Phytochemical analysis and Green synthesis of ZnO and Ag/ZnO nanocomposites from *Lansium parasiticum* for the evaluation of their toxicology

A

Dissertation submitted

In partial fulfillment of the requirements for the degree of

Masters of Science

In

Chemistry

Submitted by

Ruhisha
(Regd. No.: 301502031)

Under The Supervision of

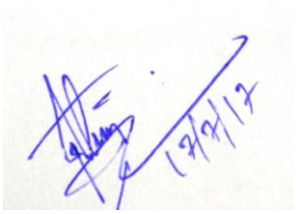
Dr. Diptiman Choudhury
Assistant professor
&

Dr. Satnam Singh
Professor
School of Chemistry and Biochemistry
Thapar University, Patiala- 147004

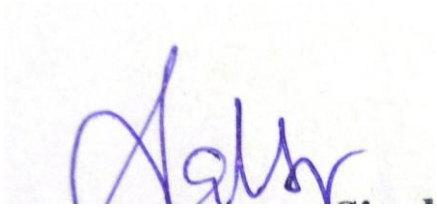
July 2017

Certificate

This is to certify that the thesis entitled "**Phytochemical analysis and Green synthesis of ZnO and Ag/ZnO nanocomposites from *Lansium parasiticum* for the evaluation of their toxicology**" being submitted by **Ms Ruhisha** in partial fulfillment of the requirements for the award of degree of Master of Science in Chemistry to the School of Chemistry and Biochemistry, Thapar University, Patiala, is an authentic record of work carried out by her under our supervision. The contents of this thesis have not been submitted for the award of any other degree or diploma.



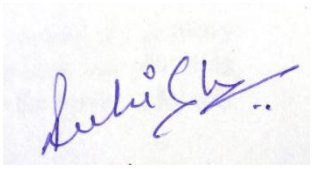
Dr. Diptiman Choudhury
Assistant professor
School of Chemistry and Biochemistry,
Thapar University, Patiala-147004



Dr. Satnam Singh
Professor
School of Chemistry and Biochemistry,
Thapar University, Patiala-147004

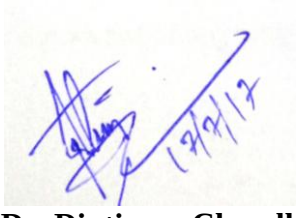
Candidate's Declaration

I hereby declare that the work being presented in the thesis entitled "**Phytochemical analysis and Green synthesis of ZnO and Ag/ZnO nanocomposites from *Lansium parasiticum* for the evaluation of their toxicology**" in partial fulfillment of the requirements for the award of degree of Master of Science in Chemistry, and being submitted to School of Chemistry & Biochemistry, Thapar University, Patiala, is my own work during the period of January 2017 to July 2017, under the supervision of Dr. Diptiman Chaudhury, Assistant Professor and Dr. Satnam Singh, Professor, School of Chemistry and Biochemistry, Thapar University, 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.

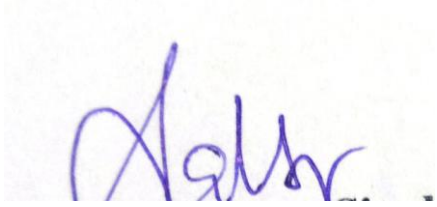


Date:17-07-17 Ruhisha

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



Dr. Diptiman Choudhury
Assistant professor
School of Chemistry and Biochemistry,
Thapar University, Patiala-147004



Dr. Satnam Singh
Professor
School of Chemistry and Biochemistry,
Thapar University, Patiala-147004

Acknowledgement

I would first like to thank my Supervisors, **Dr. Diptiman Choudhury,** Assistant Professor and **Dr. Satnam Singh**, Professor, School of Chemistry and Biochemistry**,** Thapar University, Patiala for their constructive guidance and constant support during the project. The work presented here could not have been accomplished without their selfless interest and willingness to teach. They have taught me to be concise and correct in my approach, from the formulation of ideas to the presentation of results. Their 'out of the box' thinking has always inspired me to try and learn something new every single day during my dissertation.

I would also like to owe my gratitude to **Dr. Amjad Ali,** present Head and **Dr. Bonamali Pal**, Ex. Head, School of Chemistry and Biochemistry**,** Thapar University, Patiala for providing me this opportunity in the form of this dissertation to develop my interest in research.

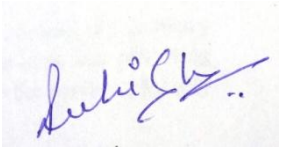
It is my duty to express gratitude to **Dr. Soumen Basu,** Associate Professor, School of Chemistry and Biochemistry**,** Thapar University, Patiala for his support, encouragement and providing required facilities throughout the period of my project and special thanks to the **teaching faculty** of the department for their cooperation and guidance.

I would also like to express my gratitude to **Ms. Geetanjali Pawha**, **Ms. Vanshita Goel and Ms. Pawandeep Kaur** who never turned me down whenever I approached them for their kind help .My heartfelt thanks to the research scholars **Mr. Gulshan Kumar, Mr. Iqbal Singh Brar, Mrs. Ruhi Mehta, Ms. Purnima Sharma, Mr.Rayees Ahmad**, **Mr.Amit Mishra** and **Mr. Aadil Bathla**who always helped me through my way out of confusions.

I am very grateful to **Thapar University** and the **School of Chemistry and Biochemistry** for providing financial support and all necessary infrastructure and laboratory facilities to carry out the experimental work.

I fall short of words to express my thankfulness to my **Parents** and friends- **Nehal Gupta**, **Nitya Chawala**, **Satinder Kaur**, **Bhavya Khurana**, **Dimple Garg**, **Shruti Prashar** and **Pranshu Puri** who have always supported me and have become a source of strength and inspiration to me during my entire period of work.

All the words of gratitude are however, only a fraction of what I owe of the **Almighty** for granting me an opportunity and strength to successfully accomplish this project.



Date:17-07- 17 **Ruhisha**

Abstract:

The increasing trend in the popularity of green synthesis owing to its cost effective and ecofriendly attributes, the unscathed plant *Lansium parasiticum* is evaluated for its phytochemical makeup and the anthelmintic activity of its extracts in various solvents. Methanolic and Aqueous plant extracts proved effective as anthelmintic agents. ZnO nanoparticles were synthesized by direct precipitation method using zinc acetate and sodium carbonate as precursors and biosynthesized using the plant extract. The Ag doped ZnO particles were also synthesized in various concentrations in a green route of synthesis. The synthesized particles were characterized by UV, XRD, SEM and DLS.The X-Ray diffraction studies reveal that both the chemically and biosynthesized ZnO have a crystalline monoclinic structure. The UV-Visible spectra shows the ZnO peak at 284 nm in case of green ZnO and the shift in the absorption band in case of different concentrations of Ag-ZnO nanocomposites. DLS studies show that the average particle size of the biosynthesized ZnO without an external capping agentwas 210 nm and that of the different concentrations of Ag-ZnO nanocomposites ranged from 45-210 nm. After the addition of an external capping agent it was reduced to 19- 30 nm. The as-synthesized ZnO and Ag-ZnO nanocomposites were also tested for their antibacterial activity against the facultative pathogenic bacteria- *E.coli*. Colony counting and broth dilution methods were used. In both the methods 200 µg/ml was found out to be the MIC.

List of Figures

List of Schemes

List of Tables

List of Abbreviations and Symbols

- °C Degree celsius
- % Percent
- gm Gram
- mg Milligram
- µg Microgram
- mmol Milimoles
- M Molarity
- L Litre
- min Minute
- nm Nanometer
- SEM Scanning Electron Microscopy
- FTIR Frontier Infrared
- UV Ultra Violet
- XRD X-ray Diffraction
- DLS Dynamic Light Scattering
- CFU Colony Forming Units
- ZnO Zinc Oxide
- Ag Silver
- w.r.t With respect to

List of Contents

Chapter 1

Introduction

1.1 Phytochemistry

Phytochemistry is the study of various secondary metabolites which are synthesized by plants. The majority of the best plant medicines owe their existence to these constituents. The plant materials show their physiological and therapeutic effects as a result of the combinations of the phytochemicals present in them. These secondary metabolites are the class of compounds which are known to show curative activity against various diseases in human beings, and therefore could explain the traditional use of medicinal plants for the treatment of some illnesses.The present studies on the constituents reveal the uses of the plants but only a small percentage of which have been investigated for their phytochemicals and only a fraction has undergone biological or pharmacological screening. With the new phytochemicals being identified and investigated, the traditional uses of the plants are being verified [1]

Phytochemicals are classified into various categories according to their chemical structures and to some amount on the basis of their function. Listed below are the most commonly known phytochemicals.

1. Alkaloids:These compounds mostly contain the basic nitrogen. Most commonly found alkaloids classified as 'True Alkaloids' are known to have nitrogen in the heterocycle for example Morphine.

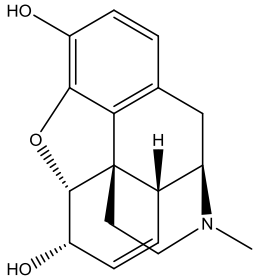


Figure 1.1 : Structure of Morphine (Alkaloid)

2. Terpenoids: These are also known as isoprenoids. Terpenoids basically contain a large no. of isoprene units arranged in a structural backbone to form different compounds. Around 60% of the known natural products are terpenoinds. Terpenoids include various classes of compounds like- saponins, xanthophylls (yellow pigments), limonene, etc.

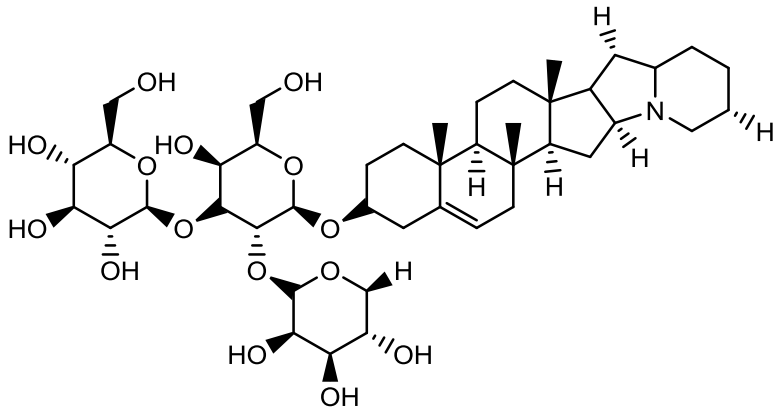


Figure 1.2: Structure of Saponin- Solanin (Terpenoid)

3. Steroids: Steroids are characterized by the presence of four carbon atom rings in their molecular structure. For example dietary lipid- cholesterol, sex harmones- estradiol and testosterone, etc.

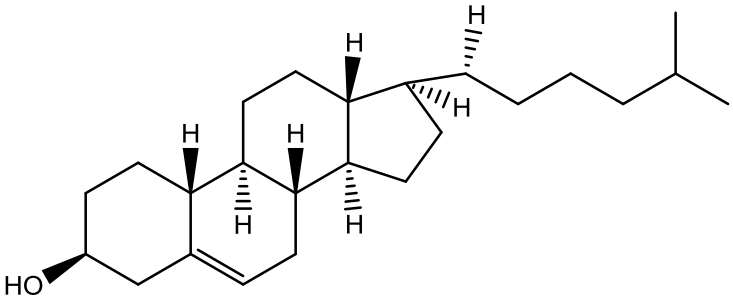


Figure 1.3: Structure of Cholesterol (Steroid)

4. Flavanoids: Flavanoids are the type of polyphenol compounds found in plants. This class of pytochemicals is responsible for the various pigments in plants. Red, blue and purple pigments of the fruits, vegetables, grains, etc. is due to the presence of flavanoids.

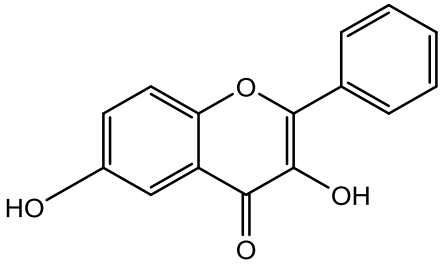


Figure 1.4: Structure of 6-Hydroxyflavone (Flavanoid)

5. Carbohydrates: Carbohydrates are the groups of compounds which cover starch, sugars and fibers. These are commonly termed as 'saccharides' which means sugar. The structure of carbohydrates typically contains carbon, hydrogen and oxygen and is included in all the commonly consumed food items like- bread, fruits, vegetables and sugars.

