CAFFEINE MEDIATED GREEN SYNTHESIS OF ZnO NANOPARTICLES AND Ag/ZnO NANOCOMPOSITES AND STUDY THEIR ANTIMICROBIAL PROPERTY

A DISSERTATION WORK SUBMITTED FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTER OF SCIENCE DEGREE IN CHEMISTRY

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BOARD OF EXAMINER AND CERTIFICATE OF APPROVAL

This dissertation is entitled "Caffeine Mediated Green Synthesis of ZnO Nanoparticles and Ag/ZnO Nanocomposites and Study Their Anti-Microbial Property" by Kabita Neupane, under supervision of Assoc. Prof. Dr. Sharmila Pradhan, Department of Chemistry, Amrit Campus is hereby submitted for the partial fulfillment of the Master of Science (M.Sc.) Degree in Chemistry. This dissertation has been accepted for the award of a Master's degree.

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This is to certify that the dissertation entitled "Caffeine Mediated Green Synthesis of ZnO Nanoparticles and Ag/ZnO Nanocomposites and Study Their Anti-Microbial Property" has been carried out by Kabita Neupane as a partial fulfillment for the requirement of a Master's Degree in Chemistry under my supervision. During the research period, Kabita Neupane has performed the work sincerely and satisfactorily. To the best of my knowledge, this work has not been submitted to any other degree in this institute.

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DECLARATION

I, Kabita Neupane, hereby declare that the work entitled "Caffeine Mediated Green Synthesis of ZnO Nanoparticles and Ag/ZnO Nanocomposites and Study Their Anti-Microbial Property" submitted to the Institute of Science and Technology Tribhuvan University as partial fulfillment for the requirements of Master of Science Degree in Chemistry has been done by myself and has not been submitted earlier in part or full in this or any other form to any other university/institute, here or elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the authors or institutions.

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Kabita Neupane

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LIST OF ABBREVIATIONS

Ag/ZnO	Silver Zinc Oxide Nanocomposite
$AgNO_3$	Silver Nitrate
CDH	Congenital Diaphragmatic Hernia
DNA	Deoxyribonucleic Acid
EGCG	Epigallocatechin Gallate
FTIR	Fourier Transform Infrared Spectroscopy
ISO	International Organization for Standardization
Nms	Nanomaterials
NNI	National Nanotechnology Initiative
Nps	Nanoparticles
NTCDB	National Tea and Coffee Development Board
ROS	Reactive Oxygen Species
pm	Rate Per Minute
SCWE	Subcritical Water Extraction
SEM	Scanning Electron Microscope
UV	Ultraviolet Spectroscopy
XRD	X-Ray Powder Diffraction
ZnO	Zinc Oxide
ZOI	Zone of Inhibition

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ABSTRACT

Green synthesis of metal nanocomposite is regarded as one of the hot topics for research in the field of nanoscience for its eco-friendly nature and convenient to perform. The present investigation involves the synthesis of ZnO and Ag/ZnO nanocomposites reacting caffeine obtained from *Camelia sinesis* leaves with zinc acetate and silver nitrate and study their biological activity. Phytochemical screening shows the presence of the secondary metabolites such as alkaloids, flavonoids, glycosides, polyphenols, terpenoids and quinones. The UV-vis absorption peak appears at 360 nm and 380 nm with band gap energy 3.44eV and 3.17eV respectively for nanoparticles and nanocomposites. The distinct bands observed at 453 and 507 cm^{-1} in the FT-IR spectrum are due to the vibration of Zn-O bond. The strong absorption band at 507 cm^{-1} and the weak bands in the region of 1305 to 1507 cm^{-1} are responsible for the biomolecules of the caffeine surrounding the Ag/ZnO nanocomposite. The average crystallite size of ZnO Nps is 27.98 nm and Ag/ZnO nanocomposite is 33.4 nm as calculated from the nonlinear fit. The result of agar well diffusion method revealed that caffeine mediated Ag/ZnO nanocomposite exhibited better antimicrobial activity against a broad-spectrum bacteria *Bacillus subtilis* (ATCC 6051) and *Escherichia. Coli* (ATCC 8739) and one fungus *Candida albicans* (ATCC 2091) when compared to the ZnO nanoparticle, due to their smaller size. Zone of inhibition of 7.4 mm for ZnO and 7.6 mm for Ag/ZnO shows an improvement against fungus *Candida albicans.*

Keywords: *Camelia sinesis*, caffeine, Ag/ZnO, UV, FT-IR, XRD, antimicrobial activity

CHAPTER I: INTRODUCTION

1.1 General Background of *Camelia Sinensis* and Its Distribution

It has been well known that humans have used natural products produced from animals, plants, microorganisms, and marine organisms, in medicines to diminish and medicate diseases since prehistoric period. Plants have been used medicinally by humans for at least 60,000 years, according to fossil records (Yuan et al., 2016). Several traditional plants were regarded as the major source of modern drugs. In order to keep using community-based methods within the medical system, ethnomedicinal knowledge is essential. Due to affordability, accessibility, and limited access to allopathic medication, many rural people rely on traditional herbal medicine. (Aziz et al., 2018).

Nepal has significant floral biodiversity with 6500 kinds of ferns and flowering plants because of the significant variations in topography, altitude and climate, of which 2000 are commonly used in traditional curing practices (Ambu et al., 2020).

The synthesis of nanomaterials using plants is gaining popularity due to its low cost and environmental friendliness. Plant-based nanomaterials are safer and more sustainable than traditionally synthesized nanomaterials, which are synthesized using chemical methods. Metal ions can be converted to nanoparticles using biomolecules found in plant extracts in a single step of green synthesis, which can be easily conducted at ambient temperature and pressure (Mittal et al., 2013).

Tea was originated by Chinese Emperor Shen Nung (divine healer) in 2737 BC (Kalauni et al., 2020). Historians believe that the Chinese Emperor gifted the seeds of tea to the then Prime minister, Janga Bahadur Rana and the first tea bushes grown in Nepal. It is believed that Mr.

Gajaraj Singh Thapa cultivated tea plants in Ilam after a trip to Darjeeling. Tea cultivation was started in 1863 and the first factory was built in Ilam in 1978 for processing of tea leaves (NTCDB). Tea is grown on 16,905 hectares area, yielding 24,118,274 kg of tea, with the help of 14,014 workers from 146 estates throughout Nepal. 11,185 tons of tea were exported from Nepal for Rs. 2.78 billion (NTCDB, 2019/2020). In Nepal tea is commonly called Chiya. It has a different name in different countries such as India: Chha, China: Cha, Russia: Chai, United State: Tea, etc (Namita et al., 2012).

Tea leaf, biologically, *Camelia sinensis* is consumed in fermented (black and red), semi-fermented (oolong tea) and unfermented (green tea) forms. Tea plants contain many secondary metabolites such as terpenoids, phenolics, polysaccharides and flavonoid having redox capacity. Because of its fine subtle and pleasant fruity flavor and good quality, Nepalese tea has a distinctive value and reputation throughout the world. This is because tea-growing regions have cold temperatures and high elevations and fertile soil (KC et al., 2020). Green tea is rich source of antioxidants (catechins). Tea leaves have antibacterial and astringent properties that help relieve dysentery and other digestive disturbances. It also contains high levels of caffeine (Vuong & Roach, 2013). The phenolic content and antioxidant activity of different teas depend on several factors, including type of tea, brewing method, age of tea, and conditions of storage. Dark tea has the highest phenolic content and antioxidant activity, followed by oolong, black and green tea. White tea has the lowest phenolic content and antioxidant activity. Antioxidant activity and phenolic content in different teas is shown in figure 1.

