inexhaustible polyphenols in the human diet. Flavonoids have the general structure of a 15-carbon skeleton, which comprises two phenyl rings (A and B) and heterocyclic ring (C). Flavonoids are the most imperative plant pigments for flower hue, creating yellow or red/blue pigmentation in petals intended to draw in pollinator creatures.

   For example: 2-phenylchromen-4-one

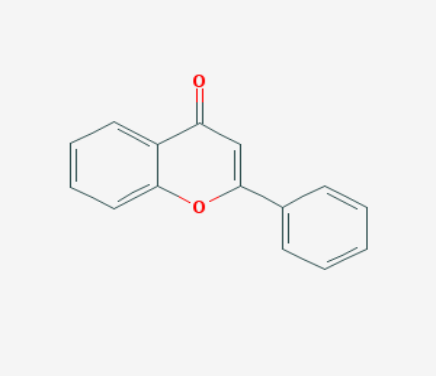


Fig. 2.4 Structure of 2-phenylchromen-4-one

Source: PubChem

5. Carbohydrates: Carbohydrates include compounds like starch, linen; sugar etc. are mostly made of atoms like carbon(C), oxygen (O) and hydrogen (H). They serve as energy sources and as essential structural components in organisms and are present in most of the food items like vegetables, fruits and bread.

   For example: Glucose

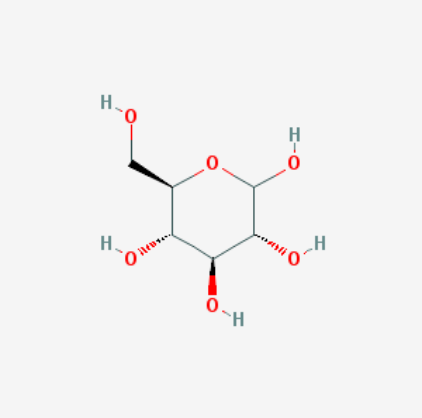


Fig. 2.5 Structure of Glucose

Source: PubChem

6. Tannins: Tannins are described as water-dissolvable phenolic blends with molecular weights going from 500 to 3000 that have the property of consolidating with proteins, cellulose, gelatin, and gelatin to shape an insoluble complex. [15] They are found in leaves, roots, seeds, buds, and stems. They are a reason behind shading, flavor, and astringency of a few sorts of tea.

   For example: gallic acid

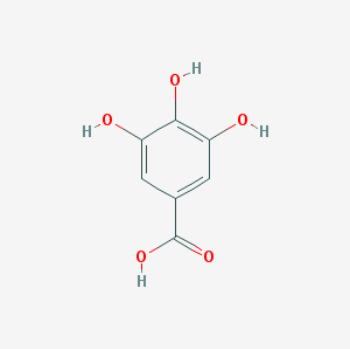


Fig. 2.6 Structure of Gallic Acid

Source: PubChem

2.3 Different activities and properties exhibited by phytochemicals

Phytochemicals are known to have a broad assortment of health benefitting properties and moreover give assurance against various chronic sicknesses. Some possible impacts showed up by phytochemicals:

Antioxidants: All the phytochemicals have cell reinforcement properties which offer protection to cells against oxidative harm and henceforth decrease the risk of working up specific sorts of malignancy. Phytochemicals indicating cell reinforcement movement are allyl sulfides, carotenoids, flavonoids, and polyphenols.

Antibacterial effect: Many phytochemicals like tannins can impede bacterial growth.

Regulating hormonal action: Isoflavones are found to mirror human estrogens and help to calm menopausal symptoms and osteoporosis.

Reduce risk of cancer: Indoles animates catalysts that reduce the effect of estrogen and in this way could diminish the hazard for the breast cancer. Different phytochemicals, which interfere with chemicals, are protease inhibitors and terpenes. Intake of specific phytochemicals has demonstrated lessened chances of the infection. [16]

Obstruction with DNA replication: Saponins have a property to intrude with the replication of cell DNA subsequently shielding the tumor cells from duplicating. Capsaicin, another phytochemical shields DNA from cancer-causing agents. [17]

2.4 Sustainable nanotechnology:

The US National Nanotechnology Initiative (NNI) describes nanotechnology as the `research and technology development in the length scale of around 1-100 nm range at the atomic, molecular and macromolecular level' to get a significant comprehension of materials at the nanoscale and favorable position of having little size is used to blend and use structures, gadgets, and frameworks that show novel properties and capacities. The ability to intentionally modify the properties of nanostructures by controlling their structure and their surface properties at a nanoscale level makes them to an awesome degree appealing probability for use in biological contexts, from fundamental scientific investigations to modernly reasonable innovations. Benign materials, for instance, polyphenols from tea, from coffee or from wine waste can work both as diminishing and topping agents. These agents give an extraordinary degree essential, one-pot, greener methodology to consolidate mass measures of nanomaterials in water without the requirement for a lot of insoluble formats. [18]

With the ongoing advances the rule of green chemistry intends to lessen or wipe out substances unsafe and hazardous s to human wellbeing and the earth in the plan,

improvement and execution of chemical procedures and items is winding up increasingly critical. To conform to the 12 standards of green chemistry, numerous specialists attempted to maintain a strategic distance or diminish the employments of dangerous chemicals and solvents, for example, utilizing natural materials rather than toxic chemicals. [19]

Thus, the requirement for the improvement of a clean, dependable, biocompatible and ecofriendly procedure to combine NP's prompt turn specialists towards green chemistry and bioprocesses. Lately, plant extracts including bark, leaves blossoms and organic products have been utilized to incorporate the metal NP's. these biogenically orchestrated NP's show greater similarity for pharmaceutical and other biomedical applications than those incorporated by compound and physical techniques. Moreover, utilization of plant for synthesis of NP's does not require high energy, temperature and is inexpensive and furthermore used to scale up for vast scale synthesis. [20]

2.5 Mode of synthesis of nanoparticles

There are two main approaches for the synthesis of nanoparticles which are discussed below:

2.5.1 Top-Down approach:

Just the name proposes, the top to down approach implies from the top (larger) to the bottom (smaller). This kind of approach is like making a statue out of stone. Just like in sculpturing of a statue, a mass or enormous bit of stonne is taken, similarly in top-down path; a heavy piece of substance is taken. At that point engraving and cutting is done until the desirable shape is accomplished. Example: Distinctive types of lithographic strategies cutting, (for example, electron beam, photo ion beam or X-ray lithography cutting), engraving, crumbling, ball milling and sol gel technique. [21]

2.5.2 Bottom-Up approach:

A bottom up synthesis method depicts that the nanostructures are manufactured onto the substrate by bundling atoms onto each other, which gives rise to crystal planes,

crystal planes further bundle onto each other, resulting in the composition of the nanostructures. A bottom-up approach can thus be portrayed as a compositional approach where the building blocks are combined onto the material to form the nanostructures. The bottom-up approach is more propitious than the top-down approach because the former has a better chance of generating nanostructures with fewer defects, more homogenous chemical composition, and better short- and long-range ordering. Example: organic semiconductors employ bottom-up processes to pattern them. [22]

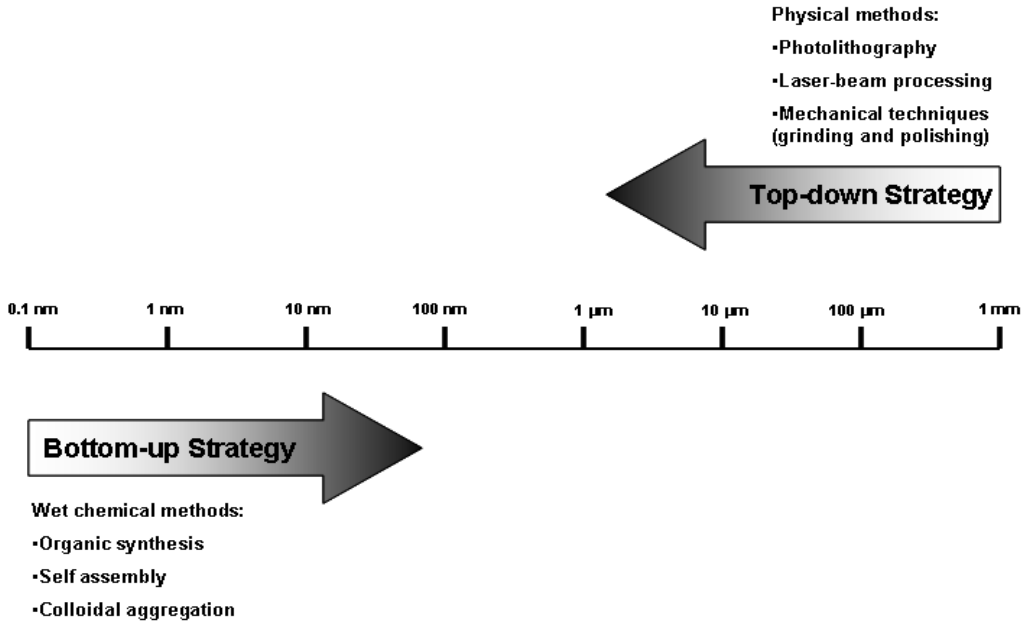


Fig.2.7 Top-Down and Bottom-Up approach of synthesis of nanoparticles [23]

2.6 Importance of phytochemicals in nanotechnology

The usage of phytochemicals in the synthesis of NP's is a fundamental framework among nanotechnology and green chemistry. In the medicinal field particularly, nanoparticles are being utilized as a part of symptomatic and supportive devices to better perceive, distinguish, & treat human ailments. Prologue to nanoparticles for medicinal uses incorporates deliberate contact or administration; thus, understanding

the properties of nanoparticles and their effect on the body is essential before clinical use can happen. [24] Thus it is essential to create NP's under nontoxic and green conditions to decrease the general lethality of NP's so they can be utilized for medicinal applications. The phytochemicals in plants can start numerous reactions in the biological systems.