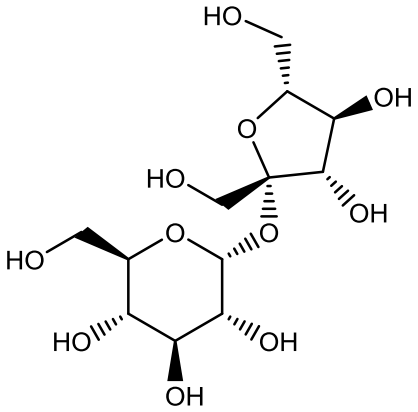


Figure: 1.5 Structure of Sucrose (Carbohydrate)

6. Tannins: Tannins are polyphenolic compounds found in almost all the plants. These are found naturally in bark, stem, wood, roots and leaves of the plants. Tannin is responsible for the astringency, color, and some of the flavor in tea.

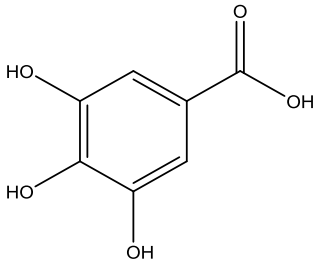


Figure :1.6 Structure of Gallic Acid (Tannin)

1.2 Various activities shown by Phytochemicals

Phtochemicals are known to possess a wide range of heath benefiting activities and also provide protection against many chronic diseases. Some possible effects shown by photochemicals:

- Antioxidant – Almost all the phytochemical have antioxidant property which in turn give protection to cells against oxidative damage and hence reduce the risk of developing certain types of cancer. Phytochemicals showing antioxidant activity are allyl sulfides, carotenoids, flavonoids and polyphenols.
- Hormonal action –Isoflavones are found to imitate human estrogens and help to soothe menopausal symptoms and osteoporosis.
- Stimulation of enzymes - Indoles stimulate enzymes that reduce the effect of estrogen and thus consequently could reduce the risk for breast cancer. Other phytochemicals, which interfere with enzymes are protease inhibitors and terpenes.
- Interference with DNA replication – Saponins have a property to interfere with the replication of cell DNA hence preventing the cancer cells from multiplying. Capsaicin, another phytochemical protects DNA from carcinogens.
- Anti-bacterial effect –Many phytochemicals have anti-bacterial properties.
- Physical action - Some phytochemicals get physically adhered to the human cell walls of which prevents the adhesion of pathogens to the walls of the cells. Proanthocyanidins are such phytochemicals which demonstrate this anti adhesion activity.

1.3 Sustainable Nanotechnology:

Nanotechnology is the study and application of extremely small i.e. nanosize (1-100 nm) compounds that can be used in various fields of science and this word brings great innovations that could be beneficial for our future. Exciting thing about nanotechnology is the extremely small size of the particles (nm). The US National Nanotechnology Initiative (NNI) defines nanotechnology as the ‘Research and technology development in the length scale of approximately 1-100 nm range at the atomic, molecular and macromolecular level, to get a fundamental understanding of materials at the nanoscale and advantage of having small size is used to synthesize and use structures, devices and systems that show novel properties and functions.

The science related to field of nanotechnology has become of the most active areas of research in material sciences. Nanotechnology is a field that makes an impact in all aspects of human life and thus creates a growing sense of knowledge and exploration in the field of life sciences with the regard to biomedical devices and biotechnology [2, 3]. With the recent advances the principle of green chemistry aims to reduce or eliminate substances dangerous and hazardous to human health and the environment in the design, development and implementation of chemical processes and products is becoming more and more important. To comply with the 12 principles of green chemistry, many researches tried to avoid or reduce the uses of hazardous chemicals and solvents, such as using natural materials instead of traditional toxic chemicals [4]. Nanotechnology has opened new theories to fight and prevent disease at atomic scale tailoring of materials. This is indeed a challenging field of research with unlimited future prospects [5,6].. Nanobiotechnology represents the intersection of nanotechnology and biotechnology, which is an emerging field dedicated to creation, improvement, and utility of nanoscale structures. The last two decades has seen tremendous rise in the demand for environmentally sociable technologies for the material synthesis. The nanoparticles (NP's) are of great interest because of their extremely small size and high surface to volume ratio. Synthesis of NP's can be performed in number of physical and chemical methods. However, altogether these methods are energy and capital intensive, and they employ toxic chemicals and non polar solvents and in the synthesis procedure and later on synthetic additives or capping agents, thus precluding their application in clinical and biomedical fields. Therefore, the need for the development of a clean, reliable, biocompatible and ecofriendly process to synthesize NP's lead to turn researchers towards green chemistry and bioprocesses. Recently plant extracts including bark, leaves, flowers and fruits have been used to synthesize the metal NP's. These biogenically synthesized NPs show more compatibility for pharmaceutical and other biomedical applications than those synthesized by chemical and physical procedures. Furthermore, use of plants for the synthesis of NPs does not require high energy, temperature and is cost effective and also used to scale up for large scale synthesis.

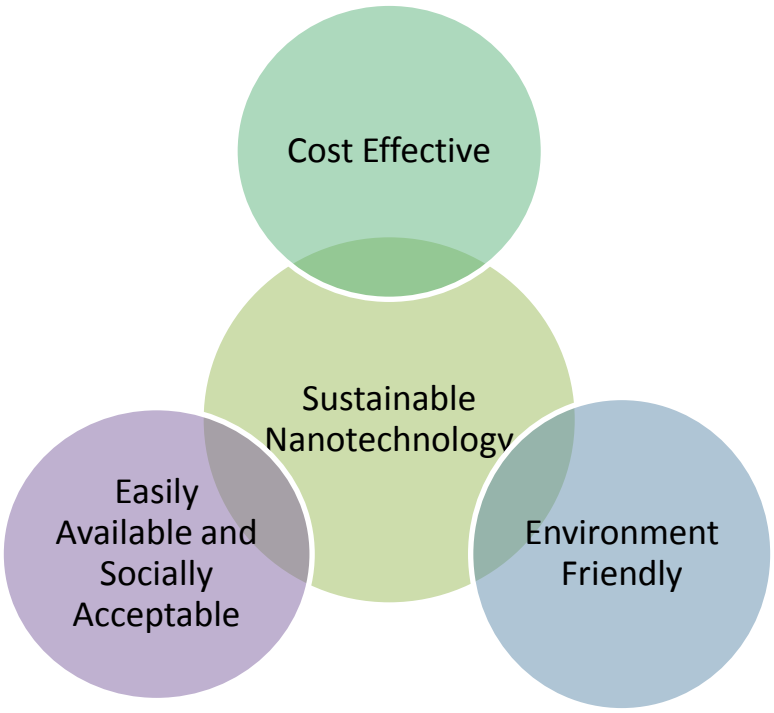


Figure 1.7: Advantages of Sustainable Nanotechnology

1.4 Role of Phytochemicals in nanotechnology:

The use of phytochemicals in the synthesis of NP's is an important bridge between nanotechnology and green chemistry [7-9]. Production of NP's under nontoxic green conditions is of vital importance to curb growing concerns on the overall toxicity of NP's for medical and technological applications [10-12]. The power of phytochemicals, which initiate varieties of chemical transformations within biological systems, is well known. For example, a high level of genistein found in soybeans is both a phytoestrogen and antioxidant [13-16], and has been extensively used to treat conditions affected by estrogen levels in the body. Polyphenolic flavonoid in tea, of which epigallocatechingallate (EGCG) is the major constituent, has anti carcinogenic activity. Many biotechnological applications such as remediation of toxic metals employ microorganisms such as bacteria and yeast for the synthesis of NP's.[17-21].

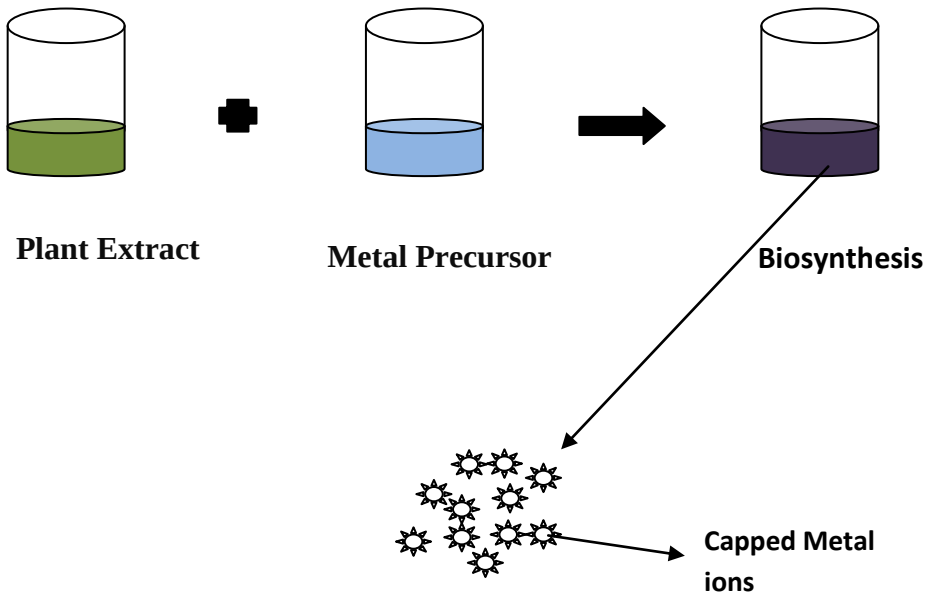
Phytosynthesis is a preferred and more advanced technique than conventional chemical and physical synthesis due to following reasons:

I. In phytosynthesis, both reducing and stabilizing agents are phytochemicals present in plant extract but in conventional synthesis we need a different reducing and stabilizing reagents.

II. Phytosynthesis is a single step reaction but conventional chemical and physical synthesis is multi step reaction.

III. There is no much need of solvents which are expensive and dangerous to discard off. As though generally water is used as an universal solvent but in conventional reactions many dangerous and corrosive solvents are used.

IV. Phytosynthesis occur at ambient temperature and pressure so drastic conditions need not be maintained which are basic requirements in conventional physical synthesis.



(Phytochemicals act as reducing and stabilizing agents)

Figure 1.8: Biosynthesis of Metal Nanoparticles using plant extracts.

Chapter 2

Literature Review

2.1 About the plant:

LATKA (BURMESE GRAPE)

Latka (*Lansium parasiticum*) also known as 'latkan' or 'latko' in West Bengal, is known as Burmese grape worldwide. It is a species of tree belonging to the Mahogany family. It is native to the South East Asian region. The tree is found wild or cultivated in the sub-Himalayan tract in eastern India from Bihar to Arunachal Pradesh and in the lower hills and valleys of Meghalaya, Assam, Nagaland, Manipur, Mizoram, Tripura and Orissa, ascending to an altitude of 900 m, and in Andaman and Nicobar Islands, chiefly in the moist tropical forests [23]. It is mainly cultivated in Cooch Behar and Jalpaiguri districts in West Bengal. It is a slow growing, evergreen, dioecious, short to medium height (20-30 m) plant species. Latka fruit is consumed fresh locally. The time of flowering of this plant is the summer months (March-April) and fruits are available during the rainy season, 3-4 months after flowering. The bearing habit of latka is cauliflory and fruits appear on bunches. Matured latka fruits are round to oval in shape, greenish when tender and turns yellow or yellowish brown in color at ripening. Fruits are sub-acid in taste having 3-4 segments and the edible portion of the fruit is the aril covered by the leathery skin. The number of seeds per fruit varies from 3-4. The average fruit weight, peel weight is 9.0 g and 3.75 g, respectively. Fruit shows around 10°Brix TSS, 4.42% total sugar and 2.1% acidity [24]. Latka fruits contain 5.5% protein, 178 mg vitamin C per 100 g of pulp, and among the minerals the fruit contains 169 mg calcium, 137 mg potassium, 177 mg phosphorous, and 100 mg iron per 100 g of fruit pulp [26]. Average yield varies from 70-80 kg/plant/year. Latka is conventionally propagated by seed. But the dioecious nature of the plant is one of the limiting factors of seed propagation. Attempts had been made to propagate latka plants by means of mature stem cutting [26]. Apart from fresh consumption the fruits are used to make wine, and have been used medicinally to treat skin problems. In Bangladesh, it is cultivated chiefly for production of valuable dye 'annatto' from seeds. Seeds contain 4.8-6% annatto dye. Annatto is used for coloring silk, cotton and other textile materials for orange color.[26] A typical ritual practice of

this locality is throwing of matured, ripe latka fruits for offerings to God Lord Jagannatha during holy RathaYatra. The peels of mature but unripe fruits yield 14.1% pectin and this pectin is useful in preparation of jellies and jams[23].

Burmese grape has been exploited to a very small extent as far as the genotype and its physico-chemical properties are concerned so till now there is very limited literature available regarding this plant

(A)

(B)

Figure 2.1: (A) Picture of Ripened Burmese Grape (*Lansium paraciticum*)

(B) Picture of the peeled fruit

2.2 Zinc Oxide:

Due to the unique physical and chemical properties ZnO have been attracting increasing attention in the modern times. Owing to its exceptional optical and electrical properties ZnO has many technological applications in the production of thin-film transistors, gas sensors, transparent conductors, biomedical and piezoelectric devices [28-30].ZnO is also being used in the synthesis of quantum dot sensitized solar cells as a fabricating agent of the cells [31-32].