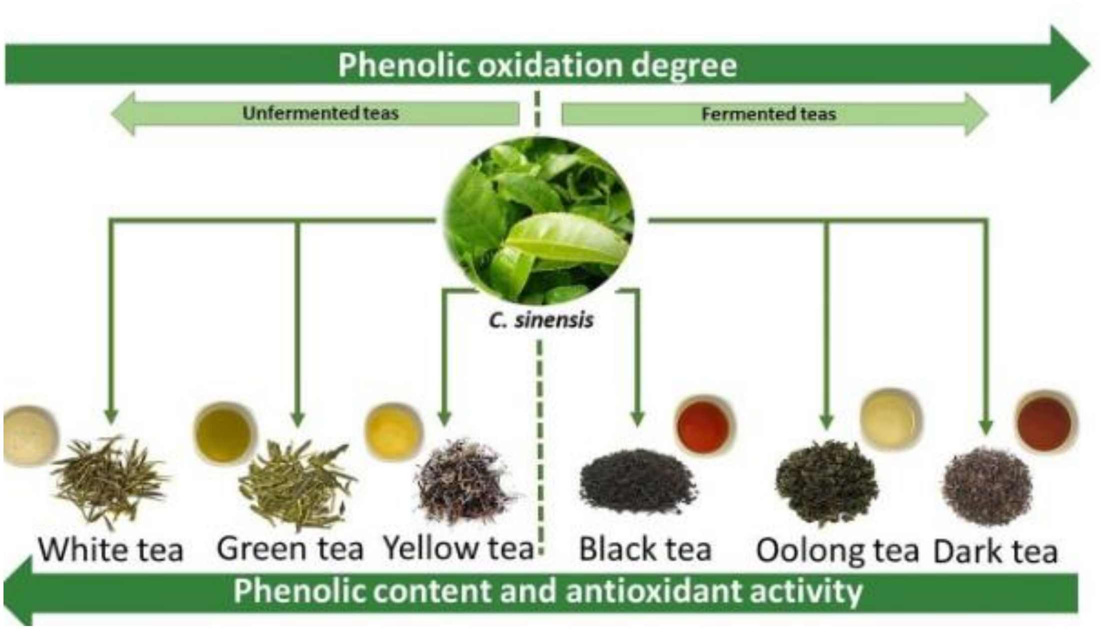


Figure 1. Phenolic content and antioxidant activity in different teas (Gonçalves Bortolini et al., 2021)

The scientific classification of *Camellia sinesis* is shown below.

Scientific Classification

Kingdom: Plantae

Subkingdom: Tracheobionta

Super division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Magnoliidae

Order: Theales

Family: Theaceae

Genus: Camellia

Species: *Camellia sinensis*

Camellia sinensis is an evergreen shrub or small tree. It is present in the tropical and subtropical areas. It belongs to family Theaceae, having toothed leathery leaves and white fragrant flowers

(Aboulwafa et al., 2019). The largest tea producing country is China with an output of 2.3 million tons followed by India, 1.2 million tons, Kenya (399,211 tons), Srilanka (328,964 tons and Turkey that occupies together of the total tea product over world (Kalauni et al., 2020). The main tea growing region in China are the Hainan, Fujian and Anhui provinces. The majority of tea plantations are in Assam and Darjeeling regions in India. In Japan, the main tea producing regions is Shizuoka prefecture. Government of Nepal revealed five eastern districts i.e., Jhapa, Ilam, Panchthar, Dhankuta and Terhathum) as tea zones in 1980s (Poudel, 2014). For optimum growth, it needs an acidic soil (pH 4.5 to 5.5), ideal average temperature (18 to 20 °C), at least 5 hours of direct sunlight, and relative humidity levels between 70% and 90%. The land from sea level to high altitudes up to 1.3 miles above sea level is suitable for tea plantation.

Camellia sinesis contains polyphenols such as alkaloid including caffeine, theophylline, theobromine and catechin including epigallocatechin, epicatechin and epigallocatechin gallate (EGCG). The main constituents present in tea leaf are tabulated in the table 1.

Table 1. Different compositions of green tea leaf (Senthilkumar and Sivakumar, 2014)

S. N	Component of tea leaf	Percentage (%)
1.	Phenolic substances	30
2.	Proteins	15
3.	Amino acids	4
4.	Carbohydrates	7
5.	Lipids	7
6.	Vitamin C, E and K	-

1.2 Economic Importance

Tea is the major export-oriented cash crop as its leaves are very important for food industry. Tea has a great export potential, which helps Nepalese farmers improve their level of living by generating foreign currency and eradicating poverty (Adhikari et al., 2017).

The *Camelia sinensis* leaves are of great significance for variety of properties such as anticancer (Boehm et al. (2004), antioxidative, antiarthritic and anti-inflammatory (Singh et al. (2018) and antimicrobial (Shah et al. (2015).

Caffeine is an alkaloid found in tea. The presence of caffeine in tea allows us to improve our physical performance, enhance memory and accelerates energy consumption, so caffeine is widely employed in non-alcoholic drinks and medications. A tea leaf contains 2–4 percentage of caffeine (Shalmashi et al., 2008). Healthy adult can consume caffeine at a dose of 400 mg (about 5.7 mg/kg bw) per day. One can drink up to 4 cups of black tea per day because a cup (220 mL) has about 50 mg of caffeine. Depending on the type of tea and the brewing technique, the quantity of caffeine in a cup of tea might vary widely. For example, a cup of black tea can contain up to 70 milligrams of caffeine, while a cup of green tea can contain up to 30 milligrams (Vuletic et al., 2021). Excessive intake of caffeine containing compounds affects the central nervous system and cardiovascular system (Cappelletti et al., 2015).

1.3 Nanomaterials

A nanomaterial is a substance that has any external dimension, internal structure, or surface structure in the nanoscale, according to the International Organization for Standardization (ISO) (Sudha et al. (2018) and nanoparticles as a nano-object with at least one of the dimensions in the nanoscale (Khan et al., 2019). Nanomaterials are crucial for having improved properties from bulk materials because of large surface to volume ratio, small size, morphology and distribution (Amatya and Joshi, 2019; Sudha et al., 2018). Nps are unique because the physical behavior of particles between 1-100 nm shifts from classical physics to quantum physics as the particle size decreases. Therefore, the physical and chemical properties of NPs change by varying its size, composition or surface (Sudha et al., 2018).

1.4 Property of ZnO Nanoparticle and Composite

ZnO is an n-type semiconductor inorganic compound having diverse properties such as electronic, optical, chemical, medicinal, catalytic and many more (Sirelkhatim et al., 2015). Wurtzite, zinc blende, and rock salt are three possible crystal formations of ZnO. Wurtzite structure is the thermodynamically stable phase at normal temperature in which each zinc atom is tetrahedrally linked to four oxygen atoms as shown in figure 2 (Espitia et al., 2016).

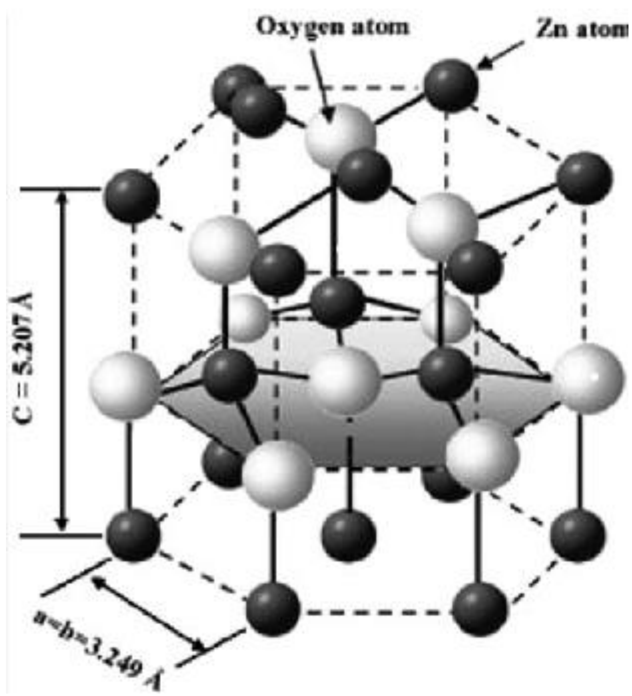


Figure 2. Wurtzite structure of ZnO (Khan & Khan, 2015)

The hexagonal wurtzite structure of ZnO is shown in figure 2. Zn ions are depicted as smaller brown spheres, while O atoms are bigger white spheres.

ZnO is a suitable material for optoelectronic purposes because of its large band gap of 3.37 eV. Its optical properties include a high refractive index (2.1) and strong optical absorption in the visible and ultraviolet regions, making it ideal for optical coatings and filters (Zheng, 2012). Some important physical properties of ZnO Nps are presented in table no. 2.