For instance: Polyphenolic flavonoids in tea of which epigallocatechin gallate (EGCG) is the second major constituent, has anticarcinogenic movement in vitro which may brace the aftereffects of the epidemiologic research on the correlation between drinking tea & the danger of morbidity from cancer. [25][26]

Biosynthesis of metal nanoparticles is advantageous than conventional chemical synthesis because of the following reasons:

a. They are proposed to be cost effective and environment friendly than other chemical and physical methods
b. They are synthesized at ambient temperatures, neutral pH, low costs and do not require any extreme conditions.
c. The reducing agents and stabilizing agents are present in the plant extract as phytochemicals while other chemical methods require other chemicals and molecules.
d. They are prepared by using water as solvent which is a universal solvent, easily available and non-toxic when discarded, while in chemical synthesis many dangerous and corrosive solvents are used.
e. They require a single step unlike the chemical synthesis which is a multistep reaction.

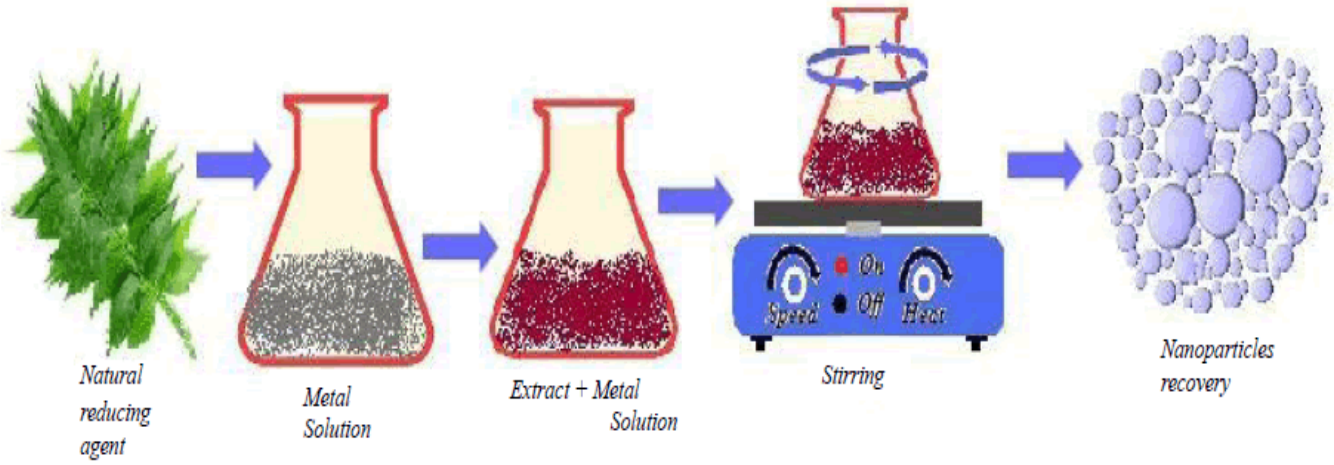


Figure 2: Synthesis of nanoparticles from plant extract.

Fig.2.8 synthesis of nanoparticles from plant extracts [27]

2.7 Elemental Silver (characteristics and sources)

It is a ductile & malleable transition metal having white metallic gloss emergence. It has the most astounding electrical-conductivity & thermal-conductivity and has the most reduced influenced defiance. 28 radioisotopes have been described, with a dominant part of them exhibiting a half-life of less than 3 min. The average concenteration of Ag in water is 0.5 ppm while its fixation in soil is around 10 ppm.

Ag happens normally in unadulterated and pure form, not only this, it is usually extracted by amalgamation & uprooting using metals such as mercury or by refining. [22]

A comprehensive survey on most recent patterns on the synthesis, global consumption, properties and future challenges of silver nanoparticles were discussed by Achmad Syafiuddin *et. al.* (2017). The three different methods of composition of silver nanoparticles using physical, chemical and biological paths were discussed. [28]

2.8 Titanium Dioxide ($TiO_{2)}$

Titanium dioxide (TiO2) is the maximum utilized white color, for instance in enamel. It has very high brightness and a very high refractive index. Apart from generating a white color in fluids, paste or as coating on sollids, TiO2 is also a very effective opacifier, making materials more opaque. E.g. paper, paints, plastics, ink etc.

a. TiO2 is also used in sunscreens. Relatively every sunscreen consists of titanium dioxide. It is a physical bloccker for UVA (bright light with a wavelength from 315 to 400 nm) and UVB (bright light with a wavelength from 280 to 315 nm) radiation. It is chemically steady and would not become decolorised under UV light.
b. It is also used in food industry. It is an inert, insoluble material normally utilized as a "whitener" and in some cases as anti-caking agent to keep items from "clumping up". Titanium dioxide can likewise be utilized to give some food items texture. [29]

2.9 Aims and Objectives

a. Phytochemical screening of *Deparia boryana.*
b. Biosynthesis of AgNP, TiO_2 nanoparticles, Ag coated TiO_2 nanoparticles, TiO_2 coated AgNP.
c. Evaluation of Bacterial toxicity of synthesized nanoparticles against ***E.coli*** and ***Bacillus subtilis.***
d. Estimation of antihelmintic activity of the extracts of *Deparia boryana.*

3. Materials and Methods

3.1 Materials

3.1.1 Apparatus

Beakers (50ml, 100ml and 250ml), glass slides, glass vials, round bottom flask, measuring cylinder, Petri plates, reagent bottles, micro tips, falcon tubes, conical flasks (100ml and 250ml), spatulas, micro pipettes, soxhlet apparatus, magnetic stirrer, magnetic beads, epprendroffs, test tubes, filter paper, tripod stand, mortar and pestle.

3.1.2 Reagents and Chemicals used

Silver Nitrate, Lead Acetate, Sodium Bicarbonate, Ferric Chloride, Potassium Iodide, Potassium Hydroxide, Sodium Chloride, Sodium Hydroxide, Copper Sulphate were purchased from **Loba Chemie** and were used without further purification.

Reagents used: Petroleum Ether, Methanol, Chloroform, Conc. HCl, Conc. H2SO4, Acetic Anhydride, Glacial Acetic Acid, TiCl4 were purchased from **Loba Chemie.**

3.1.3 Collection of the plant

The fresh ferns were collected from Kasauli, Himachal Pradesh, India. The leaves were cleaned a few times with fresh water lastly with distilled water. The leaves were then dried in daylight for 2-3 days. The leaves were then pounded to a fine powder.

3.1.4 Bacterial Culture

The reference bacteria *E.coli* strain MTCC77 and *Bacillus subtilis* strain MTCC441 was obtained from Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala. The Luria Broth used for growing and maintaining the bacterial cultures was purchased from **Himidea**.

3.1.5 Collection of Earthworms

Adult earthworms (*Pheretima posthuma)* were purchased from Shergill Farms, Patiala, India and were washed with distilled water to remove soil and fecal matter.

3.2 Instruments Used:

3.2.1 Magnetic Stirrer:

A magnetic stirrer of REMI 2MLH is a laboratory device which uses magnetic field to mix liquid samples.

3.2.2 Weighing Balance:

Accurate quantities of chemicals to be used were achieved with the help of the weighing balance (SARTORIOUS). Maximum measurement-250gm.

3.2.3 Hot Air Oven:

The hot air oven also known as digital indicator cum controller was used for the drying of the samples and apparatus. It was generally operated at 50-80^{o}C.

3.2.4 Soxhlet Apparatus:

A device for use in separating greasy or other material with an volatile solvent, (for example, ether, liquor, or benzene) comprising of a vertical glass tube shaped extraction tube that has both a siphon tube & a vapor tube, which is fitted at its upper end to a condenser & at its lower end to a flask so the solvent might be refined from the flask into the condenser whence it streams once more into the round and hollow tube and directs over into the flask to be refined once more.

3.2.5 Laboratory Centrifuge:

The centrifuge is the laboratory device which works under the rule where centripetal acceleration will make denser substance move outward the radial way, the less dense substance are uprooted and move to centre. The centrifuge used is THERMO FISHER SCIENTIFIC (SL8R).

3.2.6 Autoclave:

It is a laboratory device with extraordinary conditions-high or low pressure and temperature that can be built up for various applications. It is particularly used for sanitizing glass apparatus utilizing steam under high pressure.

3.2.7 Laminar Flow Cabinet:

It is a precautious enclosed bench which is used to avoid contamination of biological or any sensitive samples. There is a continuous air flow towards the user which is drawn through a HEP filter.

3.2.8 Incubator:

It is a device that facilitates the growth and maintenance of the microbiological cultures or cell cultures. It provides optimum temperature, humidity and atmosphere required for the optimal growth of the cultures.

3.3 Methodology

3.3.1 Preparation of plant extract

10 gm of plant powder was taken and boiled in distilled water for 60 minutes. The water extract was then cooled and separated utilizing Whatmann channel paper No. 1 and was put away at 4^{o}C for further utilization.

25gm of plant powder was extracted effectively with 200ml of each of Chloroform and Petroleum ether utilizing soxhlet device at 55-85^{o}C for 8-10 h keeping in mind the end goal to extricate polar and nonpolar compounds for the phytochemical examination.