Kumbhakar *et.el*, 2005, have investigated that ZnO has striking optical and electrical properties- Bulk ZnO has a wide band gap of 3.37 eV at room temperature and its large excitation bindingenergy of 60 meV is responsible for excitonic transitions which allows it to emit spontaneously with high radiative recombination efficiency and at the same time it acts as laser emission due to its low threshold voltage.

2.3Biosynthesis of ZnO nanoparticles:

An inclusive review on the newest trends in the synthesis and antibacterial activity of nanoparticles was provided by Moritz and Moritz (2013). Various chemical and biological methods of synthesis and mechanisms of antibacterial activity were discussed [33].

A comprehensive review on various antioxidant properties of natural products from medicinalplants was presented by Chanda and Dave (2009). In depth discussions on the various antioxidant assays, list of different plant sources studied were given with respective references [34].

The prospective aspect of 'green chemistry' to synthesize nanoparticles not only in the laboratory scale but also on the large scale was discussed and reviewed by Salam et *al.* (2012). Current and future applications of biosynthesis of nanoparticles and the factors affecting the green synthesis were also discussed [35].

Syed Baker*et et.al.* (2013) reported plants as emerging nanofactories. Factors influencing the nanoparticle synthesis, role of biomolecules, applications and characterization techniques were discussed.

The recent reports on the phytosynthesis of ZnO nanoparticles have been summarized in **Table 2.1**:

S.No.	Plant	**Part used**	**Nanoparticle**	**Size (nm)**	**Studies**	**Reference**
1	*Calotropis gigantean*	leaves	ZnO	30-35	Characterization	37
2	Aloe vera	leaves	ZnO	40	Anti microbial	38
3	Ixora coccinea	leaves	ZnO	8.48-32.51	catalytic	39
4	*Acalypta indica*	leaves	ZnO	100-200	Characterization	40
5	*Corriandrum sativu*	leaves	ZnO	100-200	Characterization	41
6	Seaweeds	whole	ZnO	36	Antibacterial	42

7	*Cassia auriculata*.	flower	ZnO	80-90	Characterization	43

2.4 Metallic Doping:

Various transition metals had been used as a dopent on ZnO. As a result of doping the dopent metal ion interacts with the Zn^{+2} ions with tetrahedral O coordination in the ZnO lattice. As a consequence of which the band gap narrows by sp-d exchange interactions between the conduction band electrons (CB made up of 4s, 4p orbitals of Zn) and the d electrons of the transition metal in consideration [44].

Composites of Co and Cu with ZnO illustrated high visible light sensitivity. Increasing the concentration of Co in the composites increased the light sensitivity. Co doped ZnO illustrated better visible light activity as compared to Ni and Mn doped ZnO catalyst owing to the narrower band gap and better crystalline structure[45].

ZnO show effective antimicrobial activity. Ag when doped on ZnO enchanced the anti microbial activity of the nanoparticles. The relationship of the particle size to the antibacterial activity shows the inverse relationship, this is due to the fact that increase in surface-to-volume ratio of the small particle increases the penetrating ability and the reactivity[46].

2.5 Research Gap:

Very less literature about the plant *Lansium parasiticum* is available as far as the recent technological studies are concerned. This plant can be considered as untouched owing to its use in the chemical synthesis provided this plant has a vast potential for the medicinal as well as physicochemical uses are considered.

2.6 Aims and Objectives:

1. Phytochemical screening of the extracts of *Lansium parasiticum*.
2. Biosynthesis of ZnO nanoparticles and Ag-ZnO nanocomposites.
3. Evaluation of Bacterial toxicity of chemically synthesized and Biosynthesized ZnO.
4. Evaluation of the effect of Ag-ZnO nanocomposites on the growth of *E.coli*.
5. Estimation of the Anthelmintic activity of the plant extracts of *Lansium parasiticum*.

Chapter 3

Materials and Methodology

3.1 Materials:

3.1.1 Apparatus

Beakers (50 ml and 250 ml), round bottom flask, measuring cylinder, soxhlet apparatus, magnetic beads, reagent bottles, micro pipettes, petri plates, tips, falcon tubes, spatulas, crucible, centrifuge tubes, filter paper, test tubes, appendorfs and glass slides.

3.1.2 Reagents and Chemicals used

Zinc acetate, sodium carbonate, silver nitrate, ferric chloride, potassium iodide, acetic anhydride, lead acetate, sodium citrate, sodium hydroxide and sodium chloride were purchased from Sigma Aldrich and Lobachemie and were used without further purification.

Reagents used- Hexane, ethyl acetate, chloroform, methanol, acetic anhydride, hydrochlotic acid, sulphuric acid and nitric acid were purchased from Sigma Aldrich and Lobachemie.

3.1.3 Collection of the plant

The fresh fruits were collected from Assam, India. These were cleaned several times with running water and subsequently with distilled water. Fruits were dried in shade for 8-10 days and then in sunlight for 2 days. The outer covering of the fruit was removed and was separated from the inner part. The outer crust was ground into a coarse powder and stored for the experimental use.

3.1.4 Bacterial culture

The reference bacterial *E.coli* strain MTCC77 was obtained from Biotechnology department, Thapar University, Patiala. The Luria-Bertini and Agar Agar medium used for growing and maintaining the bacterial cultures were obtained from Merck.

3.1.5 Collection of Earthworms

Adult earthworms (*Pheretima posthuma*) were collected form moist soil of Bharpur Garden area of Patiala, Punjab, 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- 250 gm

3.2.3 Hot Air Oven

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

3.2.4 Muffle furnace

A muffle furnace (PREFIT INDIA) is a box type oven for high temperature applications. It is used to calcinate the sample by thermal treatment process for the removal of volatile fraction. The muffle furnace can achieve a maximum temperature of 1000^{o} C.

3.2.5 Soxhlet Apparatus

The soxhlet extractor generally consist of a vertical glass cylindrical extraction tube that has both a siphon tube and a vapor tube, that is fitted at its upper end to a reflux condenser and at its lower end to a flask so that the solvent may be distilled from the flask into the condenser whence it flows back into the cylindrical tube and siphons over into the flask to be distilled again. It is used for the extraction of fatty and other material from a substance using volatile solvents like- ether, alcohol, hexane or benzene.

3.2.6 Laboratory Centrifuge

The laboratory centrifuge works under the principle where the centripetal acceleration will cause denser substances to move outward in the radial direction, the substance which are less dense are displaced and move to the centre. The centrifuge used is THERMO FISHER SCIENTIFIC (SL 8R)

3.2.7 Autoclave

It is an apparatus with special conditions- high or low pressure and temperature that can be established for different applications. It is especially used for sterilizing glass apparatus using steam under high pressure.

3.2.8 Laminar Flow Cabinet

It is a carefully enclosed bench intended to prevent the contamination of biological samples or any sensitive samples. There is a continuous laminar air flow towards the user which is drawn through a HEPA filter.

3.2.9 Incubator

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

3.3 Methodology:

3.3.1 Preparation of plant extract

10 gm of the plant powder was boiled in distilled water for 30 mins. The aqueous extract was then cooled and filtered using Whatmann filter paper no. 1 and stored at 4^{o}C for further use.

25 gm plant powder was extracted successively with 200 ml each of hexane, chloroform, ethyl acetate, methanol and water using Soxhlet apparatus at 55-85 °C for 8-10 h in order to extract the polar and non-polar compounds for the phytochemical analysis.

3.3.2 Preliminary Phytochemical assay of *Lansium parasiticum*

Various plant extracts (hexane, chloroform, ethyl acetate, methanol and aqueous) are tested for the presence of different phytochemicals. Following test are performed for the phytochemical essay.

I. **Test for Alkaloids- -** The extracts were dissolved in dil. HCl separately, filtered and filtrates were tested for the presence of alkaloids.
    - **Mayer's test**- The filtrates were treated with a few drops of Mayer's reagent (2 gm I_2 + 6 gm KI in 100 ml H_2O). Formation of yellow colored precipitates confirmed the presence of alkaloids.
    - **Wagner's test**-The filtrates were treated with a few drops of Wagner's reagent (1.36 gm $HgCl_2$ + 5 gm KI in 100 ml H_2O). Formation of reddish/brown precipitates indicates the presence of alkaloids.

II. **Test for Carbohydrates-** The extracts were dissolved in distilled water, filtered and filtrates were testes for the presence of carbohydrates.

- **Benedict's test-** The filtrate was treated with a few drops of benedict's reagent (10 gm Na_2CO_3 + 17.3 gm $Na_3C_6H_5O_7$+ 1.7 gm CuSO4). Formation of orange red precipitates indicates the presence of carbohydrates.

III. **Test for Flavanoids-**

- **Alkaline reagent test-** Each extract was tested with 2 ml of NaOH. Formation of intense yellow color which disappears on the addition of equal amount of acid indicates the presence of flavanoids.
- **Lead acetate test-** Each extract was treated with a few drops of lead acetate and the formation of yellow precipitate indicates the presence of flavanoids.

IV. **Test for Glycosides-**

- **Modified Borntrager's test-**The extracts were treated with 1-2 ml of $FeCl_3$ and the heated over the water bath for 5 minutes. This solution was allowed to cool and then extracted with equal volumes of benzene. The benzene layer was separated and treated with a few drops ammonia solution. Pink coloration indicates the presence of glycoside.

V. **Test for Saponins-**

- **Froth test-** The extracts were diluted with distilled water in a graduated cylinder up to 10 ml and this was shaken for 5 minutes. Formation od foam layer of at least 1 cm indicates the presence of saponins.
- **Foam test-** 2-3 ml of extract was shaken with water in a test tube for 5 minutes. If the foam persisted for 10 minutes, it indicates the presence of saponins.

VI. **Test for Phenols-**

- **Ferric Chloride test-** The extracts were treated with 1-2 ml of $FeCl_3$ solution. Formation of bluish black precipitates indicates the presence of phenols.

VII. **Test for Tannins-** The extracts were dissolved in 5 ml distilled water for the following tests and filtered.

- **Lead Acetate test-** To 1-2 ml of filtrate a few drops of 1% lead acetate were added. Formation of yellow precipitates infers the presence of tannins.
- **$FeCl_3$ test-** To 1-2 ml filtrates, a few drops of 5% $FeCl_3$ was added. Formation of a green precipitate indicates the presence of tannins.

VIII. **Test for Steroids-** Each extract was dissolved in 5 ml chloroform and filtered. The filtrate was analyzed for the presence of steroids.

- **Salkowaski's Test-** The filtrate was treated with a few drops of sulphuric acid, shaken for a few minutes and allowed to stand undisturbed for some time. Appearance of a golden yellow coloration indicates the presence of steroids.
- **LibermannBurchard's test-** The filtrate was treated with a few drops of acetic anhydride. The solution was boiled and then allowed to cool. To this a few drops of Conc. Sulphuric acid were added. The appearance of a brown ring at the junction indicates the presence of steroids.

IX. **Test for Protiens and Amino Acids-**

- **Xanthoprotic test-** The extracts were treated with a few drops of nitric acid. Appearance of yellow color indicates the presence of proteins.

X. **Test for Oils and Fats-**

- **Filter paper test-** Different extracts were pressed between filter papers. An oil stain on the filter paper indicates the presence of oils and fats.

3.3.3 Chromatographic analysis

TLC is the most powerful method that is employed for the separation, identification and estimation of the various components present in different extracts. Aluminum plates with precoated silica gel were used as stationary phase and the extracts to be analyzed were diluted with the respected solvents and spotting was done 2 cm above the bottom of the plate with a help of a capillary tube.

Solvent system was selected on the basis of the phytochemical components present in each extract. Various solvent systems were tried based on increasing the polarity successively. Finally the best solvent system was selected which showed the maximum separation and maximum number of components.