Table 2. Physical properties of ZnO (Sharma et al., 2020)

Appearance	White powder
Molecular formula/ mass	ZnO/ 81.37
Stable phase at 300K	Wurtzite
Density	5.66 g/cm^3
Refractive Index	2.01
Band Gap	3.37 eV, direct
Electron effective mass	0.24
Melting point	1975 °C
Excitation binding energy	60 meV
Static dielectric constant	8.656
Lattice constant	a=b=0.32495 nm and c=0.52069 nm

1.4.1 Applications of ZnO Nanoparticles and Nanocomposites

Owing to its unique physical, chemical characteristics it has variety of applications such as in optoelectronics, chemical sensing, biosensing, and photocatalysis (Bharat et al., 2019). The ZnO was studied for dye sensitized solar cells (DSSC) (Joshi, 2015) which are a type of thin-film solar cell. They are used to absorb visible light and generate electricity. The ZnO Nps can be used to form a nanocrystalline film, which increases the efficiency of the DSSC (Sharma et al., 2018). They are also used in cancer therapy and wound healing. Also, ZnO has huge potential in the biomedical sciences due to the antimicrobial, antioxidant, anticancer, drug delivery and gene delivery (Senthikumar and Shivakumar, (2014); Sharmila et al. (2018); Kumar et al. (2019). ZnO is nontoxic compound so, it is used in cosmetic and pharmaceutical products (Shaikshavali et al., 2020). It is used in the treatment of skin diseases such as psoriasis and acne (Anjum et al., 2021). Some of the important fields in which ZnO nanoparticles have been extensively used are shown in figure 3.

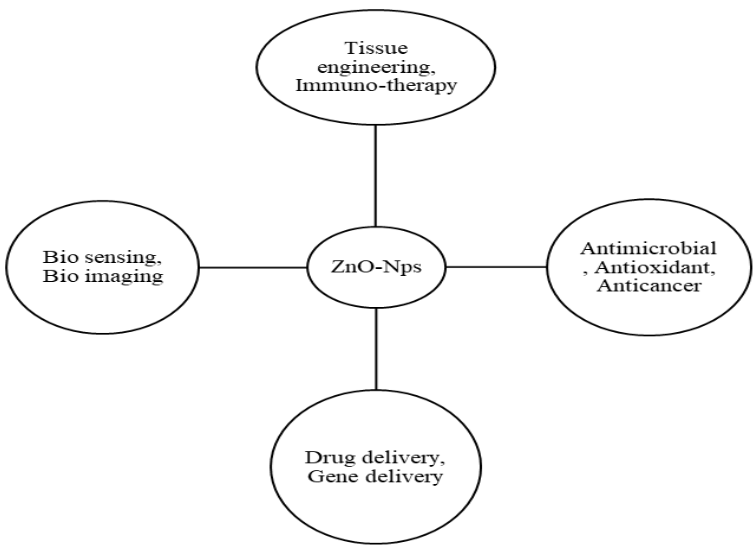


Figure 3. Diagram showing applications of ZnO Nps (Alhujaily et al., 2022)

Variety of metallic and metal oxide nanoparticles such as copper, zinc, gold, silver, and other metals, are utilized for numerous applications such as water treatment (Zhang et al. (2008), agriculture, medication delivery for medicine and so on (Singhal et al., 2023). Nanoparticles are also extremely employed in food processing and preservation. For example, TiO_2, SiO_2, ZnO, MgO and Ag and it nanocomposite have been used for food packaging (Sarfraz et al., 2020). This treatment provides a protective layer to hide various tastes and flavors (McClements et al., 2016). In the context of synthesizing nanomaterials, most researchers are showing interest in modifying nanomaterials like ZnO through metallic particles such as Au, Ag, As and Sb, where Ag is more popular for its multifunction. Ag nanoparticles are employed as inhibitors of bacterial growth, microbial infections, etc. It is employed in a numerous application, including ointments, medical devices, implants, and wound dressings. (Kumar et al., 2019).

1.5 Antimicrobial Activity

Antimicrobial activity of nanomaterial is important to provide protection against microorganisms, biological fluids, and aerosols, as well as disease transmission. Oxidative stress caused by the production of reactive oxygen species (ROS) on the surface of Nps and free metal ion toxicity caused by the dissolution of metals from the surface of Nps are the two well-known mechanisms of antibacterial action (Khatami et al., 2018). Antimicrobial responses can be influenced by porosity, specific surface area, morphology, and particle size distribution. Numerous nanoparticles, including zerovalent iron, magnesium oxide, titanium dioxide, zinc oxide, silver nanoparticles and fullerene derivatives, have shown strong antibacterial properties (Hoseinnejad et al., 2017). Ag Nps are not only bactericidal but also the treated bacteria had smaller diameters compared to untreated bacteria (Gogoi et al., 2006). The damage of the microbial cell membrane could be either by formation of ROS or by release of nanoparticles (Matai et al., 2014).

Rationale of The Study

1. Green synthesis is a rapidly growing field in nanotechnology that aims to develop environmentally friendly and sustainable materials.

2. Caffeine, is being naturally available, non-toxic compound, is assumed to be an effective green reducing agent instead of toxic chemicals for the synthesis nanoparticles and Ag/ZnO nanocomposites.
3. ZnO nanoparticles and Ag/ZnO nanocomposites have been shown to exhibit excellent bactericidal and fungicidal activities, making them ideal for use in various biomedical and industrial applications.

Objectives of The Study

General Objective

The main objective of this study is to synthesize the ZnO and Ag/ZnO using eco-friendly approach and to compare their antimicrobial properties.

Specific objectives

The specific objectives of this study are as follows:

1. Preparation of caffeine extract from tea leaves.
2. Synthetization of caffeine mediated ZnO nanoparticles and Ag/ZnO nanocomposite.
3. Characterization of as-synthesized ZnO and Ag/ZnO nanocomposite using UV-visible spectroscopy, FTIR and XRD.
4. Study of the antimicrobial property of ZnO and Ag/ZnO nanocomposites comparatively.

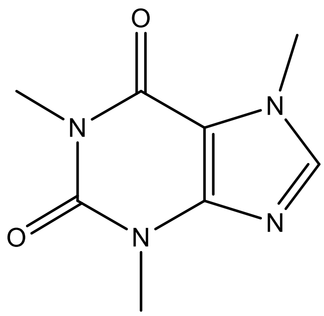
CHAPTER II: LITERATURE REVIEW

Nepal cultivates three different kinds of tea: *Camellia sinesis* for orthodox tea, *Camelia asamica* and *C. asamicaspplasiocalyx* for cut, tear and curl (CTC). A review was done to know to what extent the studies have been done on *Camelia sinesis,* caffeine, ZnO nanoparticles and Ag/ZnO nanocomposite.

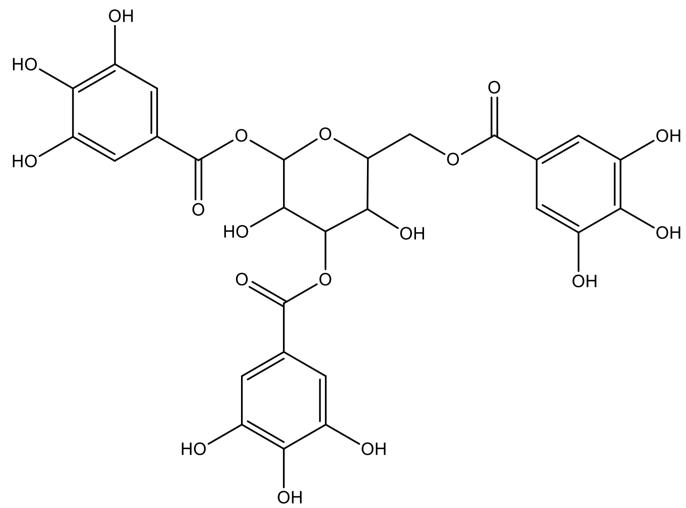
2.1 Situation of Tea in Nepal and Its Chemical Constituents

Kalauni et al., (2020) examined the export and import patterns, annual growth rate, production pattern, and future possibilities of Nepali traditional tea. Nepal's climate, soil, and geography are ideal for the cultivation of traditional tea. The production of tea is increasing, with 9.55 percent of yearly growth rate. In spite of significant domestic and international market, the country produces very little traditional tea. In addition, 90% of all orthodox tea produced was imported, largely to India. The different chemical constituents found in tea are caffeine, catechin, epicatechin, tannins, flavonoid, ascorbic acid, vitamins and theaflavin (Reto et al., 2007).