3.3.2 Preliminary Phytochemical Assay

The three plant extracts (chloroform, petroleum ether and aqueous) were tested for the presence of different phytochemicals. The following tests were performed:

1. **Test for Alkaloids**
    - **Meyer's Test:** The filtrates were treated with few drops of Meyer's reagent (2gm I2 + 6gm KI in 100ml of H2O). Development of yellow colored precipitates demonstrates the presence of alkaloids.
    - **Wagner's Test:** The filtrates were treated with few drops of Wagner's reagent (1.36gm HgCl2 + 5gm KI in 100 ml of H2O). Development of reddish brown colored precipitates affirmed the presence of alkaloids.
2. **Test for Carbohydrates:**
    - **Fehling Solution Test:** 1:1 of Fehling solution A (CuSO4.5H2O) and Fehling Solution B (Sodium Potassium Tartrate in NaOH) was taken in a

test tube and was warmed to boiling and afterward poured in aqueous ethanol extract. Colored reaction affirmed the existence of carbohydrates.

3. **Test for Flavonoids:**
    - **Alkaline Reagent Test:** Each extract was tried with 2ml of NaOH. Development of exceptional yellow color which vanishes on the addition of an equivalent amount of acid shows the presence of flavonoids.
    - **Zn/Mg test:** To each extract, a little amount of Zn or Mg strip was included, trailed by few drops of Conc. HCl. Development of reddish brown colored precipitates affirms the existence of flavonoids.

4. **Test for Glycosides:**
    - **$FeCl_3$ test:** Each of the extracts was mixed with glacial acetic acid. 1-2 drops of FeCl3 was included trailed by a couple of drops of conc. H2SO4. Presence of a dark colored ring at the interface demonstrates the presence of glycosides.

5. **Test for Saponins:**
    - **Froth Test:** The extracts were diluted with distilled water in a graduated cylinder up to10 ml. This was shaken for 5 minutes. Development of foam layer of no less than 1 cm affirms the presence of saponins.

6. **Test for Phenols:**
    - **Ferric Chloride Test:** The extracts were treated with 5ml distilled water for following tests and filtered.
7. **Test for Tannins:**

    The extracts were mixed in 5 ml of distilled water for the following tests and filtered.
    - **Lead Acetate Test:** To 1-2 ml of filtrates, a couple of drops of 1% lead acetate were included. Development of yellow precipitates affirmed the existence of tannins.

- **Ferric Chloride Test:** To 1-2 ml of filtrates, a couple of drops of 5 % FeCl3 were included. Formation of a green precipitate affirmed the existence of tannins.

8. Test for Steroids:

Each extracts were dissolved in 5 ml chloroform and filtered. The filtrate was analyzed for the existence of steroids.

- **Libermann Burchard's Test:** The filtrate was treated with a couple of drops of acidic anhydride. The solution was then boiled and allowed to cool. To this a couple of drops of conc. Sulphuric acid were included. The presence of Brown ring at the intersection affirms the existence of Steroids.

9. Test for Terpenoids :

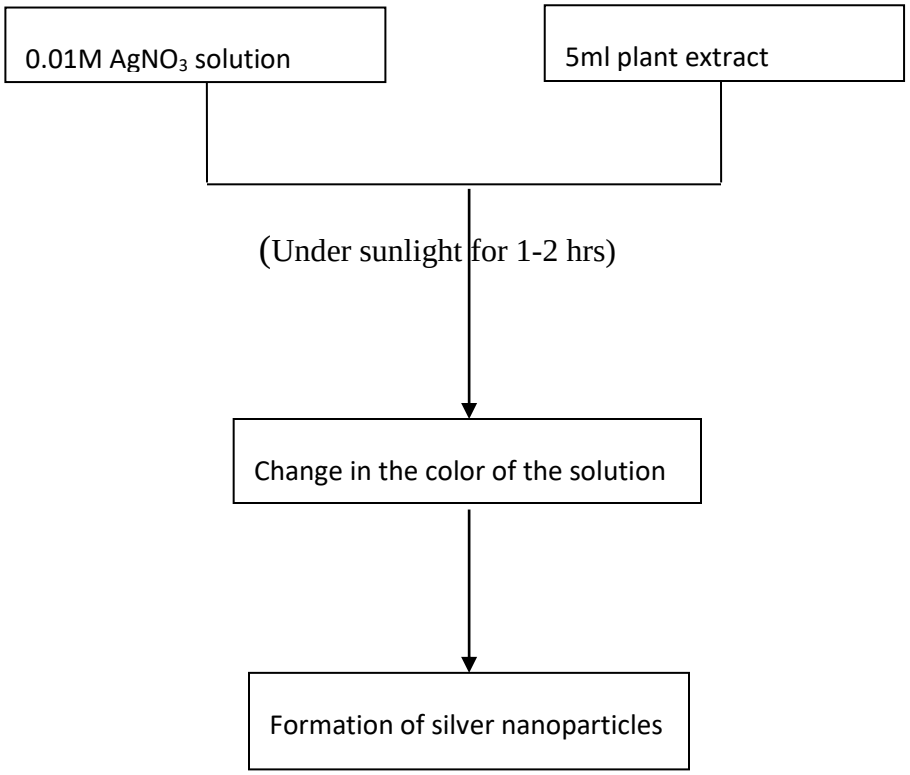
- **Acid Test:** The extracts were treated with a couple of drops of concentrated sulphuric acid. The presence of the darker ring at the interface shows the presence of Terpenoids.

10. Test for Oils and Fats:

- **Filter Paper Test:** Different Extracts were pressed between filter papers. An oil stain on filter paper affirms the presence of oils and fats.

3.3.3 Biosynthesis Of AgNP:

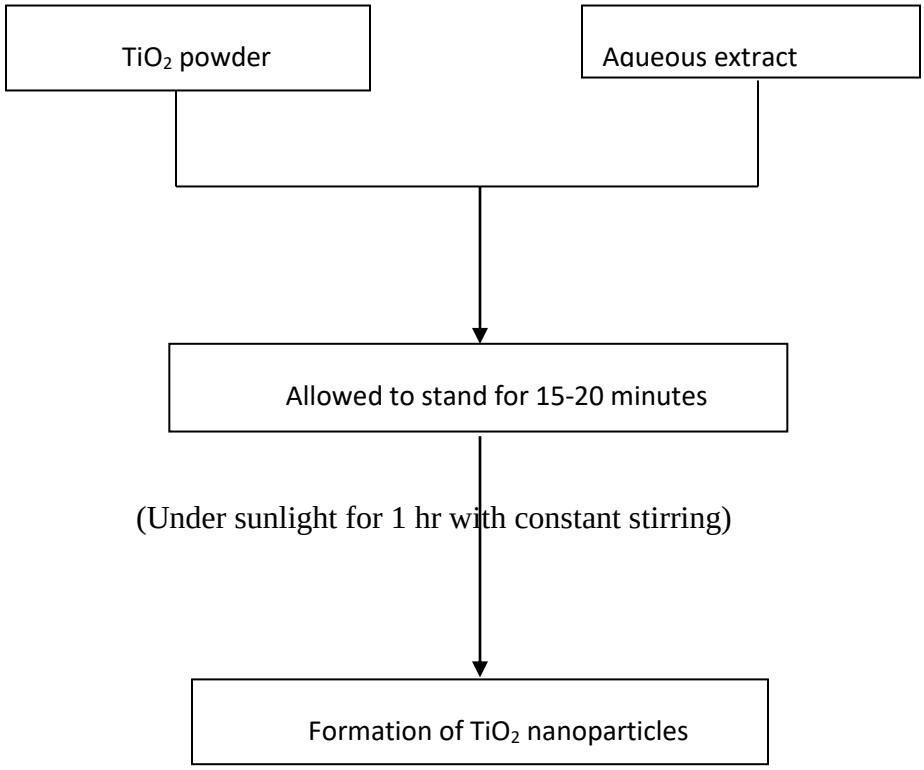
The green synthesis of Silver nanoparticles was done by using 0.01M $AgNO_3$ solution in 5 ml plant aqueous extract whose pH was maintained at 7 by adding NaOH. The mixture was then kept under sunlight for 1-2 hrs and color change was observed. The change in color indicated the formation of silver nanoparticles. The flow chart of formation of silver nanoparticles is explained briefly in the following scheme:



Scheme 3.1: Green synthesis of AgNP

3.3.4 Biosynthesis of TiO_2 Nanoparticles:

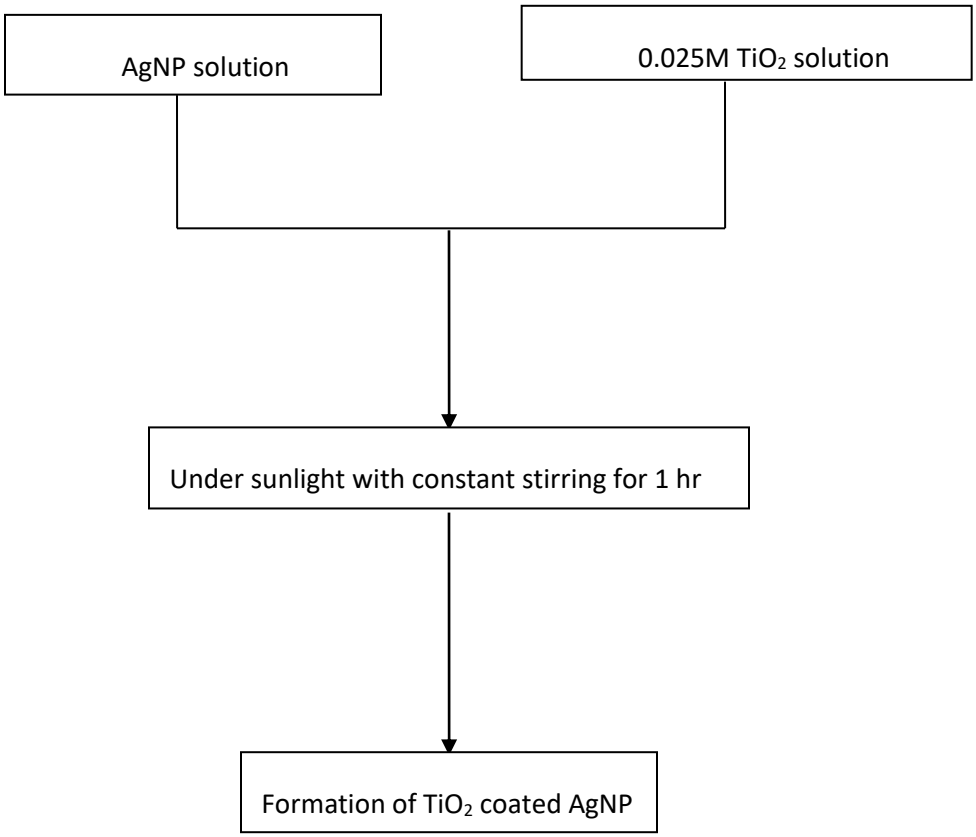
The green synthesis of TiO_2 nanoparticles was done by using TiO_2 powder and plant extract. The mixture was then allowed to settle down for 15-20 minutes and then it was kept under sunlight for 1 hr with constant stirring. The flowchart of the synthesis of TiO_2 nanoparticles is briefly described in the following scheme:



Scheme 3.2: Biosynthesis of TiO_2 nanoparticles

3.3.5 Biosynthesis of TiO_2 coated AgNP:

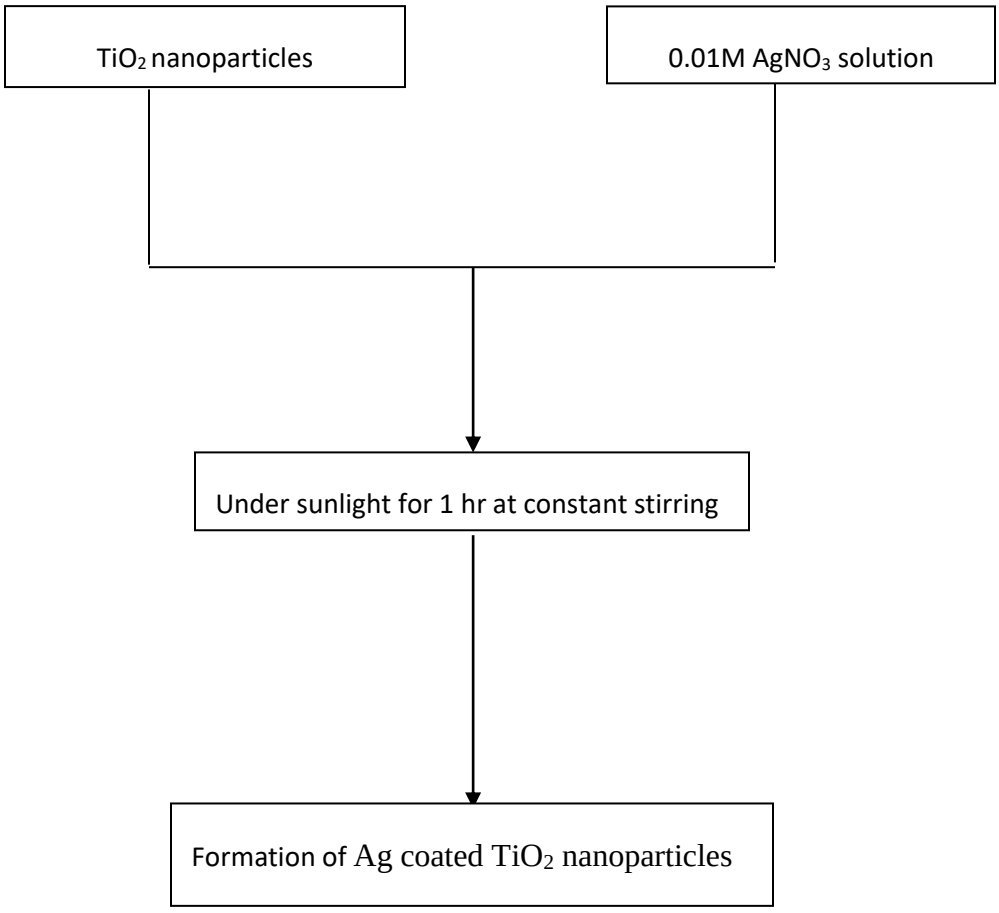
The green synthesis of TiO_2 coated AgNP was done by using the above formed silver nanoparticles and doping them with TiO_2. 0.025M TiO_2 was added to the silver nanoparticles solution and the mixture was then again kept in sunlight for 1 hr with constant stirring. The process is briefly explained in the following scheme:



Scheme 3.3: Biosynthesis of TiO_2 coated AgNP

3.3.6 Biosynthesis of Ag coated TiO_2 coated nanoparticles

Biosynthesis of Ag coated TiO_2 nanoparticles were done by using above formed TiO_2 nanoparticles and doping them with 0.01M $AgNO_3$ solution. The mixture was then kept under sunlight for 1 hr and the change in color was observed, which indicates the formation of Ag coated TiO_2 nanoparticles. The following scheme represents the scheme for the same:



Scheme 3.4: Biosynthesis of Silver coated TiO_2 nanoparticles.

3.4 Characterization

3.4.1 UV-Visible Spectroscopy

UV-Visible spectra of the above-synthesized nanoparticles and OD of the growth in the bacterial cultures were determined by UV-Visible spectrophotometer (UV 2600 spectrophotometer Schimatzu) from the range 200-800nm.

3.4.2 FT-IR (Fourier Transform Infrared Spectroscopy)

FT-IR was further done to affirm the production of the synthesized nanoparticles. FT-IR was done using Agilent Resolution Pro Carry 600 FT-IR spectrophotometer.

3.4.3 SEM-EDS

SEM-EDS analysis was carried out for investigating the morphological and elemental structure of the nanoparticles that were synthesized with the plant extract and to determine the ratios of the elements present in the samples. Powdered samples were used for SEM analysis. The micrographs were recorded using Scanning Electron Microscope (JEOL JSM-6510 LV).

3.4.4 Dynamic Light Scattering

DLS was used to determine the hydrodynamic size of the nanoparticles synthesized. It gives the average size of the maximum number of nanoparticles.

3.5 Applications:

3.5.1 **Antibacterial activity** of AgNP, TiO_2 coated AgNP, TiO_2 nanoparticles, Ag coated TiO_2 nanoparticles, Petroleum ether extract and Chloroform extract was evaluated against *E.coli* and *Bacillus subtilis.*

- **Broth Dilution Method**: Minimum inhibitory concentration (MIC) was determined by growing *E.coli* and *Bacillus subtilis* in LB (2X concentration i.e. 4 gm in 100 ml distilled water) comprising of different concentrations of nanoparticles (0, 0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, 1 mg/ml). The bacterial cultures were incubated at 37^{o}C and 300 rpm. The growth culture was determined by spectrophotometric absorbance which was recorded at 600nm.

3.5.2 Antihelmintic activity:

The antihelmintic activity was carried out using the nanoparticles against adult earthworms. The earthworms were divided into four groups containing 5 earthworms each. Group 1 was control, placed in normal saline; Group 2, 3, and 4 were treated with AgNP, Petroleum ether extract and Chloroform extract with 15mg/ml extract each. To make such doses, the extracts were measured respectively and mixed in normal saline. Observations were done for the time-taken for the worm to get paralysed (the state when worm could not resurrect in normal saline) and the time for the death of worms was observed after assuring that worms neither moved when jolted actively nor when lowered in warm water ($50^{o}C$), followed by the colors of their body fading away.

3.5.3 MTT assay:

The MTT assay was carried out using the nanoparticles against cancer cell lines (A549 lung cancer cell lines). ELISA plate was taken and the test samples were run in triplicates. Two groups of each type of nanoparticles was set up with 1% and 5% concentration each in 100µl DMEM media and was kept overnight followed by 5µl of MTT and was incubated for 2 hrs. 100µl DMSO was added and incubated for 30 minutes. After that the growth of cell lines was observed by measuring UV-Vis spectroscopy at 570 nm.

4. Results and discussions

4.1 Qualitative phytochemical screening

Following phytochemicals are found to exist in various extracts of *Deparia boryana*. The observation is represented below.

NOTE: '+' represents positive result and '-' represents negative result.

Table 4.1: Phytochemical assay of *Deparia boryana*

S.No.	Test performed	Water Extract	Chloroform Extract	Petroleum Ether Extract
1.	**Alkaloids**			
Meyer's Test		-	+	-
Wagner's Test		-	+	-
2.	**Carbohydrates**			
Fehling Solution Test		+	-	-
3.	**Flavonoids**			
Alkaline Reagent Test		-	-	-
Zn/Mg Test		-	-	-
4.	**Glycosides**			
Ferric Chloride Test		+	-	-
5.	**Saponins**			
Froth Test		+	+	-
6.	**Phenols**			
Ferric Chloride Test		-	-	-
7.	**Tannins**			
Lead Acetate Test		-	-	-
Ferric Chloride Test		-	-	-
8.	**Steroid**			
Libermann Burchard's Test		-	-	-
9.	**Terpenoids**			
Acid Test		+	-	-
10.	**Oils and Fats**			
Filter Paper Test		-	-	-

Different extracts showed the presence of phytochemicals like glycosides, saponins, Terpenoids etc.