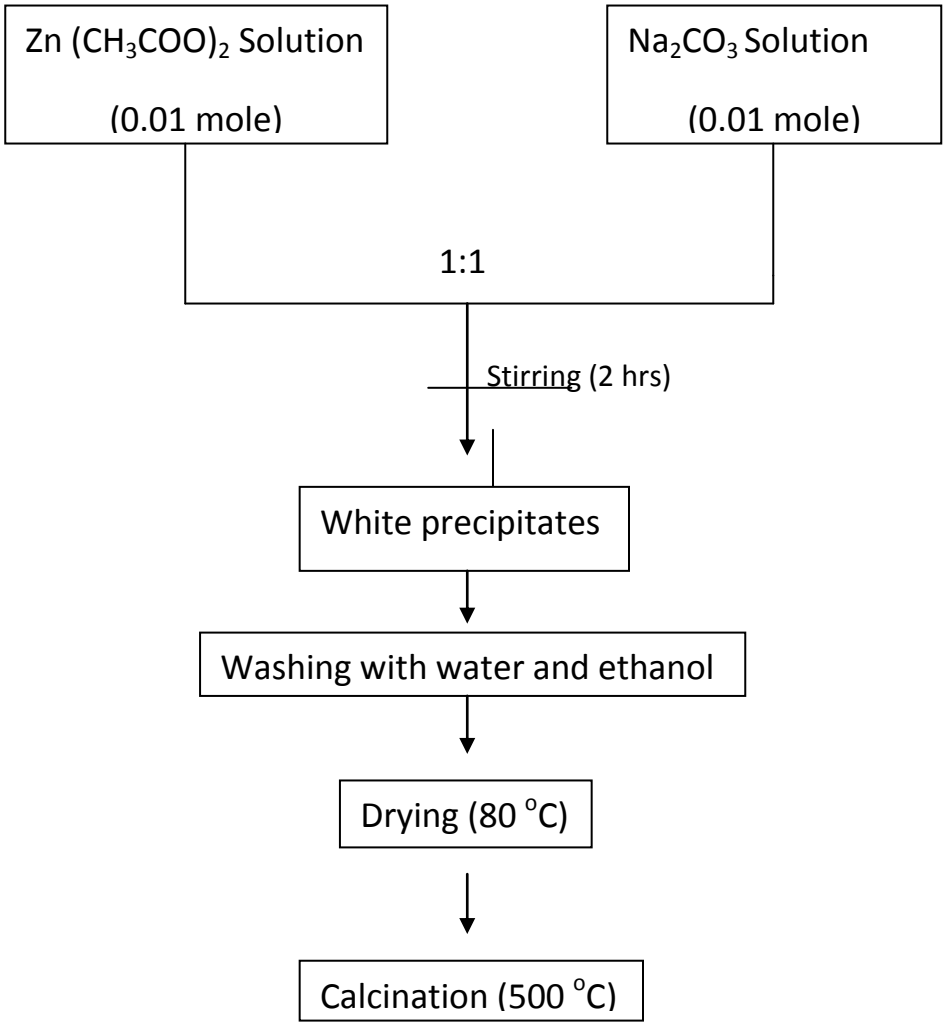
Solvent systems used:

Hexane Extract	Methanol: Chloroform (9:1)
Chloroform Extract	Methanol: Chloroform (1:9)
Ethyl Acetate Extract	Methanol: Ethyl Acetate: Hexane: Acetic Acid (1.5:6:2:0.5)
Methanol Extract	Methanol: Water (8:2)

3.3.2 Preparation of Zinc Oxide

A. Chemical method:

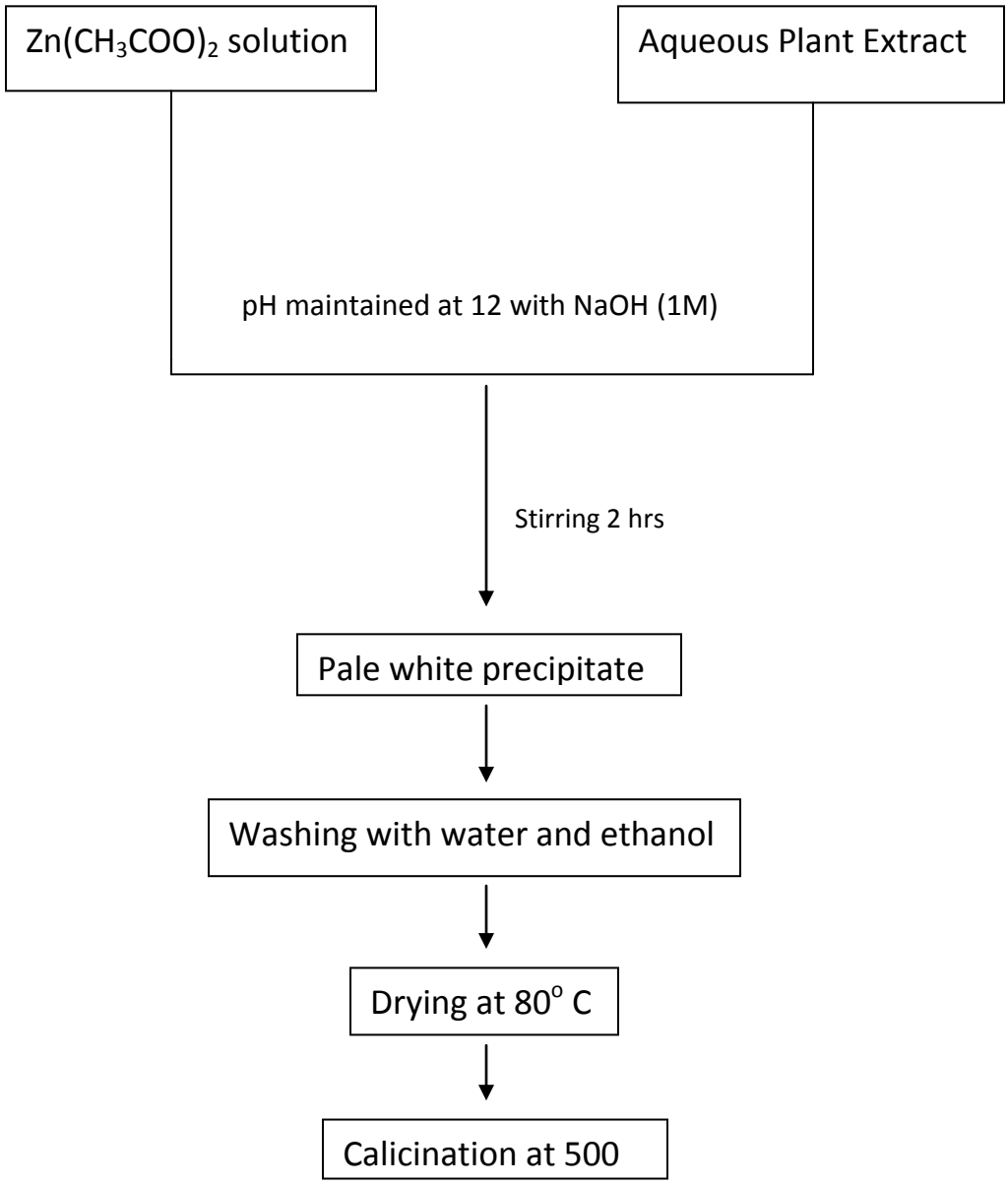
Zinc oxide was chemically synthesized by precipitation method using Zinc acetate (0.01 mole in 200 ml) and sodium carbonate (0.01 mole in 200 ml) as precursors. ZnO NP's were prepared using water as a solvent without heating under constant stirring. The flow chart of precipitation process is briefly described on the Scheme no.1.



Scheme 3.1: Chemical synthesis of ZnO

B. Biosynthesis of ZnO

ZnO was synthesized using zinc acetate (91 mM) as precursor and the pH of the solution was maintained at 12 by adding 1M NaOH solution. In this method plant extract (50 ml) acts as the reducing as well as the stabilizing agent. The flow chart of precipitation process is briefly described on the Scheme no. 2.



Scheme 3.2: Biosynthesis of ZnO

3.3.3 Biosynthesis of Ag-ZnONanocomposites

200 gms of chemically synthesized ZnO powder was added to 4 different test tubes. $AgNO_3$ solution was added in different concentrations in all the test tubes (1%, 2%, 3%, 4% respectively). Plant extract was added in the ratio 1:2 w.r.t the $AgNO_3$and the final volume was made up to 15 ml with distilled water. The samples were kept under constant stirring in a visible light source (400 W tungsten lamp).

The formed precipitates were washed 3-4 times with distilled water and once with ethanol using a centrifuge. The so formed samples were dried in the hot air oven at 80^o C.

Similar experiment was carried out with 1% Ag and 4% Ag for the biosynthesis of Ag-ZnO nanocomposites to study the effect of the addition of PVP on the particle size.

3.4 Characterization:

3.4.1 UV - Visible Spectroscopy

UV visible spectra of the synthesized ZnO and Ag-ZnO nanocomposites and the OD of the growth in bacterial cultures were analyzed by UV-Visible spectrophotometer (Model-Perkin Elmer Lambda 3500) from the range 200- 800 nm.

3.4.2 FT-IR (Fourier Transform Infrared Spectroscopy)

FT-IR was done to further confirm the formation of ZnO and Ag-ZnO nanocomposites. FT-IR was done using Agilent Resolution Pro Carry 600 FT-IT spectrophotometer.

3.4.3 Scanning Electron Microscopy

SEM analysis was carried out for investigating the morphological structure of ZnO synthesized with the plant extract. Powdered ZnO sample was used for the SEM analysis. The micrographs are recorded using Scanning electron microscope (JEOL, JSM-6510 LV).

3.4.4 X- Ray Diffraction studies

The powdered samples were characterized with XRD PAN ANALYTICAL X'PERT PRO operated at 45 kV diffractometer and Cu (Kα) radiation of wavelength 1.54 Å, in the range $2\theta = 10^o - 80^o$.

3.4.5 Dynamic Light Scattering

DLS was used to estimate the hydrodynamic size of the particles synthesized. The samples were prepared by dispersing 0.1 gm as prepared samples in distilled water and sonicating them for 15 minutes respectively.

3.5 Applications:

3.5.1 Antibacterial Activity of ZnO-Green synthesized as well as chemically synthesized ZnO was evaluated for their antibacterial activity against *E. coli*.

A. Broth Dilution Method-

Minimum inhibitory concentration(MIC) was determined by growing *E. coli* in LB media (4 gm in 100 ml distilled water) comprising of different concentrations of ZnO (0, 1, 5, 10, 25, 50, 100, 200, 250, 500, 750, 1000µg/ml.). The bacterial cultures were incubated overnight at 37^{o}C and 300 rpm. The growth of culture was determined by spectrophotometric absorbance which was recorded at 600 nm. The OD was converted to CFU per millimeter [27].

B. Pour Plate Method-

20 ml agar was poured on bacterial culture petri plates containing different concentrations of ZnO (0, 1, 5, 10, 25, 50, 100, 200 µg/ml) and the agar was allowed to solidify.Fresh overnight bacterial cultures were prepared as working culture (10^5 to 10^8 CFU/ml bacterial cells). 10 µl of the test culture was diluted in LB media to make up the final volume as 1 ml. The agar surface of different plates was streaked with a cotton swab with the diluted culture. The plates were incubated at 37^{o}C for 24 hrs. MIC was determined by colony counting.

C. Effect of ZnO and Ag-ZnO nanocomposites on the Growth of *E.coli*

The comparative effect of ZnO and Ag-ZnO nanocomposites were studied on the growth of *E.coli*. In each case around 10^6- 10^8 cells were inoculated in 100 ml LB and incubated it for 2 hrs at 37 ^{o}C with continuous shaking at 200 rpm. After this the bacterial cultures were supplemented with 100µg of ZnO and Ag-ZnO nanocomposites (1%, 2%, 3%, 4% Ag-ZnO) and one microbial culture was kept as control. Spectrophotometric absorbance was recorded at 600 nm after every 1 hour for 6 hrs. The OD values so obtained were converted to CFU [27].

3.5.2 Anthelmintic Activity

The anthelmintic activity was carried out by the method of Ajaiyeoba*et. al*, 2001 [45] with minor modifications. The earthworms were divided into three groups containing five earthworms each. Group 1 was the control, placed in normal saline, Group 2 and Group 3 were treated with methanol and aqueous plant extracts respectively with 15 mg/ml extract each. To make these doses, the extracts were measured respectively and dissolved in normal saline.

Observations were made for the time taken for paralysis (Paralysiswas said to occur when worm did not revive in normal saline) andthe time for death of worms was recorded after assuring that worms neither moved when shaken vigorously nor when dipped in warm water (50 ºC), followed with their body colorsfading away.

As the earthworm resembles anatomically and physiologically to the human intestinal round worm parasite, hence the activity is performed on the earthworm *Pheretima posthuma.*[46, 47]

Chapter 4

Result and Discussions

4.1 Preliminary Phytochemical Analysis:

4.1.1 Extraction

Table depicts the physical nature of different extracts formed by the successive extraction of the fruit- coat powder of *Lansium parasiticum*.

Table4.1: The Physical Nature of Different Extract of *Lansium parasiticum*.

S.No.	Extracts		Color	Physical Nature
1	Hexane	Successive solvent extraction in soxhlet apparatus	Yellowish	Sticky Mass
2	Chloroform		Brownish	Sticky Mass
3	Ethyl Acetate		Brown	Oily Liquid
4	Methanol		Dark Brown	Sticky Mass
5	Aqueous		Brown	Oily Liquid

4.1.2 Qualitative Phytochemical Screening

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

NOTE :‘+’ indicates positive result and ‘-’ indicates negative result.