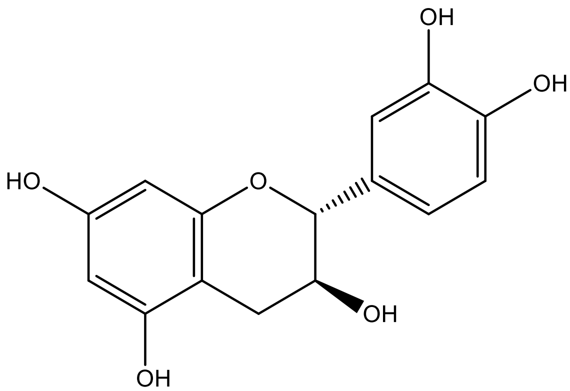
Some of the chemical constituents of tea are:



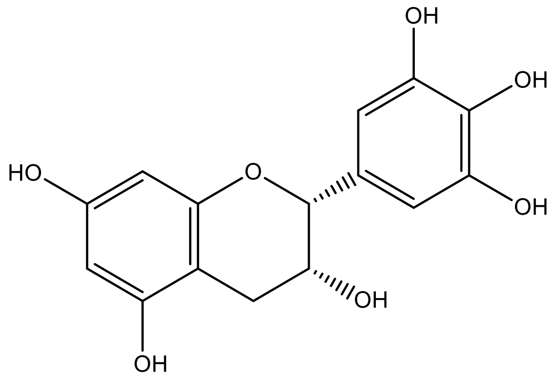
Caffeine



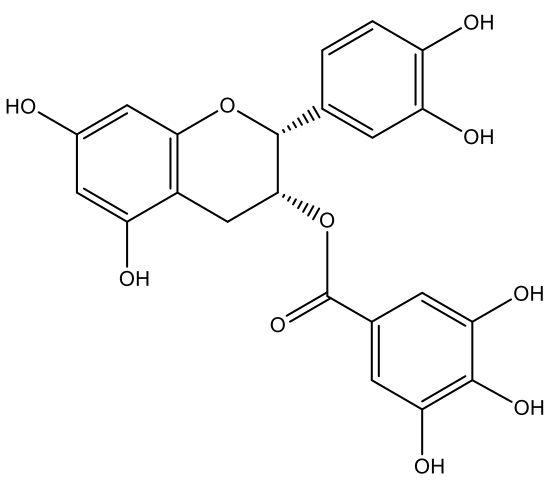
Tannin



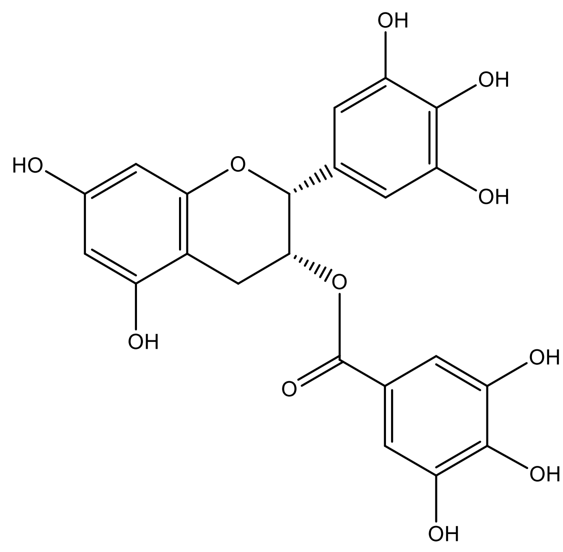
Catechin



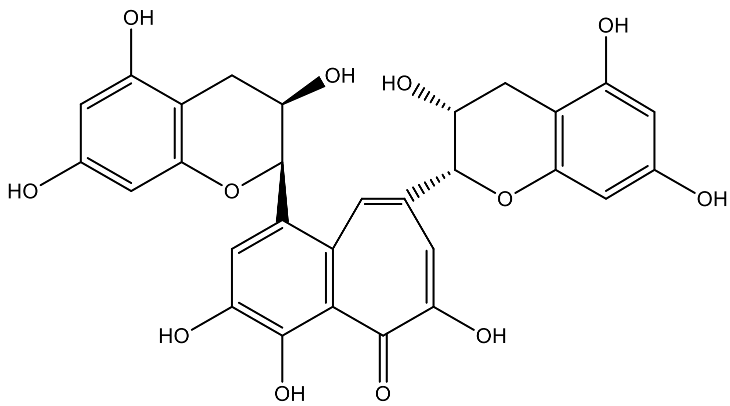
Epigallocatechin



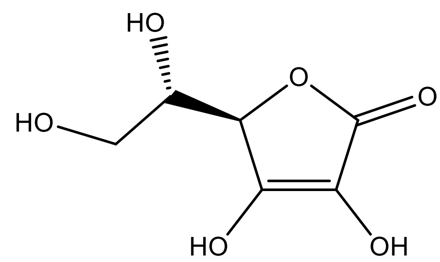
Epicatechin gallate



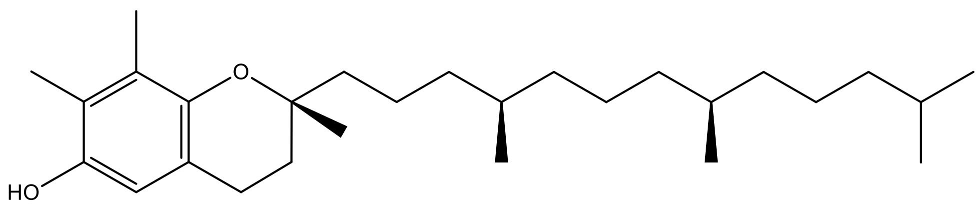
Epigallocatechin gallate



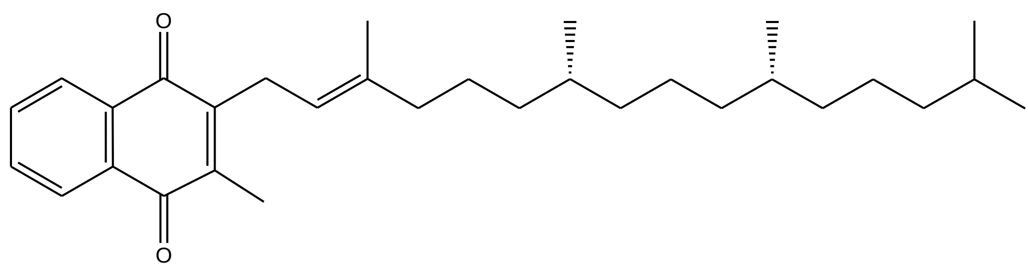
Theaflavin



Ascorbic acid



Vitamin E (Tocopherol)



Vitamin K

Figure 4. Chemical structure of some chemical constituents of tea (Reto et al., 2007)

2.2 Amount of Caffeine in Different Tea

High-quality Chinese green tea contained the highest levels of minerals as well as the proteins. Chinese black has the highest total ash and caffeine concentrations, whereas white tea has a high volatile compound content comparable to black tea, the highest water and the lowest total ash level (Czernicka et al., 2017).

The caffeine content of various tea brands was determined spectrophotometrically, including Franck's Green and Black Tea, Green Tea with Ginger and Naturavita Green, Agristar Black and Green Tea, and Dar Vitalis Eko Green Tea. Franck's Black Tea (1471.021 ppm) has the highest caffeine levels of the tea samples tested (Vuletic et al., 2021).

2.3 History of Nanomaterial and Its Importance

Romans used the nanoparticles and nanostructures in fourth century. The first known artificial nanomaterial is the Lycurgus cup. It is the oldest well known dichroic glass. The glass of the Cup appears green in direct light and reddish-purple when light transmits through the glass (Bayda et al., 2019).

Figure 5. The Lycurgus cup. The glass looks green in reflected light (A) and red-purple in transmitted light (B) (Bayda et al., 2019)

The first major development in the nanoscience and nanotechnology came in 1981 with the invention of scanning tunneling microscope (STM) by Gerd Binnig and Heinrich Rohrer. In the 1980s and 1990s, enhancements in materials science and chemistry led to development of new nanoscale materials, such as carbon nanotubes and quantum dots. In 2000, National Nanotechnology Initiative (NNI) was launched in United States, with the goal of coordinating and funding research in nanoscience and nanotechnology. Since then, the study of nanoscience and nanotechnology has developed and grown, leading to new advancements in areas like medicine, electronics, and energy as well as numerous new applications. Currently, nanoscience and nanotechnology are seen as key areas for future innovation and development. The historical development of nanoscience and nanotechnology is presented in table 3.

Table 3. Historical development of nanoscience and nanotechnology (Bayda et al., 2019)

Year	Event	Scientists
4^{th} century	Lycurgus cup	Bayda et al., 2019
1857	Synthesis of colloidal ruby gold nanoparticles	Michael Faraday
1908	Light scattering nanoparticles	Gustav Mie
1931	Discovery of TEM	Max Knoll and Ernst Ruska
1953	Discovery of DNA	James Watson and Francis Crick
1970	Suggested the icosahedron shape of C_{60}	Eiji Osawa
1974	First used the term Nanotechnology	Norio Taniguchi
1981	Invention of STM	Gerd Binnig and Heinrich Rohrer
1983	Discovery of colloidal Quantum Dots	Louis Brus
1986	Invention of AFM	Gerd Binnig, Christoph Gerber and Calvin F. Quate
1991	Discovery of Multi-wall Carbon nanotubes	Sumio Iijima
1993	Discovery of single-wall carbon nanotubes	Sumio Iijima and Donald Bethune
2018	Shrinking objects to the nanoscale	Oran et al., 2018

Recent research has highlighted the enormous potential of nanotechnologies in biomedicine for the diagnosis and treatment of a wide range of human disorders.