4.2 UV-Visible Spectroscopy

The reduction of Ag^{+} to Ag^{0} is analyzed by UV-Visible spectrophotometer. The nanoparticles were synthesized in green way. The band for AgNP was observed around 400- 412 nm, which is a characteristic for silver nanoparticles. Plant leaf extract was able to synthesize the silver nanoparticles by the indication of suitable surface Plasmon resonance (SPR) with peaks near visible spectrum around 400 -412 nm. The peak indicates the stability and uniformity of silver nanoparticles synthesis. [30]

The band for TiO_2 nanoparticles was observed at 280-320 nm. The band for TiO_2 coated AgNP was observed from 405-427 nm, because of the large dielectric function of TiO_2, a shift to the longer wavelengths was observed for the Plasmon resonance maximum of coated silver nanoparticles. [31]

The band for Ag coated TiO_2 nanoparticles were observed at 284-328 nm.

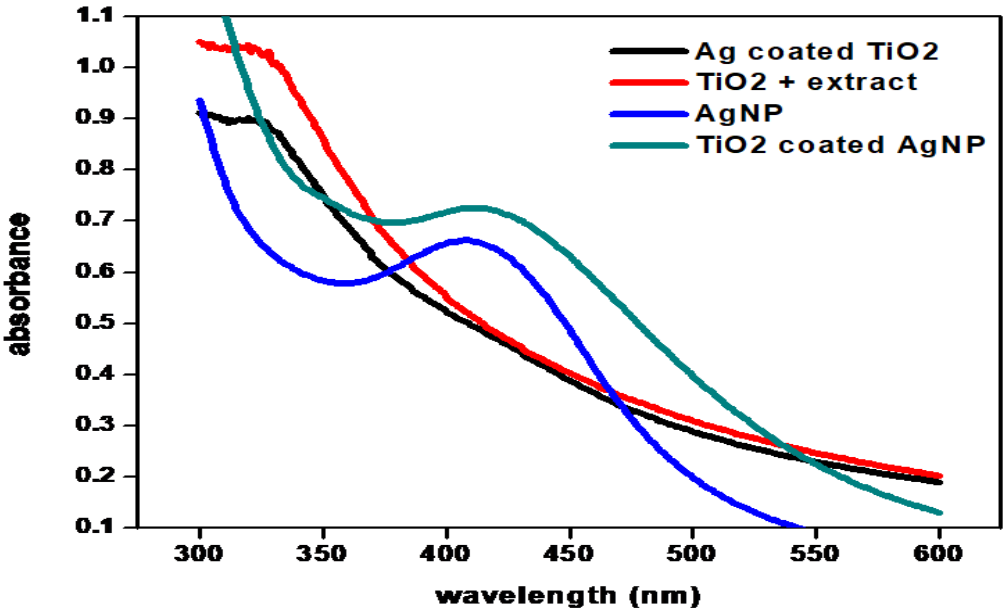


Fig. 4.1 UV-Visible spectra of synthesized nanoparticles

4.3 FT-IR Spectroscopy

FTIR analysis carried out for different nanoparticles is shown in fig 4.2. Appearance of sharp band in Ag coated TiO_2 nanoparticles (B) at 469 cm^{-1} and in TiO_2 nanoparticles (D) at 479 cm^{-1}, indicates the formation of TiO_2 nanoparticles which arises from Ti-O. Other absorption peaks are observed are 3389 cm^{-1} (B and C), 3400 (A and D): for N-H stretching; 3148 cm^{-1} (B and D), 3159 cm^{-1} (A), while no such bend is observed at silver nanoparticles: for O-H stretching (alcohol); 2919 cm^{-1} (B and D), 2930 cm^{-1} (A and C): for C-H stretching; 2375 cm^{-1} : for O=C=O stretching; 1608 cm^{-1} : for C=C stretching, 1421 cm^{-1} : O-H stretching (phenol); 1256 cm^{-1} (A and C), 1245 cm^{-1} (B), 1234 cm^{-1} (D) : for C-O stretching; 1016 cm^{-1} (B and D), 1071 cm^{-1} (A), 1016 cm^{-1} (D): for S=O stretching; 676 cm^{-1} (A), 666 cm^{-1} (C), 633 cm^{-1} (D), while so such bend is observed for Ag coated TiO_2 nanoparticles: for C-Cl stretching. These additional peaks can be attributed to the presence of phytochemicals in the plant extract used to synthesize the nanoparticles.

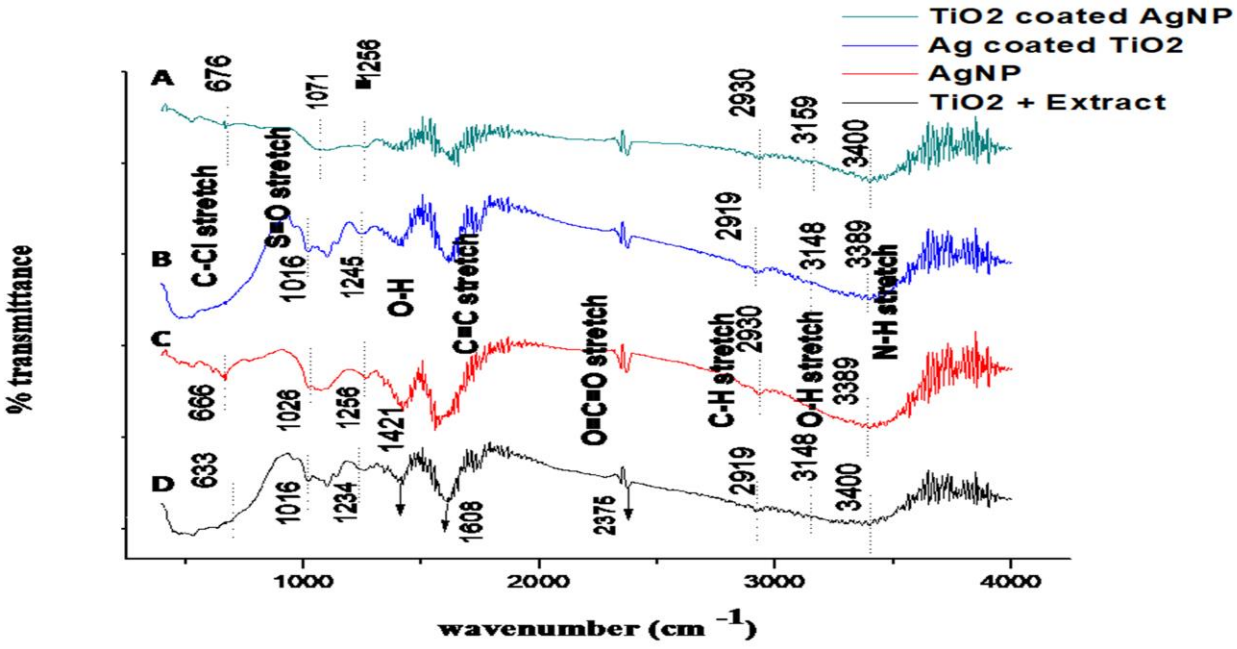


Fig. 4.2 FTIR Spectrum of (A) TiO_2 coated AgNP, (B) Ag coated TiO_2 , (C) AgNP, (D) TiO_2 nanoparticles.

Functional groups	TiO_2 coated AgNP	Ag coated TiO_2	AgNP	TiO_2 nanoparticles
C-Cl stretch (halo compound)	676	-	666	633
S=O stretch (sulfoxide)	1071	1016	1028	1016
C-O stretch (alkyl aryl ether)	1256	1245	1256	1234
O-H stretch (phenol)	1421	1421	1421	1421
C=C stretch (α, β unsaturated ketone)	1608	1608	1608	1608
O=C=O stretch (carbon dioxide)	2375	2375	2375	2375
C-H stretch (alkane)	2930	2919	2930	2919
O-H stretch (primary alcohol)	3159	3148	-	3148
N-H stretch (primary amine)	3400	3389	3389	3400

Table 4.2 FTIR data of biosynthesized nanoparticles

4.4 Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS)

The biosynthesized nanoparticles were subjected to SEM-EDS to get profound insight of the shape and size of the nanoparticles formed and to check its elemental composition. EDS relies upon the quantity of identified x beams produced from constituent components, impacts of spectral interference and background disturbances The size of the nanoparticles formed was more than the nano-range i.e. from 1-100 nm. The size of the nanoparticles was found to be in nano to micron range.