Table 4.2: Phytochemical essay of Lansium paraciticum						
S. No.	**Test Performed**	**Hexane Extract**	**Chloroform Extract**	**Ethyl Acetate Extract**	**Methanol Extract**	**Aqueous Extract**
1	**Alkaloids**					
Mayer's Test		-	+	+	+	+
Wagner's Test		-	+	+	+	+
2.	**Carbohydrates**					
Benedict's Test		-	+	-	+	+
3.	**Flavanoids**					
Alkaline R. Test		-	+	-	+	-
Lead Acetate Test		-	+	-	+	-
4.	**Glycosides**					
Borntrager's Test		-	-	-	-	-
5.	**Saponins**					
Froth Test		-	-	-	+	+
Foam Test		-	-	-	+	+
6.	**Phenols**					
$FeCl_3$ Test		-	-	-	-	-
7.	**Tannins**					
Lead Ac. Test		-	-	-	-	-
$FeCl_3$ Test		-	-	-	-	-
8.	**Steroids**					
Salkowaski Test		+	+	+	-	+
Libermann's Test		+	+	+	-	+
9.	**Protiens**					
Xanthoprotic Test		-	-	-	-	+
10.	**Oils and Fats**					
Filter Paper Test		-	-	-	+	+

Different extracts showed the presence of phytochemicals like- alkaloids, carbohydrates, flavanoids, saponins, steriods and oils and fats.

4.1.3 Chromatographic Analysis

TLC studies of hexane, chloroform, ethyl acetate and methanol extracts are shown in table 4.3

Table 4.3 :TLC studies of various extracts of *Lansium paraciticum*

S.No.	Extract	Solvent System	No. of spots	Rf Value
1	Hexane	Chloroform:Methanol (1:9)	3	0.20, 0.44, 0.50
2	Chloroform	Chloroform:Methanol (9:1)	4	0.13, 0.35, 0.48, 0.92
3	Ethyl Acetate	Methanol:EthylAcetate:Hexane:Acetic Acid (1.5:6:2:0.5)	2	0.29, 0.71
4	Methanol	Methanol:Water (8:2)	2	0.20, 0.76

4.2 UV-Visible Spectroscopy:

The reduction of Zinc acetate to Zinc oxide is analyzed by UV-Visible spectrophotometer. In case ZnO synthesized in green way (Fig. 4.1) the band is observed around 280- 350 nm. This is the 'Surface Plasmon Resonance' band and this band is ascribed to the valence electrons of ZnO arranged in nanoparticles.

As compared to ZnO, Ag-ZnO composites exhibited a red shift in the absorption band and an enhancement in the weak excitation peak exhibiting a broad absorption peak in the range 350-500 nm (Fig. 4.2), which is a characteristic of Ag nanoparticles. As the concentration of Ag is increased, the peak broadening is observed, this is due to the increase in crystalline size.

The red shift in Ag-ZnO nanocomposites may be observed due to the strong interfacial coupling between Ag and ZnO.

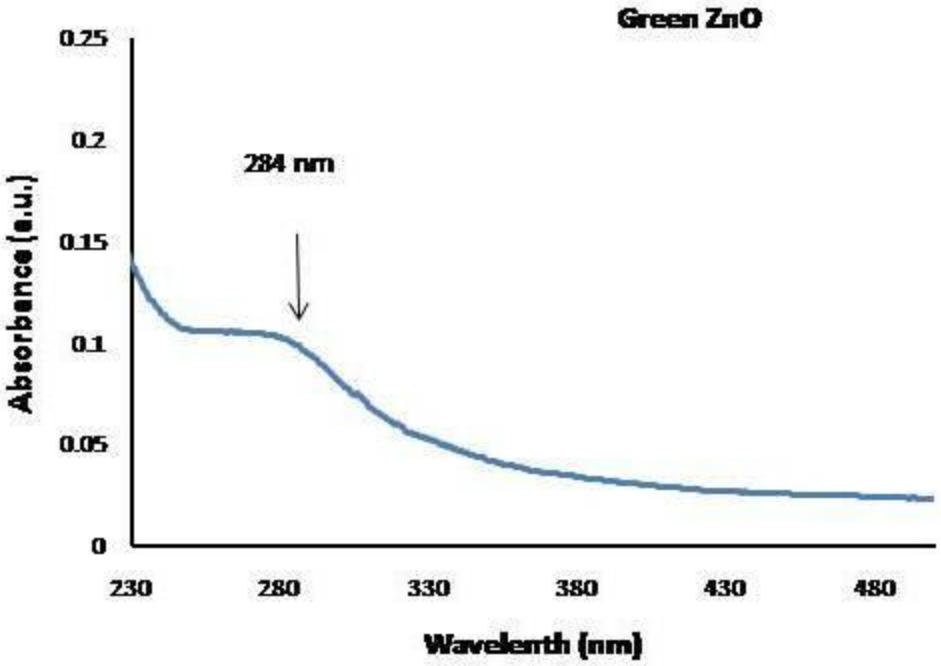


Figure 4.1: UV-Visible absorption spectra of Biosynthesized ZnO

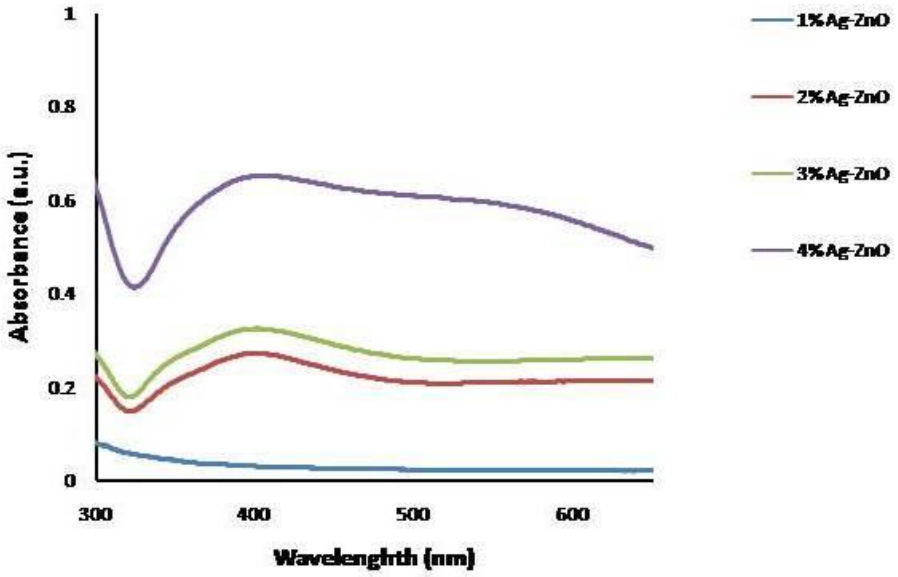


Figure 4.2: UV-Visible absorption spectra of Biosynthesized Ag-ZnO nanocomposites

4.3 FT-IR Spectroscopy:

The FTIR spectrum of biosynthesized ZnO and Ag-ZnO nanocomposites is shown in Fig. 4.3. Appearance of a sharp band at 495.26 cm^{-1} in the FTIR spectrum is the indication of the formation of ZnO which arises from the Zn-O stretching [31]. Other absorption peaks obtained in the spectrum of biosynthesized ZnO are : 3376.19 cm^{-1} for N-H stretching,2985.95 cm^{-1} for C-H stretching, 2261.68 cm^{-1} for $C\equiv N$ stretching, 2002.17 cm^{-1} for $C\equiv C$, (1577-1495 cm^{-1}) N-O stretching, 1397.70 cm^{-1} for C-H bending and 1017.78 cm^{-1} for C-N bending. These additional peaks can be attributed to the phytochemicals present in the plant extract used in the synthesis.

In case of the FTIR spectrum of the nanocomposites, the Zn-O stretching peak is observed at 495.10 cm^{-1}. An additional peak appears in all the concentrations of the nanocomposites i.e at 910 cm^{-1}, it can be related to the van der Walls force of interaction between Ag and C-C bonds [32].

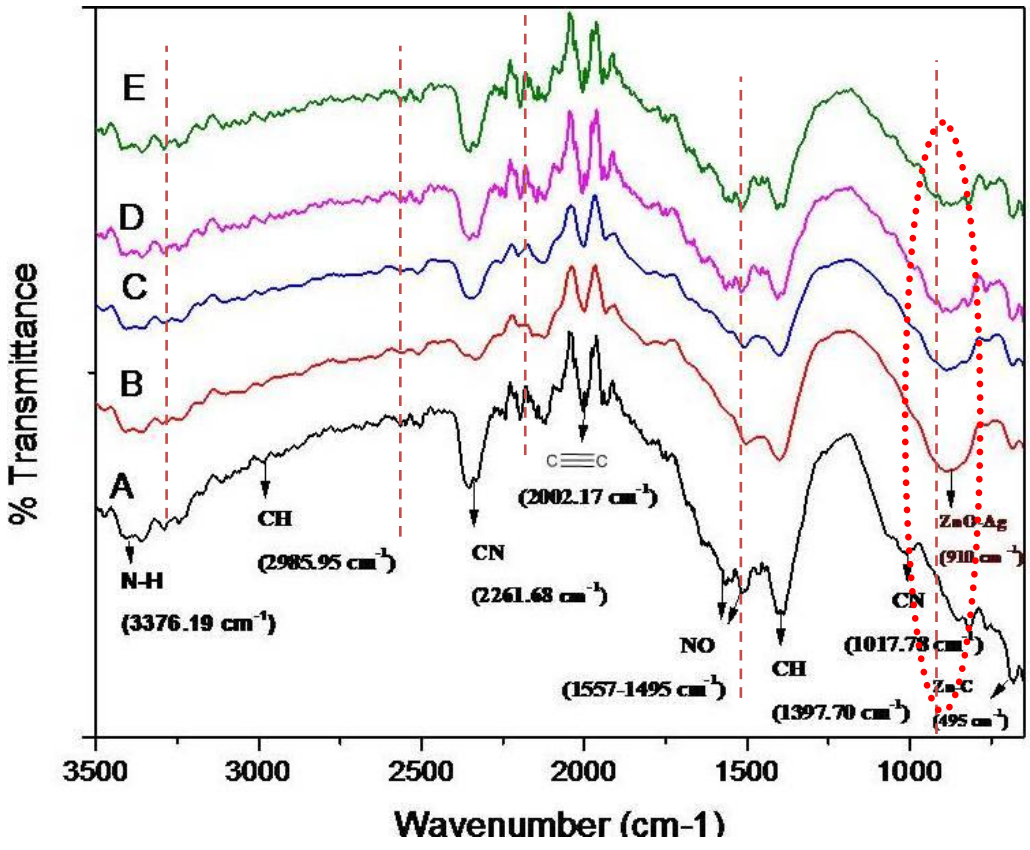


Figure 4.3: FTIR spectrum of (A)- Biosynthesized ZnO, (B)-1% Ag-ZnO, (C)-2% Ag-ZnO, (D)- 3% Ag-ZnO and (E)- 4% Ag-ZnO

4.4 Scanning Electron Microscopy (SEM):

The chemically synthesized ZnO particles were subjected to SEM to get a profound insight of the shape and size of the compound formed. The as formed ZnO with precipitation method was found to have a size from nano to micron range. The size of the particles so formed was more than the nano range i.e 1-100 nm; this is because no capping agent was added in the synthesis which resulted in the agglomeration and bigger size of the particles formed.