2.4 Synthetic method of metal nanoparticles

Generally, chemical methods and physical methods have been used to synthesize ZnO and Ag/ZnO nanocomposite including epitaxy, laser/vapor deposition, sol-gel, sonochemical, pyrolysis, electrodeposition, solvothermal and hydrothermal, etc. (Khatami et al., 2018). Such techniques require expensive equipment, toxic material and create dangers to the environment (Chandrasekaran et al., 2016; Dhandapani et al., 2014). Synthetic methods are usually classified in terms up and down approaches, which are presented as in figure 6.

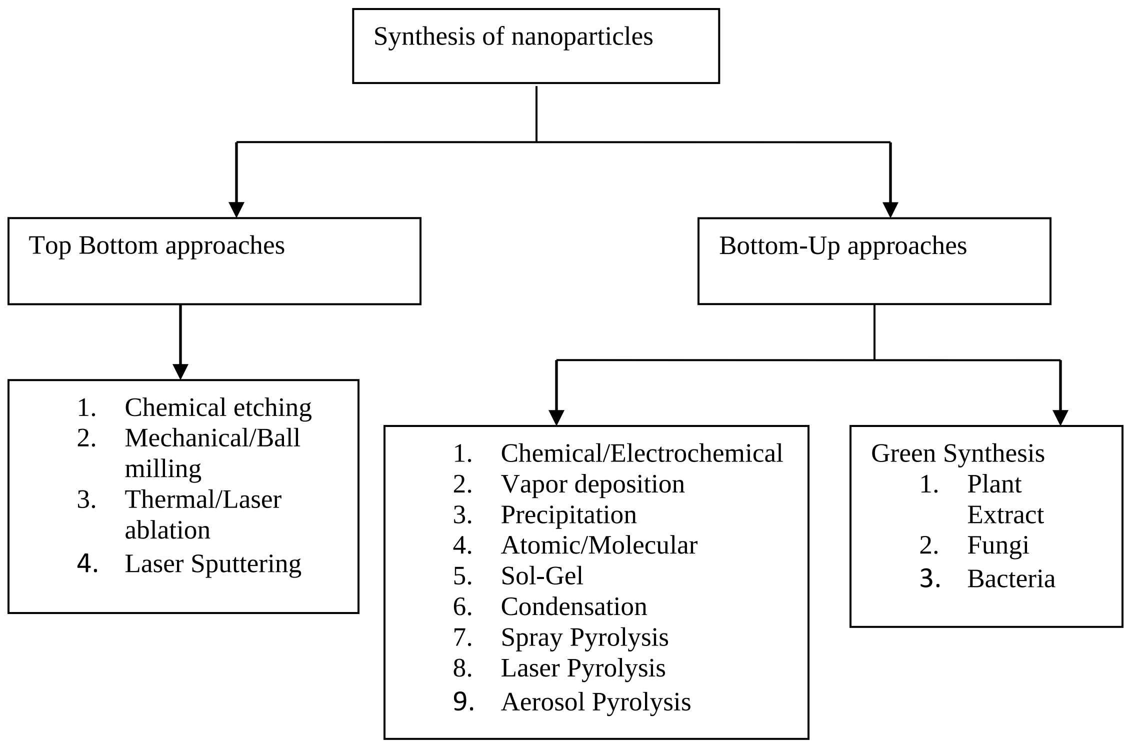


Figure 6. Schematic diagram different approaches for synthesis of nanoparticles (Baig et al., 2021)

Hence, the green synthesis technique using plant extract delivers the benefit of facilitating large-scale nanoparticle synthesis.

2.4.1 Green Synthesis of Metal Nanoparticles

Green synthesis is an increasingly popular technique for producing hybrid materials, metal or metal oxide nanomaterials, and other nanoparticles in an environmentally friendly way. This process is energy efficient, as it does not require high pressure or high energy.

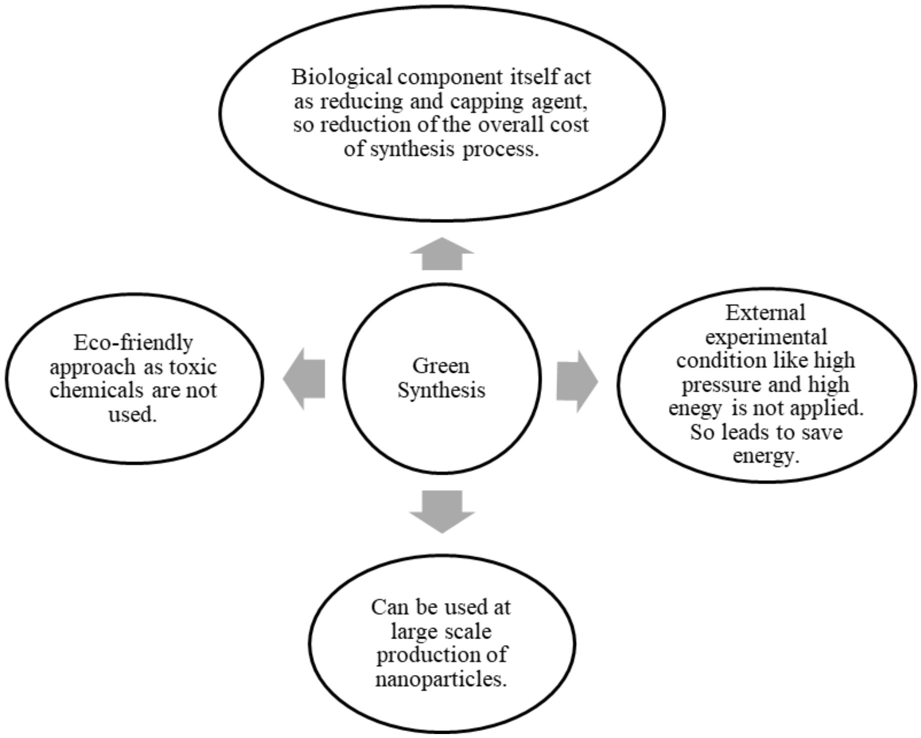


Figure 7. Merits of green synthesis (Singh et al., 2018)

It also ensures the availability of natural phytochemicals that serve as reducing and capping agents for the stability of nanoparticles without requiring toxic chemical, extreme pressure and temperature (Ramesh et al., 2015).

Green synthesis of nanoparticles is achieved by using plant-derived materials, such as plant extracts, which are rich in natural reducing and stabilizing agents. Three key stages are involved in the synthesis of metal nanoparticles in plant extracts:

1) activation stage, in which the metal ions are reduced

2) growth stage, in which nearby small nanoparticles spontaneously combine with larger ones. Nanoparticles gather to form different regularly or irregularly shaped nanoparticles as the time of the growth phase increases.

3) termination stage, in which the shape of the nanoparticles is determined (Makarov et al., 2014).

The use of plant-derived materials in green synthesis of nanoparticles helps to reduce the toxicity of the synthesized nanoparticles. In addition, this method helps to eliminate the need for expensive instrumentation and laboratory equipment (Iravani, 2011).

Various literatures have revealed about the green route for synthesizing ZnO nanocomposites conveniently.

According to Jain et al. (2011), green tea and the plants used to make a herbal extract work together effectively. Antioxidant properties have been reported in *Punica granatum, Phyllanthus emblica L., Vitis vinifera, Camellia sinensis Linn* and *Cinnamomum cassia.* Individual and combined antioxidant activity was measured in vitro using nitric oxide, super oxide free radical scavenging procedure and 1,1-diphenyl-2-picrylhydrazyl (DPPH). All of these plants can increase antioxidant activity and lower cholesterol when combined with green tea.

Several plant species, including mustard, alfalfa, and sunflower, have been reported to produce zinc, nickel, cobalt, and copper nanoparticles (Iravani, 2011). Many plants [including alfalfa (*Medicago sativa*), oat (*Avena sativa*), lemon (*Citrus limon*), tulsi (*Ocimum sanctum*), neem (*Azadirachta indica*), mustard *(Brassica juncea*), coriander (*Coriandrum sativum*) lemon grass (*Cymbopogon flexuosus*) and aloe vera (*Aloe barbadensis Miller*, Kuponiyi et al. (2014) have been used to obtain gold and silver nanoparticles (Soltys et al., 2021).