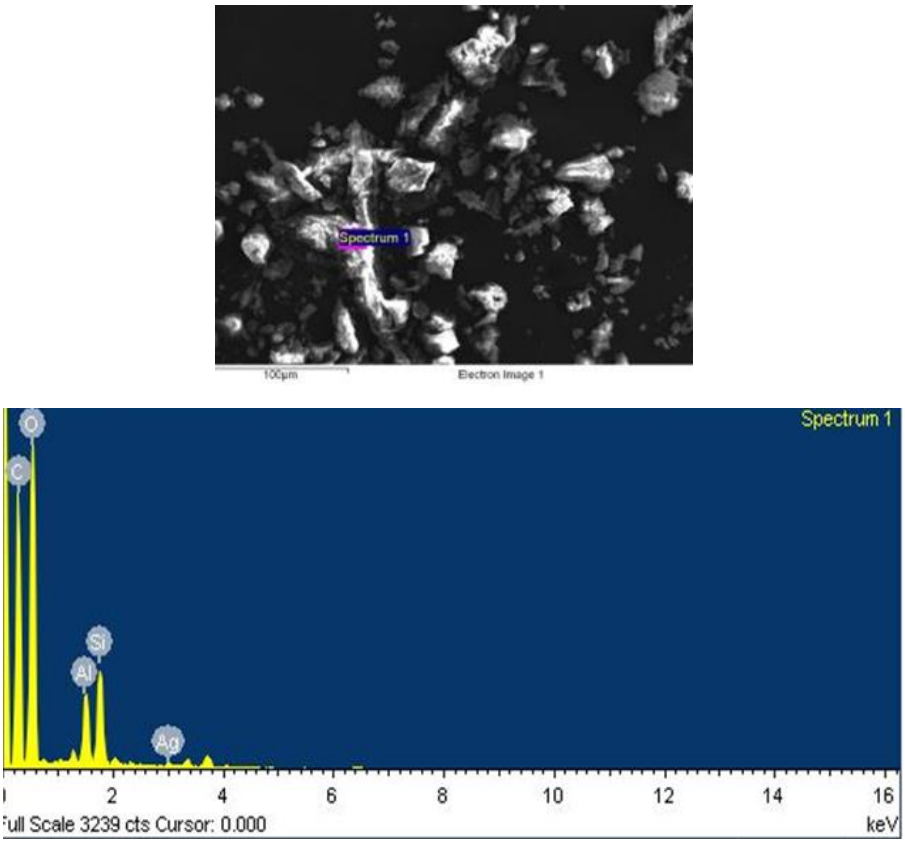
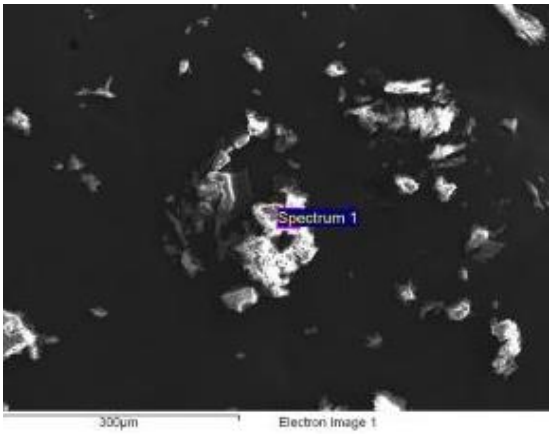


Fig. 4.3(a) SEM-EDS of AgNP

Elements	composition
C	**41.31**
O	**51.44**
Al	**2.73**
Si	**4.16**
Ag	**0.37**

Table 4.3 Elemental composition of AgNP



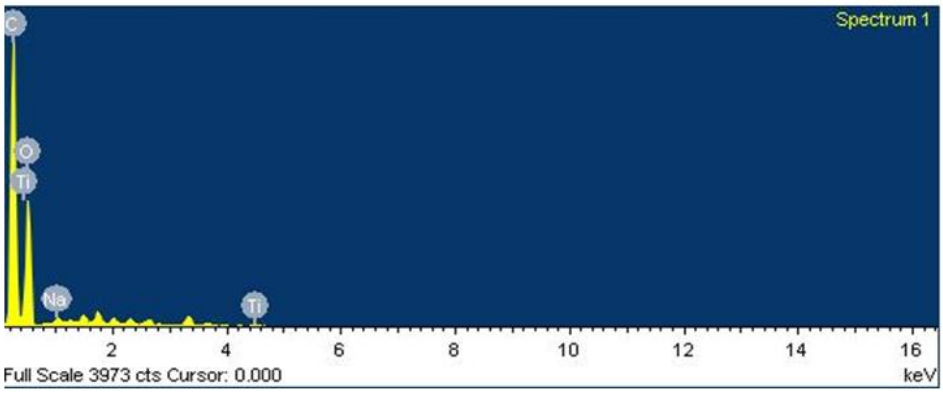
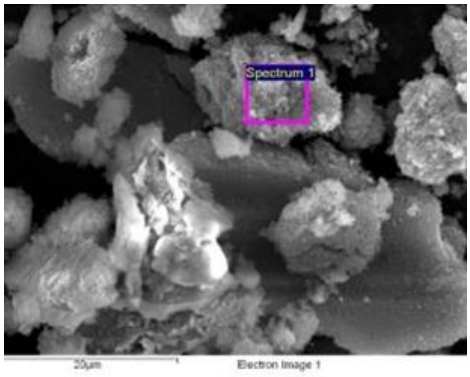


Fig. 4.3(b) SEM-EDS of TiO_2 nanoparticles

Elements	composition
C	**54.03**
O	**44.81**
Na	**0.21**
Ti	**0.95**

Table 4.4: elemental composition of TiO_2 nanoparticles



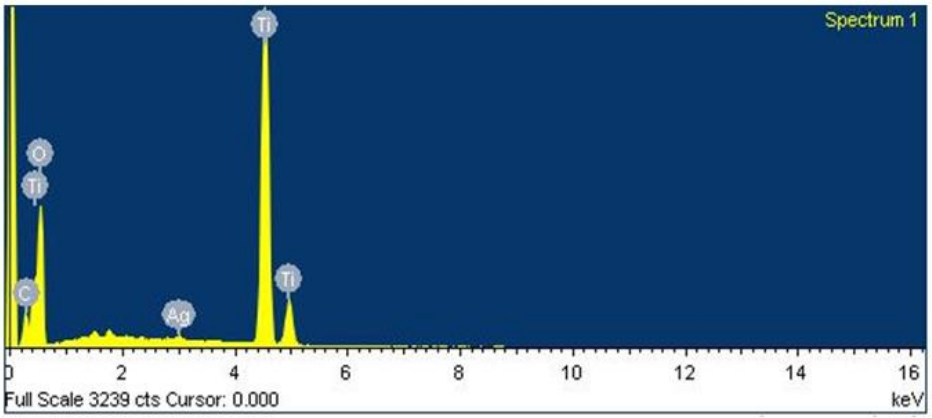
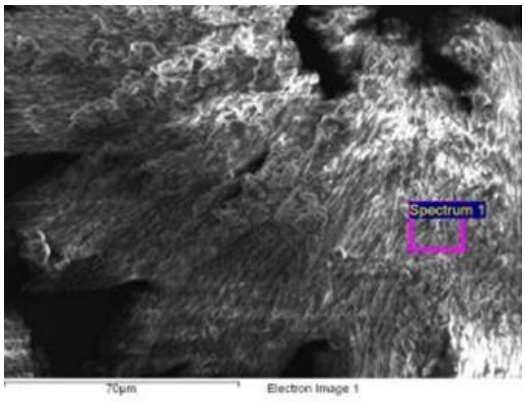


Fig. 4.3(c) SEM-EDS of Ag coated TiO_2 nanoparticles

Elements	composition
C	**4.55**
O	**43.47**
Ti	**49.62**
Ag	**2.36**

Table 4.5: elemental composition of Ag coated TiO_2 nanoparticles



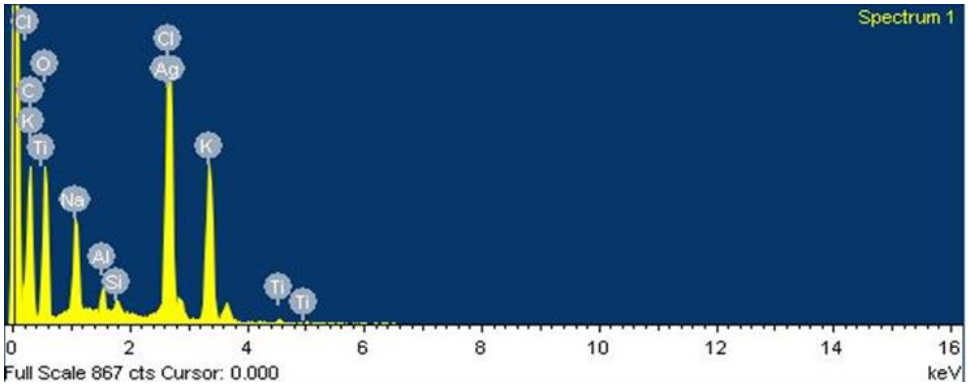


Fig. 4.3(d) SEM-EDS of TiO_2 coated AgNP

elements	composition
C	**21.31**
O	**37.32**
Na	**13.30**
Al	**1.72**
Si	**0.94**
Cl	**17.46**
K	**4.86**
Ti	**2.33**
Ag	**0.77**

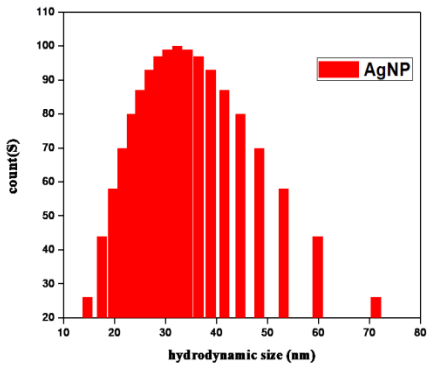
Table 4.6: elemental composition of TiO_2 coated Ag nanoparticles

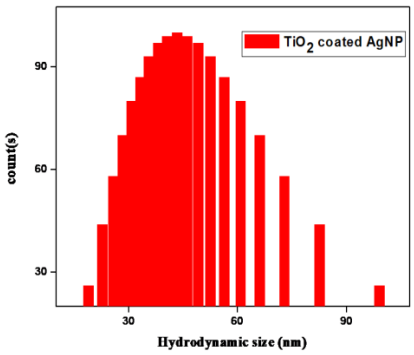
4.5 Dynamic Light Scattering (DLS)

DLS is the technique to determine the particle size. Figures below show the DLS data of biosynthesized silver nanoparticles (AgNP), TiO_2 coated silver nanoparticles **Fig. 4.4 (a) and (b)**; TiO_2 nanoparticles, Ag coated TiO_2 nanoparticles **Fig. 4.5 (a) and (b)** respectively. Maximum particle size of AgNPs were in range 30-40 nm, thus the particles formed were in nano range.