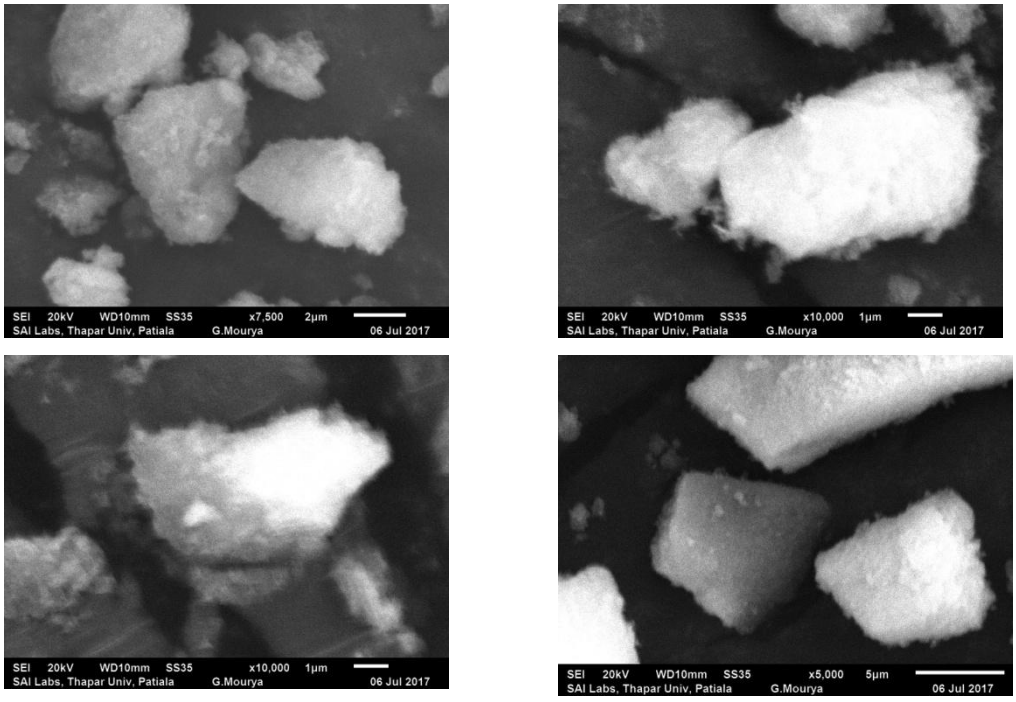


Figure 4.4: SEM images of chemically synthesized ZnO

4.5 X-Ray Diffraction studies (XRD):

X-ray diffraction is used for the identification and quantitative elucidation of the crystalline forms known as 'phases' present in the different powdered samples synthesized. Various calculations are made on the basis of Bragg's Law. Bragg's law is represented by the following relationship:

$$n\lambda = 2d \sin\theta$$

where,

n =0,1,2,3……

λ = wavelength of the X-ray

d = interplaner distance

θ = diffraction angle

Figure 4.5shows XRD pattern of the ZnO synthesized by precipitation method using zinc acetate dehydrate and sodium carbonate as precursors. The XRD pattern shows orientation and crystalline structure of ZnO. The peak positions with 2θ values of 32.212°,34.592°, 36.611°, 47.789°,56.747°,63.146°,66.405°,68.264°,69.104° and 77.222° are indexed as (100), (002), (101), (102),(110),(103), (002),(112),(201) and (202) and planes which are in good agreement with those of the powdered ZnO obtained from the International Centre of Diffraction data card (JCPDS-36-1451) confirming the crystalline monoclinic structure of ZnO.

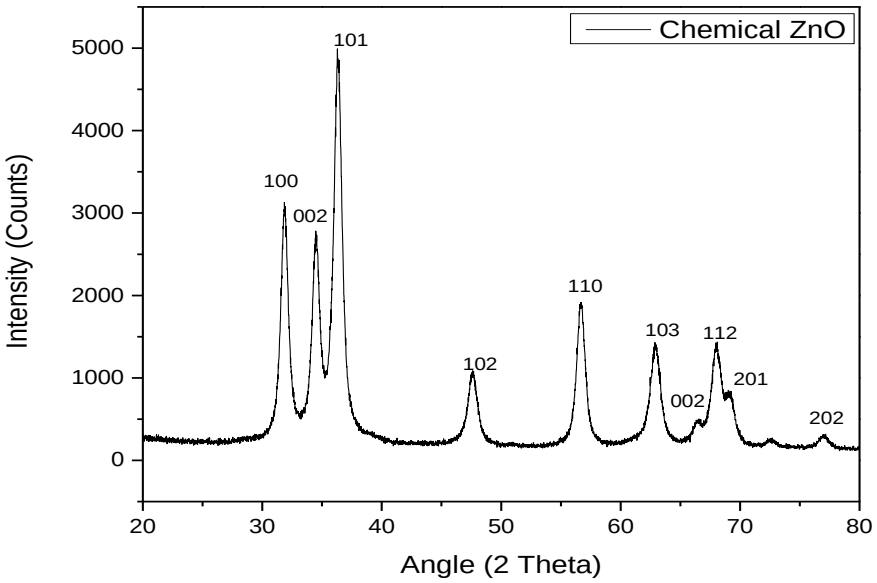


Figure 4.5: XRD pattern of ZnO synthesized by Chemical Method

Figure 4.6shows XRD pattern of the biosynthesized ZnO by using zinc acetate and aqueous plant extract as precursors. The XRD pattern shows orientation and crystalline structure of ZnO. The peak positions with 2θ values of 31.712°,34.352°, 36.471°, 47.749°,56.547°,62.906°,66.425°,68.004°,69.064° and 77.002° are indexed as (100), (002), (101), (102),(110),(103), (002),(112),(201) and (202) and planes which are in good agreement with those of the powdered ZnO obtained from the International Centre of Diffraction data card (JCPDS-36-1451) confirming the crystalline monoclinic structure of ZnO.

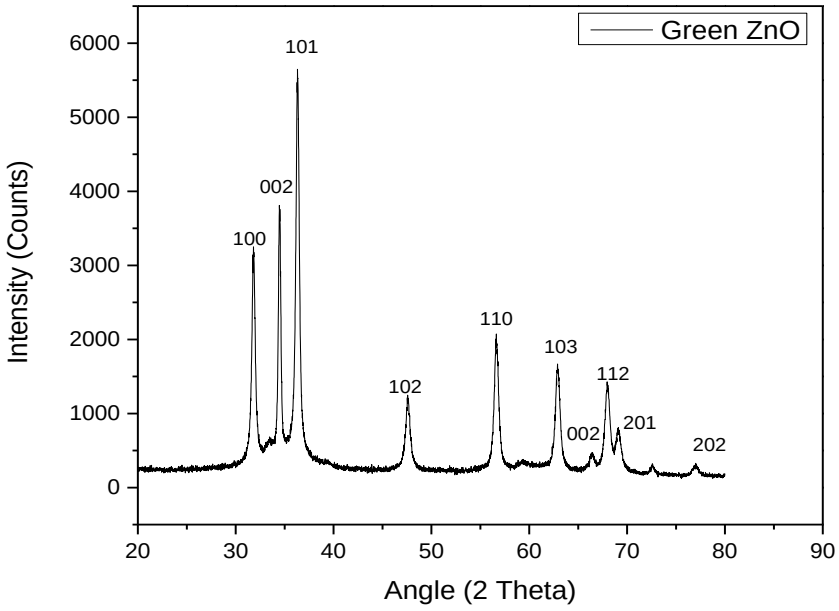


Figure 4.6: XRD pattern of Biosynthesized ZnO

Figure 4.7 shows the XRD patterns of 1% Ag-ZnO and 4% Ag-ZnO in comparison to the Biosynthesized ZnO. The XRD pattern shows some additional peaks corresponding to Ag and hence confirms the doping of Ag in the crystalline structure of ZnO.

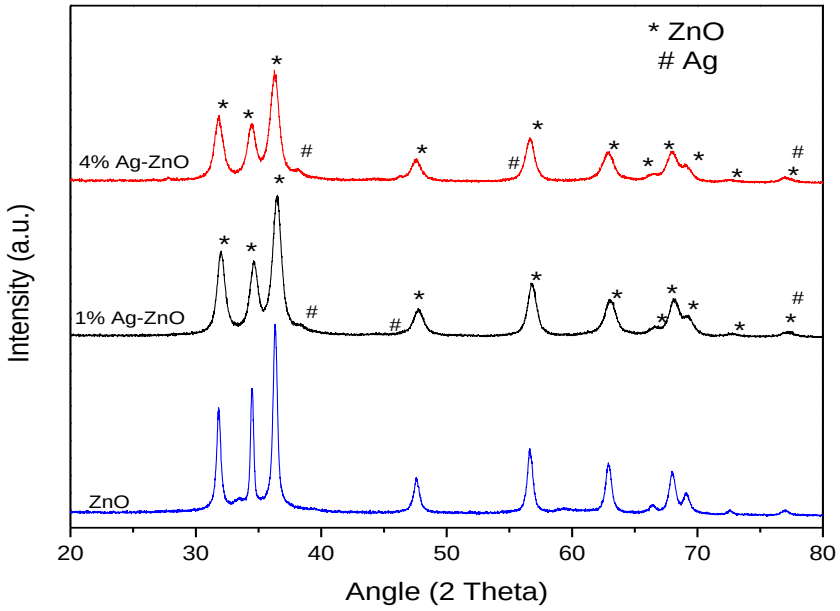


Figure 4.7: XRD pattern of Ag-ZnO nanocomposites and Biosynthesized ZnO

4.6 Dynamic Light Scattering:

DLS is a technique to commonly used to determine the particle size. Figure 16 shows the DLS data of the ZnO synthesized by chemical precipitation method (A) and biosynthesized ZnO (B). Maximum particles in chemical ZnO were in the range 200.53- 226.56 nm, from which it can be concluded that the particle so formed are micro- sized.

In ZnO biosynthesized with the help of the plant extract, particles were in the range 147.79-195.35 nm. The decrease in the particle size of the biosynthesized ZnO points towards the ability of the phtochemicals present in the aqueous plant extract to act as reducing and capping agents.

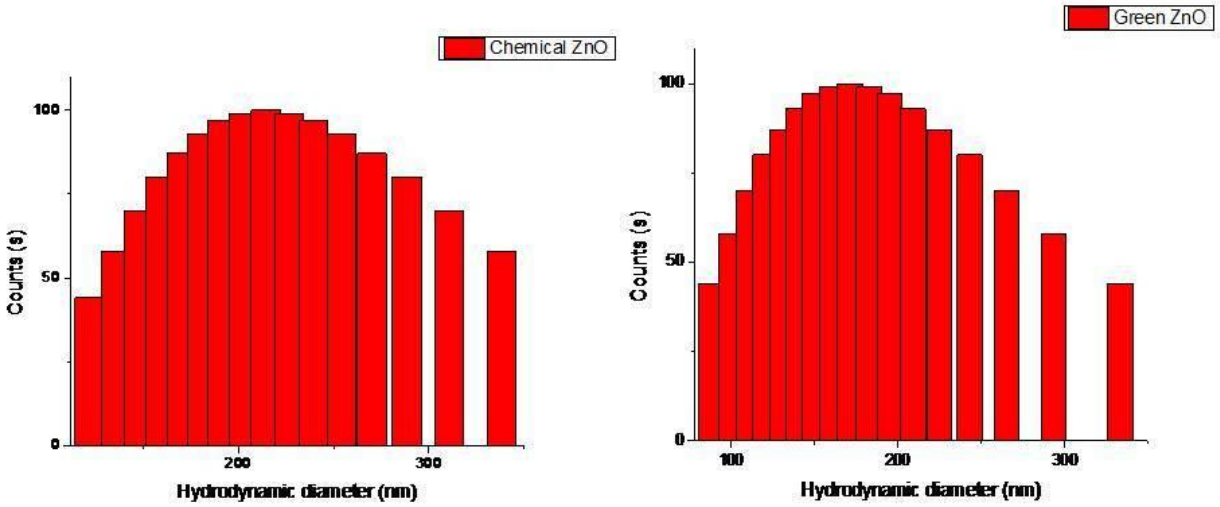


Figure 4.8: Particle size distribution of ZnO particles.

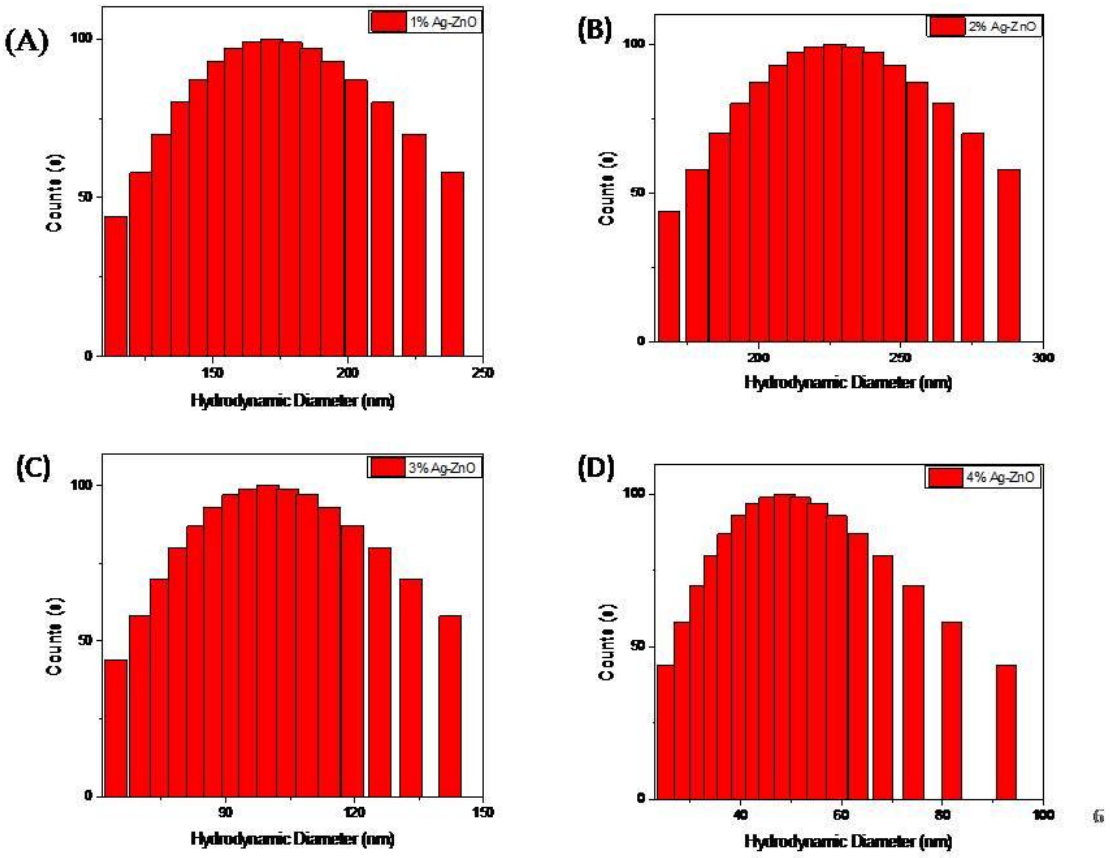
(A)- Chemical ZnO, (B)- Biosynthesized ZnO

Figure 17 illustrates the particle size distribution of the Ag-ZnO nanocomposites (1%, 2%, 3% and 4% Ag). It is observed that the particle size decreases as the percentage of Ag and consequently the quantity of plant extract added increases. The particles ranged from 45-210 nm in various concentrations. This reconfirms the reducing and capping agent capability of the plant extract.