Jan et al. (2013) used a simple wet chemical procedure to create ZnO and Sn/ZnO Nps in various shapes, such as nanorods and nanospheres. Nps were heated for two hours at 600 °C. Single phase wurtzite structure of ZnO has been established by structural analysis. As a result of Sn/ZnO nanocomposite, the geometry of ZnO can change from spherical to rod-shaped. ZnO nanostructures with Sn nanocomposite used in creams or lotions as a UV filter in sunscreen prevent bacterial infections of *S. aureus*, particularly on the skin.

Senthilkumar and Sivakumar (2014) conducted research on the synthesis of ZnO from green tea leaves. The spectra of UV-vis showed absorption peak at 325 nm. The structure of ZnO corresponds to hexagonal wurtzite, which is 16 nm in size, according to the XRD data. The antibacterial and antifungal properties of selected pathogenic species were investigated using the agar well-diffusion technique. Synthesized ZnO Nps have antibacterial properties similar to synthetic drug actions.

Sun et al. (2017) revealed the possibility of artificial hybrid nanostructures containing heated Co/ZnO nanocomposite with graphene oxide as the supporting material for fuel cell applications. The nanocomposite was found to be stable, tolerant to methanol, and exhibited superior electrocatalysis behavior compared to Platinum. The study demonstrated that these nanostructures could be used to develop an effective and cost-efficient fuel cell.

Similarly, Irshad et al., (2018) studied antimicrobial activity of green synthesized ZnO Nps. The result of agar well diffusion method showed clear zone of inhibition against *Escherichia coli*, *Staphylococcus aureus* and *Aspergillus nigher* as 36.15 mm ± 0.304, 40.05 mm ± 0.137, and 40.10 mm ± 0.050 respectively. UV visible spectral peak at 350 nm and XRD diffractogram revealed characteristic peak with size range 30-40 nm.

Ag Nps have recently been effectively bound to ZnO Nps using a variety of plant extracts, such as potato peel, Alharthi et al. (2020), *Trigonella foenum graecum* leaf extract, Noohpisheh et al. (2020) *Valeriana officinalis* root extract, Faal et al. (2017) *Usnea florida* and *Pseudevernia furfuracea* lichen extract, Koca et al. (2022), *Colocasia esculenta*, Kiran et al. (2019) and *Azadirachta indica*, Slathia et al., 2021).

2.5 ZnO and Ag/ZnO Nanocomposites

Literature reveals that synthesis of Ag/ZnO is quite difficult and challenging because of the self-compensation effect. Ai et al. (2021) reported that forming Ag/ZnO leads to increase crystallinity and minimize the optical band gap of Ag/ZnO thin films.

Ag is commonly used in p-type doping as a group IB element. For example, Fernandez et al. (2016) used a solvothermal technique to create Ag/ZnO nanocomposites. The literature revealed that the addition of Ag not only enhanced the open circuit voltage in ZnO, but also resulted in some Ag deposition in the composite. The photocatalytic impact of the Ag/ZnO nanocomposites was effective as an outcome of the methylene blue degradation in the catalytic experiment.

Ag^{+} has a significantly wider ionic radius than Zn^{2+}. Additionally, Ag has better self-generation performance compared to other valuable elements. Therefore, Ag/ZnO nanocomposite research is crucial for creating Nps and high-quality ZnO thin films (Qian et al., 2016).

Ag and ZnO nanoparticles synthesized from *Prosophisfracta* and coffee were determined using UV–vis, XRD, and SEM. The average size of the green produced Ag and ZnO nanoparticles from *Prosophifracta* is around 16 and 26 nm respectively. Cotton wound bandages were filled with Ag and ZnO nanoparticles, as well as combined Ag/ZnO nanoparticles. Both nanoparticles demonstrated better antimicrobial activity of bandages. Bandages with an Ag nanoparticle liquid solution had higher antibacterial activity than Ag/ZnO and ZnO nanoparticles (Khatami et al., 2018). The highest level of toxicity against *E. coli* has been demonstrated for several nanosized metal oxides, including ZnO, CuO, Al_2O_3, Fe_2O_3, SnO_2, and TiO_2 (Hu et al., 2009). ZnO and Ag/ZnO nanocomposites have also been synthesized from *Silybum marianum*. The UV absorption peak of the Ag/ZnO heterostructures appears at ∼374 nm corresponds to the Zinc oxide and at ∼420 nm corresponds to the Ag Nps on the surface of zinc oxide. ZnO Nps indicated slightly lower antibacterial potential than Ag/ZnO Nps (Hameed et al., 2019).

Kumar et al., (2019) synthesized the ZnO and Ag/ZnO employing leaf extract of *Colocasia esculenta* through microwave method. Nanoparticles were characterized using UV-vis, XRD, FTIR, and SEM. Due to substitution of ZnO lattice with Ag, FT- IR showed the mild change in the position of UV-vis bands in Ag/ZnO.

Moringa oleifera seed extract was used to synthesize an Ag/ZnO nanostructure using green combustion method. XRD and FESEM were used to examine the physical and structural features of nanostructures. The Scherrer and Williamson-Hall methods yielded crystallite sizes of 54.1 nm and 36.187 nm, respectively. Antimicrobial activity against different harmful bacteria in humans (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhii*, *Klebsiella pneumonia, MRSA*) and *Candida albicans* were identified. When compared to other microorganisms, nanostructures had the largest inhibition zone against *Staphylococcus aureus* (17 mm) and an 18 mm inhibition zone against *Candida albicans*. Doped nanoparticle showed effective antimicrobial activity compared to undoped nanoparticles (Swati et al., 2020).

The hydrothermal technique was used to create Ag/ZnO composite from *Trigonella foenum-graecum* leaf extract. The produced Ag/ZnO had an average size of 75 nm. The presence of Ag/ZnO is indicated by large band UV-vis absorption at 369 nm for ZnO and broad band absorption at 450 nm for Ag (Noohpisheh et al., 2020).

Silver decorated zinc oxide was synthesized from *Pseudevernia furfuracea* and *Usnea florida* lichen extracts. The UV-vis absorption peak of *P. furfuracea* and *U. florida* Ag/ZnO was 339 nm and 361 nm respectively (Koca et al., 2022). Ag/ZnO had greater antibacterial activity against *Escherichia coli* than ZnO, indicating that incorporation of Ag Nps enhances ZnO's antibacterial effect (Cuadra et al., 2022).

Overviewing literatures, studies regarding ZnO modified by silver using ecofriendly techniques are still not at satisfactory label. This leads that further investigation on Ag/ZnO nanocomposite is necessary.

CHAPTER III: MATERIALS AND METHOD

3.1 Materials

3.1.1 Collection of Plant Material

Firstly, the starting material, fresh leaves of tea (*Camelia sinensis)* was collected from Ilam, Nepal. Then fresh leaves were washed and allowed to dry in shade for fifteen days. The dried leaves were grinded into powder form with the help of an electric grinder and stored in air tight jar until further work. Some snaps of material preparation are shown in figure 8.

tea leaves dried leaves grinder leaves powder

Figure 8. Process of making tea leaves powder

3.1.2 Equipment

Electric grinder, digital weighing balance, hot air oven, burettes, pipettes, separating funnel, beakers, volumetric flasks, Whatmann filter paper 41, heating mantle and spectrophotometer.

3.1.3 Solvents and Chemicals

Chemicals and solvents used are of laboratory grade as well as analytical grade. Zinc acetate (Qualigens), Silver nitrate (Qualigens), sodium carbonate (Merck), dichloromethane (Qualigens), Ethanol and methanol (Fishner scientific) were of analytical grade. Distilled water, Muller

Hinton Agar, Nutrient broth, were available in the laboratory. Reagent and solvents used during phytochemical analysis such as Dragendorff's reagent, Meyer's reagent, Molisch's reagent etc. were made in the laboratory with the chemicals provided in the laboratory.

3.2 Method

3.2.1 Caffeine Extraction from Tea Leaves

50 g of tea leaf powder and 600 mL of distilled water was taken in 1L beaker. It was stirred and boiled at 75 °C for 30 minutes in heating mantle. 20 g sodium carbonate was added to hot solution. It was cooled to room temperature and filtered. To the residue, 200 mL of distilled water was added and the same process was repeated. The filtrate was transferred into separating funnel and 15 mL of the dichloromethane was added and shaken slowly. The knob was opened and the lower layer was collected in beaker. The knob was closed and again 15 mL of dichloromethane was added to it. The process was repeated for 3 times. The lower layer was transferred into the beaker. The filtrate was again transferred to separating funnel. It was washed with 15 mL distilled water and 0.15 g calcium chloride was added and shaken slowly. The lower layer was collected in beaker and it was evaporated in hot water bath at 70 °C. Different steps of preparing caffeine are shown in figure 9.