Maximum particle size of TiO_2 coated silver nanoparticles were in range 30-60 nm, thus the particles formed were in nano range.

Whereas the maximum particle size of TiO_2 nanoparticles and Ag coated TiO_2 nanoparticles were in range 100-200 nm and 100-140 nm range respectively, from which it can be concluded that the some of the nanoparticles with size >100 nm were in micro range and those with size <100 nm were in nano range. The nanoparticles were formed due to the presence of phytochemicals in plant extract which act as reducing agent and capping agents.

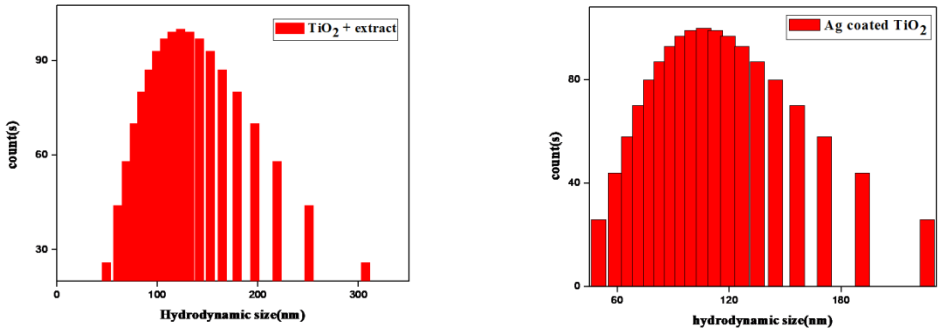




(a) **(b)**

Fig 4.4(a): particle size of AgNP

Fig 4.4(b): TiO_2 coated AgNP Nanoparticles



(a) **(b)**

(b)

Fig. 4.5(a): particle size of TiO_2 + extract

Fig. 4.5(b): Ag coated TiO_2 nanoparticles

4.6 Antibacterial Activity

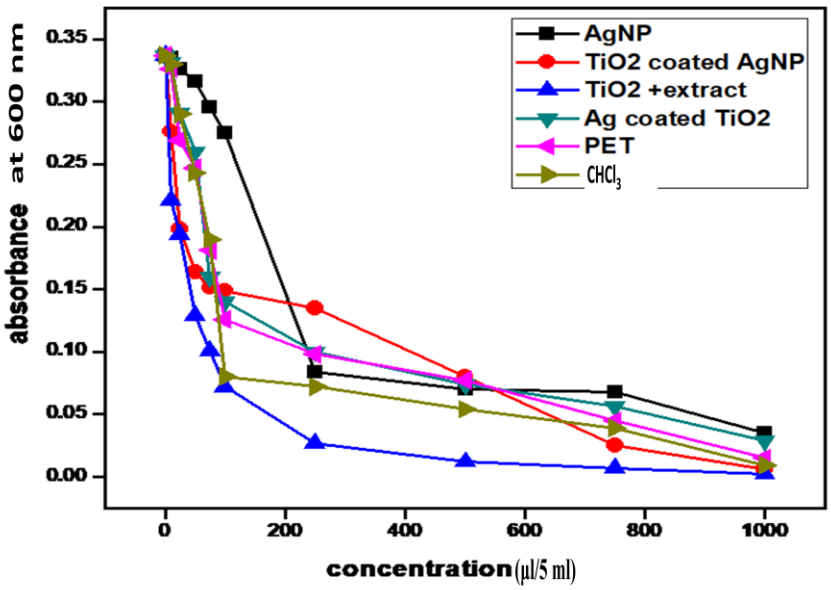


Fig 4.6: Antibacterial activity against *E.coli*

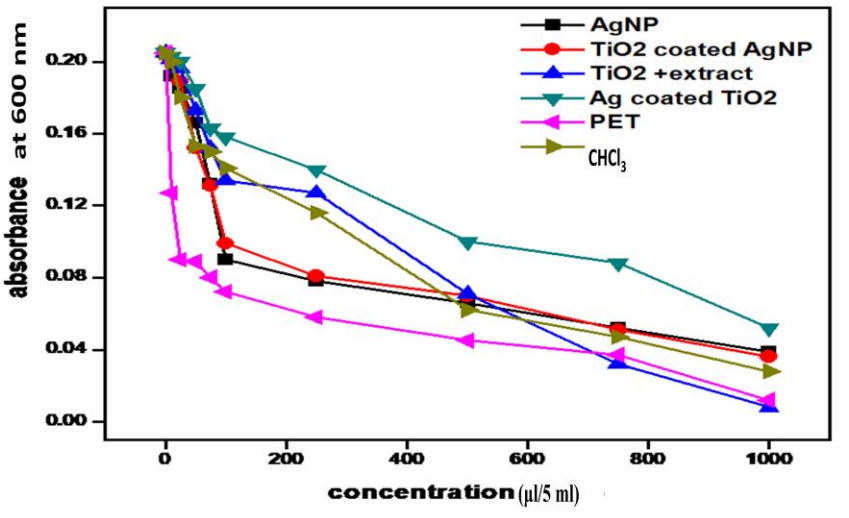


Fig. 4.7: Antibacterial activity against *Bacillus subtilis*

Antibacterial activity of different extracts **(AgNP, TiO_2 nanoparticles, Ag coated TiO_2 nanoparticles, TiO_2 coated AgNP, chloroform extract, PET extract)** were checked by broth dilution method. And the minimum inhibition concentration (MIC) was calculated by this method. The test tubes with 0.75 and 1 mg/ml concentrations showed less visual turbidity which is an indication of reduced growth of the bacteria. This was further confirmed by UV-Visible spectrophotometer and by calculating IC50 values for each sample. Lower the value of IC50 more potent is the test sample. The lowest IC50 value against *E.coli* as well as against *B. subtilis* was observed for TiO_2 coated silver nanoparticles.

4.7 Antihelmintic Activity

In antihelmintic activity the nanoparticles and the plant extracts of *Deparia boryana* exhibited the paralysis & the death of the worms. As shown in below table, chloroform extract took shortest time for paralysis & the death of the worms with 15mg/ml concentration followed by petroleum ether extract. The evaluation of the antihelmintic activity was compared with reference to the worms in the normal saline. The worms in the normal saline were alive even after 24 hrs of the experiment. The constituents responsible for the antihelmintic activity were present in the all three test samples.

Table 4.7: Antihelmintic activity of various extracts				
S.No.	**Test Sample**	**Concentration (mg/ml)**	**Time taken for paralysis (min)**	**Time taken for death(min)**
1.	**Control**	-	-	-
2.	**Petroleum ether**	15	91±5	124±5
3.	**Chloroform**	15	70±5	117±5
4.	**AgNP**	15	220±5	239±5

Fig 4.8 Antihelmintic activity of various extracts of *Deparia boryana* (Zero minute)

Fig. 4.9 Antihelmintic activity of various extracts of *Deparia boryana* (After 240 minutes)

4.8 MTT assay

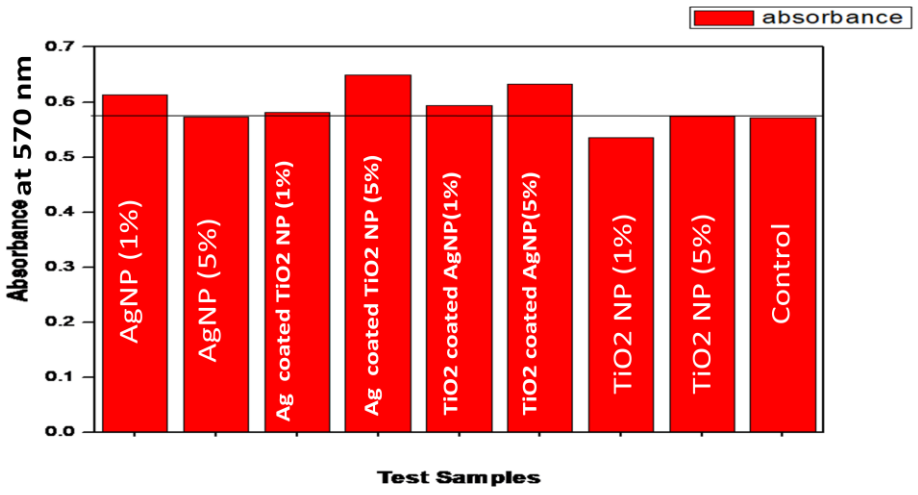


Fig. 4.10 Absorbance for the toxicity to the cancer cell lines

Ag	Silver nanoparticles
ATNP	Silver coated TiO_2 nanoparticles
TANP	TiO_2 coated silver nanoparticles
TNP	TiO_2 nanoparticles

This assay helps in the identification of intrinsic toxicity of plants and the effects of acute toxicity. It could also provide the indication of cytotoxic properties of tested plant extracts. This assay is based on the reduction of MTT by mitochondrial dehydrogenase by purple formazan product. It is used as the in vitro model system to measure toxic effects of variety of toxic substances and plant extracts against cancer cell lines.[33] It can be observed from the graph that the maximum absorbance is comparable to the absorbance of the control, which shows that the test samples show very less toxicity to the cancer cell lines, which shows that the biosynthesized nanoparticles are non-toxic to the animal cell lines but are harmful to the bacteria and hence can act as novel antibacterial agents.