- **Effect of PVP on the size of the nanocomposites:**

  DLS studies of the samples biosynthesized along with the addition of PVP show that the size of the samples reduced considerably 19.38 nm for 1% Ag-ZnO and

26.36 nm for 4% Ag-ZnO. This shows that the external addition of a capping agent enhances the capping ability of the phytochemical for the synthesis of the



efficient nano sized particle formation.

Figure 4.9: Particle size distribution of Ag-ZnO nanocomposites.

(A)- 1% Ag-ZnO, (B)- 2% Ag-ZnO, (C)- 3% Ag-ZnO and (D)- 4% Ag-ZnO

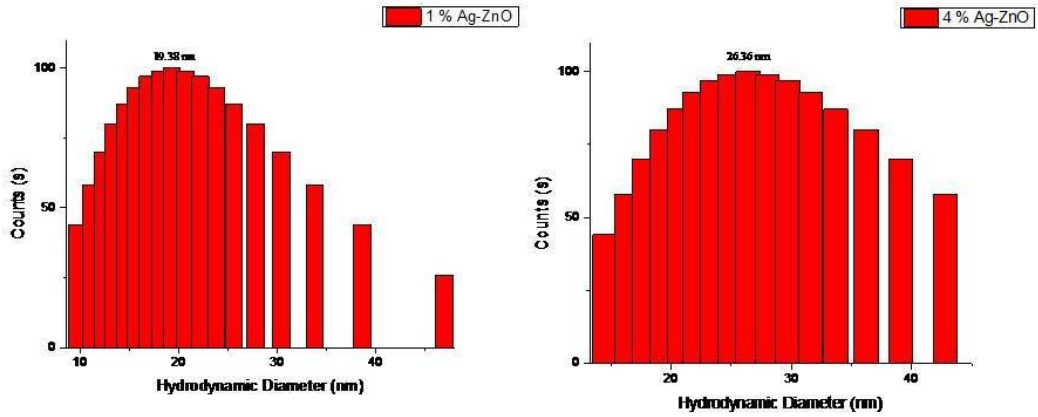


Figure 4.10 Effect of PVP on the particle size of Ag-ZnO nanocomposites

4.7 Antibacterial Activity:

A. Broth dilution Method

This method was used to calculate the MIC of chemically and biosynthesized ZnO against *E. Coli*. The test tubes with 100 and 200 µg/ml concentrations showed less visual turbidity which is an indication of a reduced amount of bacterial growth. This was further confirmed by analyzing the samples with UV-Visible spectrometer.

The spectrophotometry confirmed minimum growth in the samples containing 200 µg/ml ZnO.

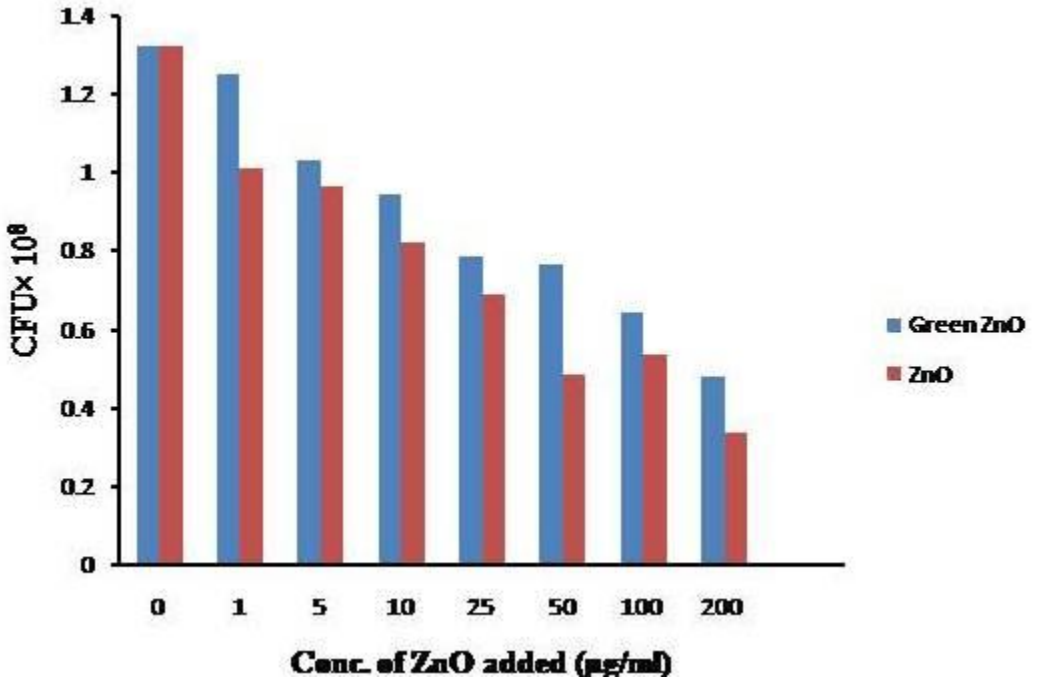


Figure 4.11: Effect of various concentrations of ZnO on the growth of *E. Coli*

B. Pour Plate Method:

After 24 hrs of incubation the plates were analyzed for the bacterial colonies. The number of isolated colonies formed in each plate were counted and noted and the anti bacterial effect was determined in reference to the colonies formed in the control plate.

The MIC was considered for a plate showing negligible or very less bacterial growth.

In this experiment, the plate supplemented with 200 µg/ml ZnO showed no bacterial colonies and was taken as MIC.

Table 4.4: Effect of Biosynthesized ZnO on *E.coli* by pour plate method		
S.No.	**Concentration of ZnO added (µg/ml)**	**No. of colonies**
1	-	750+
2	1	11
3	5	7
4	10	7
5	25	5
6	50	3
7	100	1
8	200	0

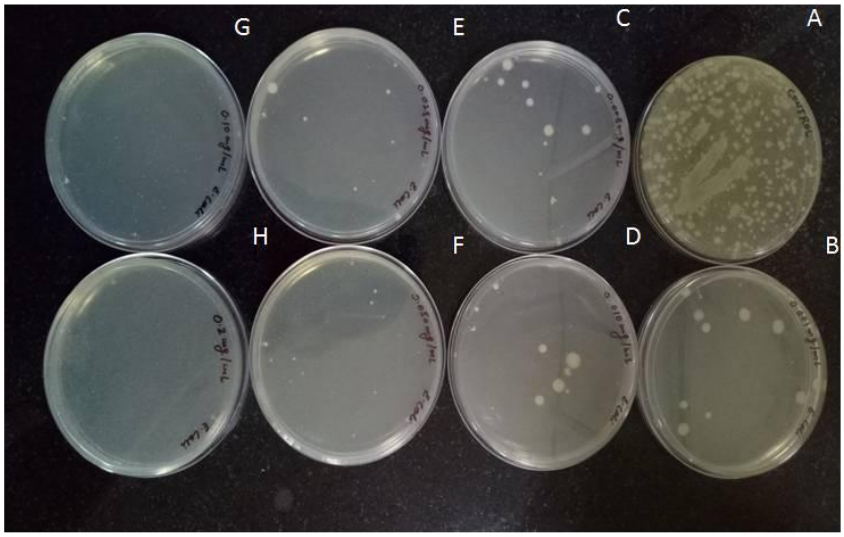


Figure 4.12: Pour plate method of Antibacterial activity of ZnO

A- Control, B- 1µg/ml, C- 5 µg/ml, D- 10 µg/ml, E-25 µg/ml, F-50 µg/ml, G-100 µg/ml, H-200 µg/ml ZnO.

C. Effect of Ag-ZnO nanocomposites on the Growth of *E.coli*

The bacterial growth kinetics was monitored with the 100 µg/ml dosage of ZnO and Ag-ZnO nanocomposites. Unlike control, the bacterial cultures supplemented with nanoparticles showed prolonged lag phase. Hence the exponential phase was greatly reduced to almost negligible.

The increased effect was observed in the nanocomposites with the higher concentration of Ag. Maximum effect was observed in 4% Ag-ZnO followed by 3% Ag-ZnO, 2% Ag-ZnO and 1% Ag-ZnO. The chemically and biosynthesized ZnO showed somewhat similar effect on the growth of *E.coli*.

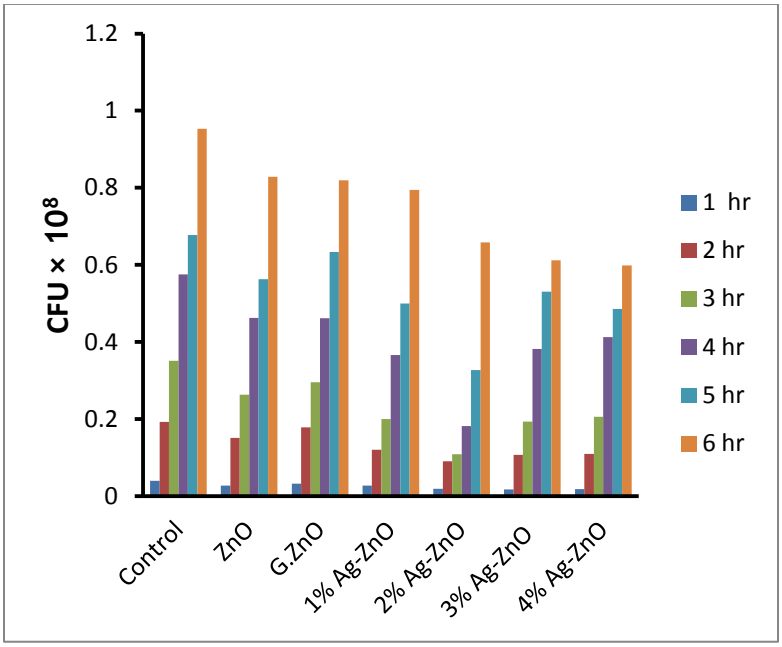


Figure 4.13: Graphical representation of the effect of 100 µg/ml ZnO and Ag-ZnO nanocomposites on Growth of *E.coli*

4.8 Anthelmintic Activity:

In the anthelmintic activity the plant extracts of *Lansium parasiticum* exhibited paralysis as well as death of the worms. As shown in Table-2, the aqueous extract depicted shortest time of paralysis and death with 15 mg/ml concentration followed by the methanolic extract. The

evaluation of the anthelmintic activity was compared with reference to the worms in normal saline. The worms in normal saline were alive even after 24 hrs from the time of experiment.

It can be concluded that the active constituents responsible for the anthelmintic activity are present in both the extracts.

Table 4.5: Anthelmintic activity of Aqueous and Methanol extract of *Lansium parasiticum*

S. No.	Test Extract	Concentration (mg/ml)	Time taken for Paralysis (min)	Time taken for Death (min)
1.	Control	-	-	-
2.	Aqueous	15	30 ± 5	43 ± 5
3.	Methanol	15	115 ± 5	135 ± 5

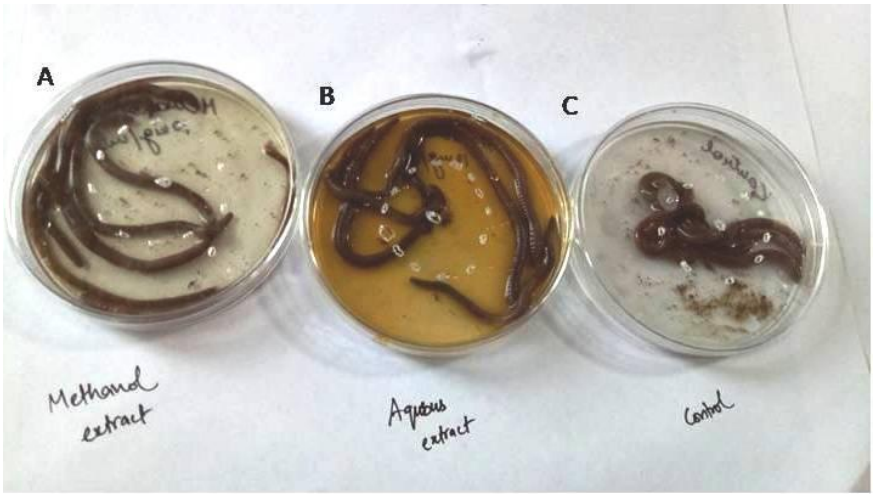


Figure 4.14: Anthelmintic Activity of Plant Extracts of *Lansium paraciticum*(Zero min)

Figure 4.15: Anthelmintic Activity of Plant Extracts of *Lansium paraciticum* (After 120 mins)

Chapter 5

Conclusions

Lansium parasiticum was partially elucidated for the phytochemicals present in its fruit peel. Various extracts showed the presence of different phytochemicals like- alkaloids, saponins, carbohydrates, oils and fats, etc.Due to the presence of theses active components aqueous and methanol plant extracts illustrated excellent anthelmintic activity carried out on earthworms.