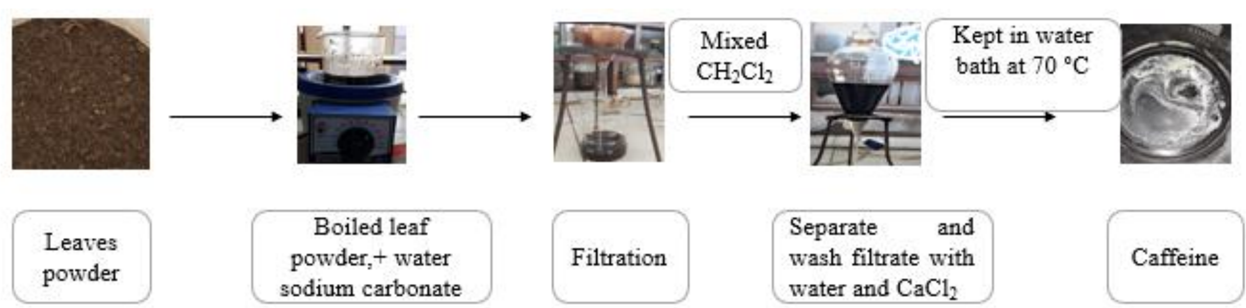


Figure 9. Preparation of caffeine from tea leaves powder

3.2.2 Preparation of Caffeine Extract

The caffeine was extracted according to the procedure put forwarded by Senthilkumar and Sivakumar (2014) with slight modification. 0.4 g of caffeine was added to 100 mL water and was stirred at 70 °C for 2 hours.

3.2.3 Preparation of Zinc Acetate Dihydrate (0.2M)

10.975 g of zinc acetate (Mol. Wt. 219.50) was dissolved in 250 mL distilled water and filled it up to mark.

3.2.4 Preparation of Silver Nitrate (0.1M) Solution

1.69 g of $AgNO_3$ was dissolved in 100 mL of distilled water in a volumetric flask to produce 0.1M $AgNO_3$ solution.

3.2.5 Preparation of ZnO Nanoparticle

Nanoparticles were synthesized using green method following the previously published protocols (Senthilkumar and Sivakumar, 2014 and Kumar *et al*., 2019) with slight modifications.

30 mL of the caffeine extract and 70 mL of zinc acetate was taken in a beaker and further stirred for 20 minutes at 70 °C. It was transferred to oven and kept for 10 hours at 60 °C. Then, the mixture was allowed to cool and the precipitate formed was collected after repeating the process of washing with distilled water and ethanol by centrifuging at 3000 rpm for 10 minutes. Then, solids were dried in oven at 60 ºC for 12 hours. The residue was calcined at 150 °C for 2 hours and further calcined at 500 °C for 1.5 hours using the muffle furnace. Finally, the samples so prepared were cooled and preserved at 4 º C in vials until further work.

Reactions Involved:

$(CH_3COO)_2Zn$ + Caffeine Extract ⟶ $Zn(OH)_2$ + CH_3COOH + unreacted extract

Zinc acetate

white colour solution

↓ Heat at high temperature

ZnO

Zinc oxide
White ppt

When zinc acetate reacts with caffeine extract, Zinc hydroxide is formed which on dehydration at high temperature gives zinc oxide.

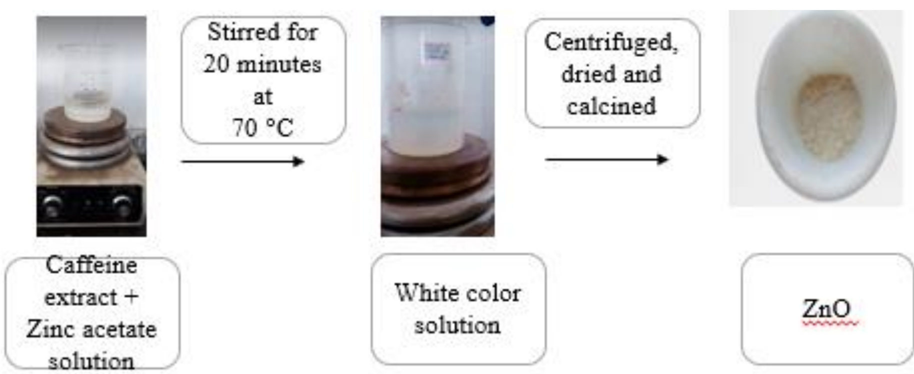


Figure 10. Preparation of ZnO

3.2.6 Synthesis of Ag/ZnO Nanocomposite

The initial steps for synthesizing Ag/ZnO nanocomposite using caffeine was same as for the nanoparticles. Firstly, 30 mL of caffeine extract was added to 70 mL of zinc acetate (0.02M). Then, 20 mL $AgNO_3$ (0.1M) solution was slowly added till brown color was formed and the mixture was stirred for 20 mins at 70 ºC. The mixture was kept into the oven and heated for 10 hours at 60 °C. The solids were then cooled, centrifuged and finally dried in the muffle furnace using the same techniques. Pictures of essential steps of preparation of Ag/ZnO nanocomposites are shown in figure 11.

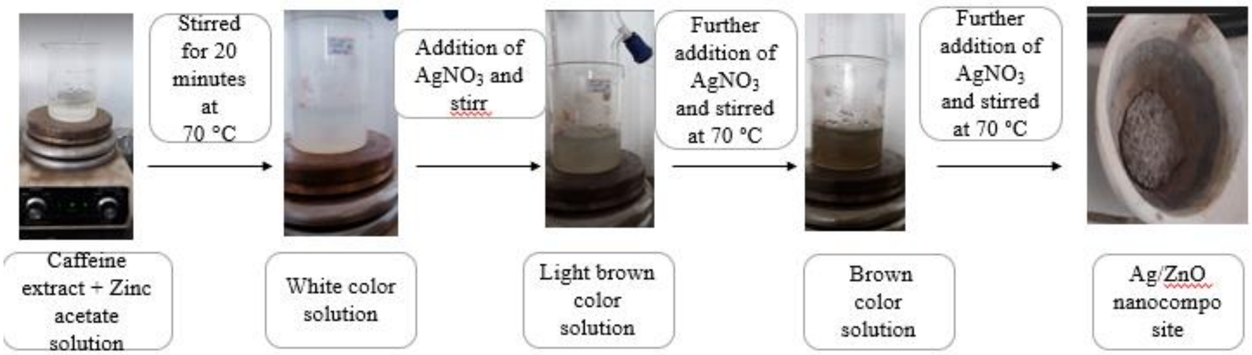


Figure 11. Preparation of Ag/ZnO nanocomposite

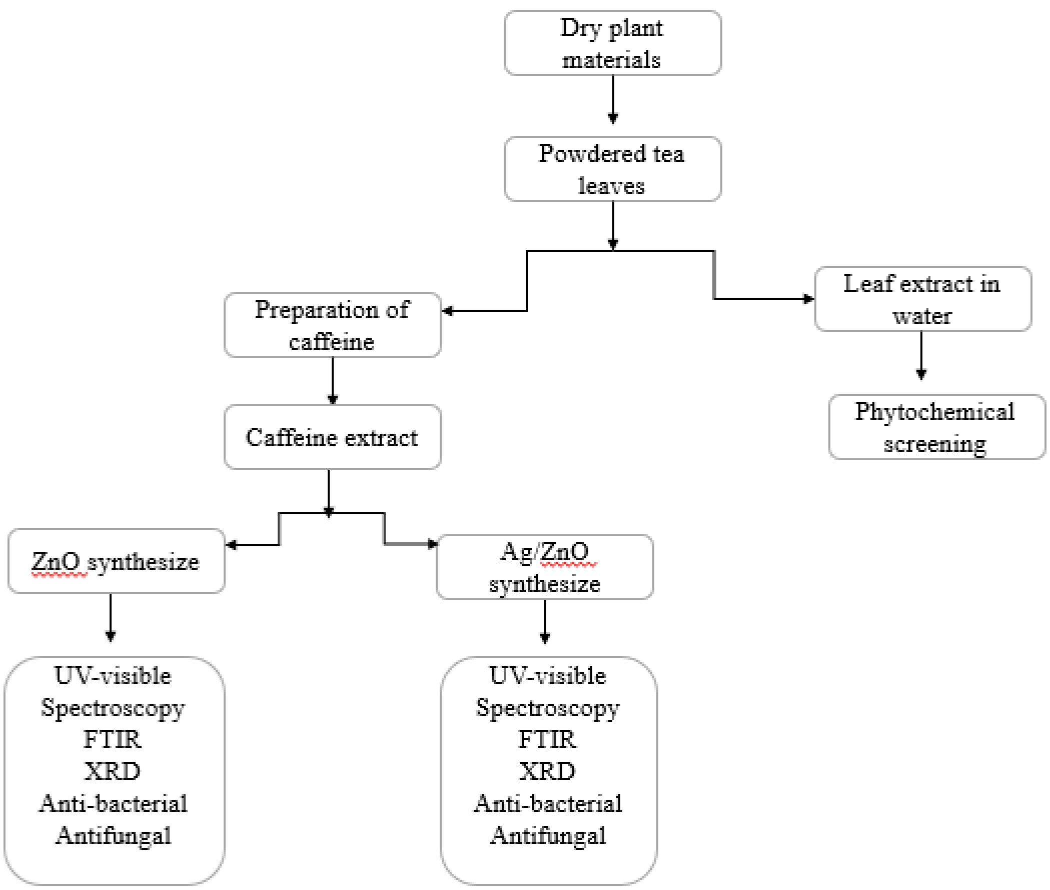


Figure 12. Schematic diagram for synthesizing nanoparticles, characterization and their biological activity