5. Conclusion

Deparia boryana was made clear for the phytochemicals present in its leaves. Aqueous extract showed maximum number of phytochemicals like, Terpenoids, saponins, glycosides etc. as compared to chloroform and petroleum ether extract. These extracts exhibited excellent antihelmintic activity carried out on earthworms. The synthesis of nanoparticles like AgNP, TiO_2 nanoparticles, TiO_2 coated Ag nanoparticles and Ag coated TiO_2 nanoparticles was done by green synthesis and the synthesized nanoparticles were characterized by UV-Visible spectroscopy, FTIR, DLS,SEM-EDS. The characterization results reveal the ability of the plant extract to act as reducing and capping agent. The experiment results also proved that the synthesized nanoparticles are active agents for antibacterial activity carried out on *E.coli* and *Bacillus subtilis.* IC50 values for each sample were calculated. The lowest IC50 value against *E.coli* as well as against *B. subtilis* was observed for TiO_2 coated silver nanoparticles at 10% and 15% concentration respectively.

Future Perspectives:

Since the new microbes are very much exposed to the environment there is a dire need to create awareness about the medicinal plants which even being the conventional way still are one of the best way to develop the antimicrobial agents, and will be easily accessible in the poor and developing countries due to their less cost. These medicinal plants can be used to make some other bimetallic nanoparticles like Zn or Se with the extract of *Deparia boryana*, because of easy administration and less toxicity of nanoparticles; they can be proved to be very effective. Also, just like we have determined antibacterial and antihelmintic activity we can also verify the extracts for antifungal, antioxidant, catalase and superoxide mutase activity. Also, we can study the effect of different concentrations of silver in nanoparticles because silver itself being antiseptic can be very effective to kill microbes.

6. References

1. Paramanantham. M, P.; Murugesan. A, M. Green Synthesis Of Silver Nanoparticles From Physalis Angulatta: Synthesis And Antimicrobial Activity. International Journal of Scientific Research 2012, 3, 40-42.]
2. Abdel-Raouf, N.; Al-Enazi, N.; Ibraheem, I. Green Biosynthesis Of Gold Nanoparticles Using Galaxaura Elongata And Characterization Of Their Antibacterial Activity. Arabian Journal of Chemistry 2017, 10, S3029-S3039.
3. Kumar, V.; Yadav, S. Plant-Mediated Synthesis Of Silver And Gold Nanoparticles And Their Applications. Journal of Chemical Technology & Biotechnology 2009, 84, 151-157.
4. Mehmood, A.; Murtaza, G.; Bhatti, T.; Kausar, R. Phyto-Mediated Synthesis Of Silver Nanoparticles From Melia Azedarach L. Leaf Extract: Characterization And Antibacterial Activity. Arabian Journal of Chemistry 2017, 10, S3048-S3
5. EFFECTS AND APPLICATIONS OF SILVER NANOPARTICLES IN DIFFERENT FIELDS Prasanta Kr. Biswas1 and Subhadip Dey2, International Journal of Recent Scientific Research Research Vol. 6, Issue, 8, pp.5880-5883, August, 2015
6. Sharma, V.; Yngard, R.; Lin, Y. Silver Nanoparticles: Green Synthesis And Their Antimicrobial Activities. Advances in Colloid and Interface Science 2009, 145, 83-96.
7. Sahoo, S.; Parveen, S.; Panda, J. The Present And Future Of Nanotechnology In Human Health Care. Nanomedicine: Nanotechnology, Biology and Medicine 2007, 3, 20-31.
8. ANTIBACTERIAL EFFECT OF TiO2 NANOPARTICLES ON PATHOGENIC STRAIN OF E. coli*; International Journal of Advanced Biotechnology and Research* ISSN 0976-2612, Online ISSN 2278–599X, Vol 3, Issue 3, 2012, pp 621-624.
9. Das.B, Dash S.K, Mandal D, Ghosh D, Chattopadhyay S, Tripathy S, Das S, Dey SK, Das D, Roy S, Green synthesized silver nanoparticle destroy multidrug resistant bacteria via reactive oxygen species mediated membrane damage, Arabian journal of chemistry (2015) 1878-5352.]

10. Li W.R, Xie X.B, Shi Q.S, Zeng H.Y, OU-Yang Y.S, Chen Y-B, Antibacterial activity and mechanism of silver nanoparticle on *Escherichia coli*, Appl Microbiology Biotechnology (2010) 85; 1115-1122
11. Miller JH1, Novak JT, Knocke WR, Young K, Hong Y, Vikesland PJ, Hull MS, Pruden A, Effects of silver nanoparticle and antibiotics resistance genes in anaerobic digestion, (2013) 85; 411-21.
12. A taxonomic and ecological analysis of two forest Chlorophytum taxa (Anthericaceae) on Mount Kilimanjaro, Tanzania Charlotte S. Bjorå, *Plant Systematics and Evolution*, Vol. 274, No. 3/4 (September 2008), pp. 243-253.
13. Fern https://www.cs.mcgill.ca/~rwest/wikispeedia/wpcd/wp/f/Fern.htm (accessed Jul 5, 2018
14. Y. Vaghasiya, R. Dave and S. Chanda, 2011. Phytochemical Analysis of Some Medicinal Plants from Western Region of India. *Research Journal of Medicinal Plants, 5: 567-576.*
15. Advances in Applied Microbiology Volume 44, 1997, Pages 215-260.
16. Phytochemicals Role in Good Health http://www.todaysdietitian.com/newarchives/090313p70.shtml (accessed Jun 19, 2018)
17. Phytochemicals | alive https://www.alive.com/food/phytochemicals/ (accessed Jun 19, 2018).
18. [*Green Chemistry by Nano-Catalysis.* V. Polshettiwar, R.S. Varma: *Green Chem.*, **12**, 743-754 (2010).]
19. K. Govindraju, V. Kiruthiga and G. Singaravelu, "Evaluation of Biosynthesized Silver Nanoparticles against Fungal Pathogens of Mulberry Morus Indica," Journal of Biopesticides, Vol. 1, 2008, pp. 101-104.
20. Huang, J.; Li, Q.; Sun, D.; Lu, Y.; Su, Y.; Yang, X.; Wang, H.; Wang, Y.; Shao, W.; He, N. et al. Biosynthesis Of Silver And Gold Nanoparticles By Novel Sundried Cinnamomum Camphora Leaf. Nanotechnology 2007, 18, 105104.
21. Iqbal, P.; Preece, J.; Mendes, P. Nanotechnology: The "Top-Down" And "Bottom-Up" Approaches. Supramolecular Chemistry 2012.

22. Biswas, A.; Bayer, I.; Biris, A.; Wang, T.; Dervishi, E.; Faupel, F. Advances In Top–Down And Bottom–Up Surface Nanofabrication: Techniques, Applications & Future Prospects. Advances in Colloid and Interface Science 2012, 170, 2-27

23. Sci. Tot. Environ., Vol. 400 (1-3), Ju-Nam, Y. and Lead, J.R., Manufactured nanoparticles: An overview of their chemistry, interactions and potential environmental implications, pp396-414, Copyright 2008 with permission from Elsevier
24. Lewinski, N.; Colvin, V.; Drezek, R. Cytotoxicity Of Nanoparticles. Small 2008, 4, 26-49.
25. Nune, S.; Chanda, N.; Shukla, R.; Katti, K.; Kulkarni, R.; Thilakavathy, S.; Mekapothula, S.; Kannan, R.; Katti, K. Green Nanotechnology From Tea: Phytochemicals In Tea As Building Blocks For Production Of Biocompatible Gold Nanoparticles. Journal of Materials Chemistry2009, 19, 2912.]
26. Espín, J.; García-Conesa, M.; Tomás-Barberán, F. Nutraceuticals: Facts And Fiction. Phytochemistry 2007, 68, 2986-3008.
27. Ingale AG, Chaudhari AN (2013) Biogenic Synthesis of Nanoparticles and Potential Applications: An Eco-Friendly Approach. J Nanomed Nanotechol 4:165. doi:10.4172/2157-7439.1000165
28. Syafiuddin, A.; Salmiati; Salim, M.; Beng Hong Kueh, A.; Hadibarata, T.; Nur, H. A Review Of Silver Nanoparticles: Research Trends, Global Consumption, Synthesis, Properties, And Future Challenges. Journal of the Chinese Chemical Society 2017, 64, 732-756.
29. Khan, S.; Qazi, I.; Hashmi, I.; Awan, M.; Zaidi, N. Synthesis of Silver-Doped Titanium TiO2Powder-Coated Surfaces and Its Ability to InactivatePseudomonas aeruginosaandBacillus subtilis (accessed Jun 27, 2018).
30. Sadhan Kumar Chaudhuri, Shivani Chandela and Lalit Malodia. Plant Mediated Green Synthesis of Silver Nanoparticles Using Tecomella undulata Leaf Extract and Their Characterization. Nano Biomed. Eng. 2016 8(1), 1-8.

31. Kumbhar, A.; Chumanov, G. Synthesis And Characterization Of Titania-Coated Silver Nanoparticles. Journal of Nanoscience and Nanotechnology 2004, 4, 299-303.
32. Phytochemical Analysis And Green Synthesis Of Zno And Ag/Zno Nanocomposites From Lansium Parasiticum For The Evaluation Of Their Toxicology. Post Graduate, Thapar Institute of Engineering and Technology, 2017.
33. Ghagane, S.; Puranik, S.; Kumbar, V.; Nerli, R.; Jalalpure, S.; Hiremath, M.; Neelagund, S.; Aladakatti, R. In Vitro Antioxidant And Anticancer Activity Of Leea Indica Leaf Extracts On Human Prostate Cancer Cell Lines. Integrative Medicine Research 2017, 6, 79-87.

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