ZnO and Ag-ZnO nanocompositeswere synthesized by the green route using aqueous extract of Lansium parasiticum. The characterization of various samples synthesized was done by the techniques such as XRD, UV, FTIR, SEM and DLS. The characterization results reveal the ability of the plant extract to act as reducing as well as a capping agent in the synthesis of metallic nanoparticles. This ability was noticed to be enhanced by adding an external capping agent like PVP.

The experiment results indicate that the ZnO and Ag-ZnO nanoparticles are active agents for the antibacterial activity carried out on E.coli.

Future perspectives:

- To estimate and characterize the total phenolic and alkaloid content of *Lansium parasiticum*.
- To study the effect of different external capping agents on the particle size in the green synthesis of ZnO.
- To study the variation of particle size with the external capping agent concentration in the green route of synthesis.
- To synthesize other metal nanoparticles like Ag, TiO_2 etc. with the extract of *Lansium paraciticum*.
- Evaluation of anti fungal activity of the plant extract of *Lansium parasiticum*.

REFERENCES

1. Arnason, J. T.; Mata, R.; Romeo, J. T., *Phytochemistry of medicinal plants*. Springer Science & Business Media: 2013; Vol. 29.
2. Poliakoff, M.; Anastas, P., A principled stance. *Nature* **2001,** *413*, 257.
3. Gross, R. A.; Kalra, B., Biodegradable polymers for the environment. *Science* **2002,** *297*, 803-807.
4. Govindraju, K.; Kiruthiga, V.; Singaravelu, G., Evaluation of biosynthesized silver nanoparticles against fungal pathogens of mulberry Morus indica. *Journal of Biopesticides* **2008,** *1* , 101-104.
5. Govindaraju, K.; Kiruthiga, V.; Kumar, V. G.; Singaravelu, G., Extracellular synthesis of silver nanoparticles by a marine alga, Sargassum wightii Grevilli and their antibacterial effects. *Journal of Nanoscience and Nanotechnology* **2009,** *9*, 5497-5501.
6. Huang, J.; Li, Q.; Sun, D.; Lu, Y.; Su, Y.; Yang, X.; Wang, H.; Wang, Y.; Shao, W.; He, N., Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum camphora leaf. *Nanotechnology* **2007,** *18*, 105104.
7. Gardea-Torresdey, J. L.; Peralta-Videa, J. R.; De La Rosa, G.; Parsons, J., Phytoremediation of heavy metals and study of the metal coordination by X-ray absorption spectroscopy. *Coordination chemistry reviews* **2005,** *249*, 1797-1810.
8. Gardea-Torresdey, J.; Tiemann, K.; Parsons, J.; Gamez, G.; Herrera, I.; Jose-Yacaman, M., XAS investigations into the mechanism (s) of Au (III) binding and reduction by alfalfa biomass. *Microchemical journal* **2002,** *71*, 193-204.
9. Hardman, R., A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. *Environmental health perspectives* **2006,** *114*, 165.
10. Curtis, J.; Greenberg, M.; Kester, J.; Phillips, S.; Krieger, G., Nanotechnology and nanotoxicology. *Toxicological reviews* **2006,** *25*, 245-260.
11. Lewinski, N.; Colvin, V.; Drezek, R., Cytotoxicity of nanoparticles. *small* **2008,** *4*, 26-49.
12. Espín, J. C.; García-Conesa, M. T.; Tomás-Barberán, F. A., Nutraceuticals: facts and fiction. *Phytochemistry* **2007,** *68*, 2986-3008.
13. Rochfort, S.; Panozzo, J., Phytochemicals for health, the role of pulses. *Journal of agricultural and food chemistry* **2007,** *55*, 7981-7994.
14. Setchell, K. D.; Brown, N. M.; Desai, P.; Zimmer-Nechemias, L.; Wolfe, B. E.; Brashear, W. T.; Kirschner, A. S.; Cassidy, A.; Heubi, J. E., Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. *The Journal of nutrition* **2001,** *131*, 1362S-1375S.
15. Magee, P. J.; Rowland, I. R., Phyto-oestrogens, their mechanism of action: current evidence for a role in breast and prostate cancer. *British Journal of Nutrition* **2004,** *91*, 513-531.
16. Limer, J. L.; Speirs, V., Phyto-oestrogens and breast cancer chemoprevention. *Breast Cancer Research* **2004,** *6*, 119.
17. Bandele, O. J.; Osheroff, N., (–)-Epigallocatechin gallate, a major constituent of green tea, poisons human type II topoisomerases. *Chemical research in toxicology* **2008,** *21*, 936-943.
18. Shankar, S.; Ganapathy, S.; Srivastava, R. K., Green tea polyphenols: biology and therapeutic implications in cancer. *Front Biosci* **2007,** *12*, 51.
19. Dannemann, K.; Hecker, W.; Haberland, H.; Herbst, A.; Galler, A.; Schäfer, T.; Brähler, E.; Kiess, W.; Kapellen, T. M., Use of complementary and alternative medicine in children with

type 1 diabetes mellitus–prevalence, patterns of use, and costs. *Pediatric diabetes* **2008,** *9*, 228-235.
20. Suppapitiporn, S.; Kanpaksi, N., The effect of cinnamon cassia powder in type 2 diabetes mellitus. *Journal of the Medical Association of Thailand= Chotmaihet thangphaet* **2006,** *89*, S200-5.
21. Chakrabarty, T.; Gangopadhyay, M., The genus Baccaurea (Euphorbiaceae) in the Indian subcontinent. *J. Econ. Taxon. Bot* **1997,** *21*, 525-534.
22. Bhowmicka, N., Some Lesser Known Minor Fruit Crops of Northern Parts of West Bengal.
23. Bhowmick, N.; Pal, R.; Suresh, C.; Paul, P.; Ghosh, S., Status and prospects of litchi cultivation in Cooch Behar district of West Bengal. *Environment and Ecology* **2009,** *27*, 771-776.
24. Kermasha, S.; Barthakur, N.; Mohan, N.; Arnold, N., Chemical composition and proposed use of two semi-wild tropical fruits. *Food chemistry* **1987,** *26*, 253-259.
25. Abdullah, A.; Hossain, M.; Bhuiyan, M., Propagation of Latkan (Baccaurea sapida Muell. Arg.) by mature stem cutting. *Research Journal of Biological Sciences* **2005,** *1*, 129-134.
26. Sondi, I.; Salopek-Sondi, B., Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *Journal of colloid and interface science* **2004,** *275*, 177-182.
27. Nomura, K.; Ohta, H.; Ueda, K.; Kamiya, T.; Hirano, M.; Hosono, H., Thin-film transistor fabricated in single-crystalline transparent oxide semiconductor. *Science* **2003,** *300*, 1269-1272.
28. Nakada, T.; Hirabayashi, Y.; Tokado, T.; Ohmori, D.; Mise, T., Novel device structure for Cu (In, Ga) Se 2 thin film solar cells using transparent conducting oxide back and front contacts. *Solar energy* **2004,** *77*, 739-747.
29. Lee, S. Y.; Shim, E. S.; Kang, H. S.; Pang, S. S.; Kang, J. S., Fabrication of ZnO thin film diode using laser annealing. *Thin Solid Films* **2005,** *473*, 31-34.
30. Wang, J.; Sallet, V.; Jomard, F.; do Rego, A. M. B.; Elamurugu, E.; Martins, R.; Fortunato, E., Influence of substrate temperature on N-doped ZnO films deposited by RF magnetron sputtering. *Thin Solid Films* **2007,** *515*, 8785-8788.
31. Wang, Z. L., Zinc oxide nanostructures: growth, properties and applications. *Journal of Physics: Condensed Matter* **2004,** *16*, R829.
32. Moritz, M.; Geszke-Moritz, M., The newest achievements in synthesis, immobilization and practical applications of antibacterial nanoparticles. *Chemical Engineering Journal* **2013,** *228*, 596-613.
33. Dave, R., In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. *African Journal of Microbiology Research* **2009,** *3*, 981-996.
34. Mishra, V.; Sharma, R.; Jasuja, N. D.; Gupta, D. K., International Journal of Green and Herbal Chemistry. **2012**.
35. Baker, S.; Rakshith, D.; Kavitha, K. S.; Santosh, P.; Kavitha, H. U.; Rao, Y.; Satish, S., Plants: emerging as nanofactories towards facile route in synthesis of nanoparticles. *BioImpacts: BI* **2013,** *3*, 111.
36. Vidya, C.; Hiremath, S.; Chandraprabha, M.; Antonyraj, M. L.; Gopal, I. V.; Jain, A.; Bansal, K., Green synthesis of ZnO nanoparticles by Calotropis gigantea. *Int J Curr Eng Technol* **2013,** *1*, 118-120.

37. Gunalan, S.; Sivaraj, R.; Rajendran, V., Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. *Progress in Natural Science: Materials International* **2012,** *22*, 693-700.
38. Nagajyothi, P.; An, T. M.; Sreekanth, T.; Lee, J.-i.; Lee, D. J.; Lee, K., Green route biosynthesis: Characterization and catalytic activity of ZnO nanoparticles. *Materials Letters* **2013,** *108*, 160-163.
39. Gnanasangeetha, D.; Thambavani, D. S., Biogenic production of zinc oxide nanoparticles using Acalypha indica. *Journal of Chemical, Biological and Physical Sciences (JCBPS)* **2013,** *4*, 238.
40. Gnanasangeetha, D.; Thambavani, S. D., Facile and eco-friendly method for the synthesis of zinc oxide nanoparticles using Azadirachta and Emblica. *International Journal of Pharmaceutical Sciences and Research* **2014,** *5*, 2866.
41. Ramesh, P.; Rajendran, A.; Meenakshisundaram, M., Green syntheis of zinc oxide nanoparticles using flower extract cassia auriculata. *Journal of NanoScience and NanoTechnology* **2014,** *2*, 41-45.
42. Naeem, M.; Hasanain, S.; Mumtaz, A., Electrical transport and optical studies of ferromagnetic cobalt doped ZnO nanoparticles exhibiting a metal–insulator transition. *Journal of Physics: Condensed Matter* **2007,** *20*, 025210.
43. Ekambaram, S.; Iikubo, Y.; Kudo, A., Combustion synthesis and photocatalytic properties of transition metal-incorporated ZnO. *Journal of alloys and compounds* **2007,** *433*, 237-240.
44. Shah, A.; Manikandan, E.; Ahmed, M. B.; Ganesan, V., Enhanced bioactivity of Ag/ZnO nanorods-a comparative antibacterial study. *Nanomedicine and Nanotechnology* **2013**, 4-3.
45. Ajaiyeoba, E.; Onocha, P.; Olarenwaju, O., In vitro anthelmintic properties of Buchholzia coriaceae and Gynandropsis gynandra extracts. *Pharmaceutical Biology* **2001,** *39*, 217-220.
46. Chatterjee, K. D., ParasitoIogy (protozoology and helminthology) in relation to clinical medicine. *ParasitoIogy (protozoology and helminthology) in relation to clinical medicine.* **1969,** (Edn 7).
47. Vidyarthi, R.; Pandey, P., *A Textbook of Zoology:(A Textbook for Senior Secondary, Intermediate, ISC, Pre-Medical, Pre-University and 1st Year of TDC)*. S. Chand: 2006.
48. Khan, S. B.; Faisal, M.; Rahman, M. M.; Jamal, A., Low-temperature growth of ZnO nanoparticles: photocatalyst and acetone sensor. *Talanta* **2011,** *85*, 943-949.
49. Azizi, S.; Mohamad, R.; Rahim, R. A.; Moghaddam, A. B.; Moniri, M.; Ariff, A.; Saad, W. Z.; Namvab, F., ZnO-Ag core shell nanocomposite formed by green method using essential oil of wild ginger and their bactericidal and cytotoxic effects. *Applied Surface Science* **2016,** *384*, 517-524.