3.3 Biological Activity

Biological studies involve the study of the effect of materials like crude plant extract or any nanomaterials of various dose in a species of organism and prediction of its effect throughout the entire dose range. Present study is based on the analysis of caffeine extract from tea leaves mediated ZnO and Ag/ZnO nanocomposites and study their antimicrobial activity.

3.3.1 Antimicrobial Assay

Agar well diffusion was used to explore the antimicrobial properties of caffeine-mediated ZnO and Ag/ZnO nanocomposites against two bacterial strains, *Bacillus subtilis* (ATCC 6051) and *Escherichia coli* (ATCC 8739), and one fungus, *Candida albicans* (ATCC 2091) species. Zone of inhibition (ZOI) was used to determine the inhibition of microbial growth.

3.3.1.1 Collection of Test Organisms

The microbial strains were obtained from the Himalayan Research Institute of Biotechnology Private Limited. Name of the microorganisms used for the study is tabulated below in Table 4.

Table 4. Name of the micro-organisms used for antimicrobial assay.

Microorganism	Name of microorganisms	Type
Bacteria	*Bacillus subtilis* (ATCC 6051)	Gram positive
Bacteria	*Escherichia. coli* (ATCC 8739)	Gram negative
Fungi	*Candida albicans* (ATCC 2091)	Pathogenic fungus

3.3.1.2 Preparation of Nutrient Agar

Nutrient agar was prepared by mixing 9.5g of Muller Hinton Agar in 250 mL distilled water. The mixture was then heated to boiling with continuous shaking to dissolve completely. It was then autoclaved for 15 minutes at 121 ºC and 15 1b pressure to sterilize it. The agar was cooled at about 50 °C and poured into petri-plates in 20 mL/plate quantities. The plates were allowed to solidify.

3.3.1.3 Preparation of Nutrient Broth

The nutrient broth solution was made by boiling 13 g of the nutrient broth in 1000 mL of distilled water. It was then cooled at room temperature and 10 mL of broth was poured into the test tubes, capped tightly with cotton plugs and autoclaved for 15 minutes at 121 ºC and 15 1b pressure. Thus, prepared nutrient broth was used in the preparation of microbial inoculums.

3.3.1.4 Preparation of Standard Culture Inoculums

Standard culture inoculums were prepared from primary cultures plates. Using an inoculating loop from a primary culture plate, the colonies of the organisms to be examined having similar appearance were properly touched and then transferred to tubes containing 10 mL of sterile nutritional broth. The tubes were incubated overnight at 37 ºC.

3.3.1.5 Determination of Antimicrobial Activity

The as-synthesized ZnO Nps were evaluated for antimicrobial activity. In order to remove excess moisture from the surface of the media, the prepared sterile Mueller- Hinton Agar (MHA) plates were dried in a hot air oven. The agar plates for assay were labelled with the name of the microbes and nanoparticles. The inoculums of bacteria and fungus were transferred into the Mueller Hinton Agar plates having wells of 6 mm. Gram-negative bacteria *Escherichia coli (E. coli)* and gram-positive bacteria *Bacillus subtilis* were equally distributed on nutrient agar plates with 6 mm wells for the antibacterial experiment. Little amount of ZnO and Ag/ZnO

Nps was added to the wells under sterile conditions. They were then incubated at 37 °C for 12 hours. Using a ruler scale, the zone of inhibition (mm) around wells was measured, and digital photos of the plates were taken. Same procedure was applied for pathogenic fungus: *Candida albicans*. The standard antibiotic used against microbial strains was Kanamycin (5 mg/mL). The zone of inhibition (mm) was measured.

3.4 Characterization Techniques

As-synthesized ZnO and Ag/ZnO nanocomposites were subjected to several sophisticated instruments for characterization. In this context, UV-vis spectrophotometer was especially used for confirmation of nanoparticle and for studying optical properties. Similarly, the phase morphology and structural characterization of the nanomaterials were explored via Fourier Transform Infrared (FT-IR) Spectroscopy and X-ray Diffraction (XRD).

3.4.1 Visual Observation

At first the detection was carried out by visual observation based on the color of the samples.

3.4.2 UV- visible Spectroscopy

UV-visible double beam spectrophotometer was used to analyze nanoparticles. UV-vis spectroscopy is an essential tool for characterizing such materials that exhibit optical properties sensitive to refractive index, concentration, shape and size near the nanomaterial surface (Pentassuglia et al., 2018). The amount of light absorbed by the sample was measured using a UV-vis spectrophotometer (LABTRONIC, Model LT-2802), double beam at wavelength range of 200 to 800 nm. It was carried out in the research laboratory on the Amrit campus. Little amount of caffeine, ZnO Nps and Ag/ZnO nanocomposites were dissolved in ethanol and sonicated it for 10 minutes for the dispersion of each sample. The solution was kept in a cuvette and placed in the cavity of the UV-visible spectroscopy for characterization.

3.4.3 Fourier Transform Infrared (FT-IR) Spectroscopy

FTIR is used to determine the interaction of reducing and stabilizing agents on nanomaterial surfaces and the functional groups present in the sample. Green synthesis of nanomaterials proceeds with essential biomolecules obtaining from plant such as caffeine extracts which assumed as reducing and stabilizing agent preventing coagulation (Hind et al., 2001). Thus, FT-IR is important tool for determining the associated phytochemicals during green synthesis. The FT-IR spectra of nanoparticles were obtained using an FT-IR spectrophotometer (FTIR Elmer Spectrum 10.6.2) with a wavelength range of 500-4000 cm^{-1}. The FTIR analysis was carried out in Amrit campus research laboratory.

3.4.4 X-Ray Diffraction (XRD)

X-ray diffraction (BRUKETD-2 PHASE) was used to analyze the phase morphology of the produced ZnO Nps and Ag/ZnO nanocomposites. It was carried out in Nepal Academy of Science and Technology.

CHAPTER: IV RESULTS AND DISCUSSION

4.1 Visual Observation

Preliminarily, the glimpse of formation of nanoparticles was perceived from the direct observation with eyes.

The green synthesized ZnO nanoparticles were observed to be white. Generally ZnO nanoparticles were observed to be of white colour as reported by the literatures (Kavitha et al., 2017, Dhanemozhi et al., 2017, Kumar et al., 2019). Ag/ZnO nanocomposites were observed to be brown in color, which was similar to the color found in various literatures (Yeganeh-Faal et al., 2017, Kyomuhimbo et al., 2019). Picture of sample of ZnO and Ag/ZnO are depicted in figure 13.

Figure 13. Picture of samples: a) ZnO Nanoparticles, b) Ag-ZnO nanocomposites

Samples are then further characterized by UV-vis spectroscopy, FT-IR and XRD.

4.2 UV- Visible Spectroscopy

In Ultraviolet (UV)-visible spectroscopy, the molecule absorbs UV-visible light. Electrons excite from lower to higher energy levels when UV-visible radiation is absorbed. Only some functional groups (chromophores) in organic compounds with valence electrons with low excitation energy can absorb UV and visible photons (Pentassuglia et al., 2018). UV-visible spectroscopy is quick, cheap and simple characterization technique commonly used in the research of Nms (Faraji et al., 2021).

The UV-visible spectroscopic results of caffeine for the formation of caffeine of biosynthesized ZnO and Ag/ZnO nanoparticles are shown in figure 14.

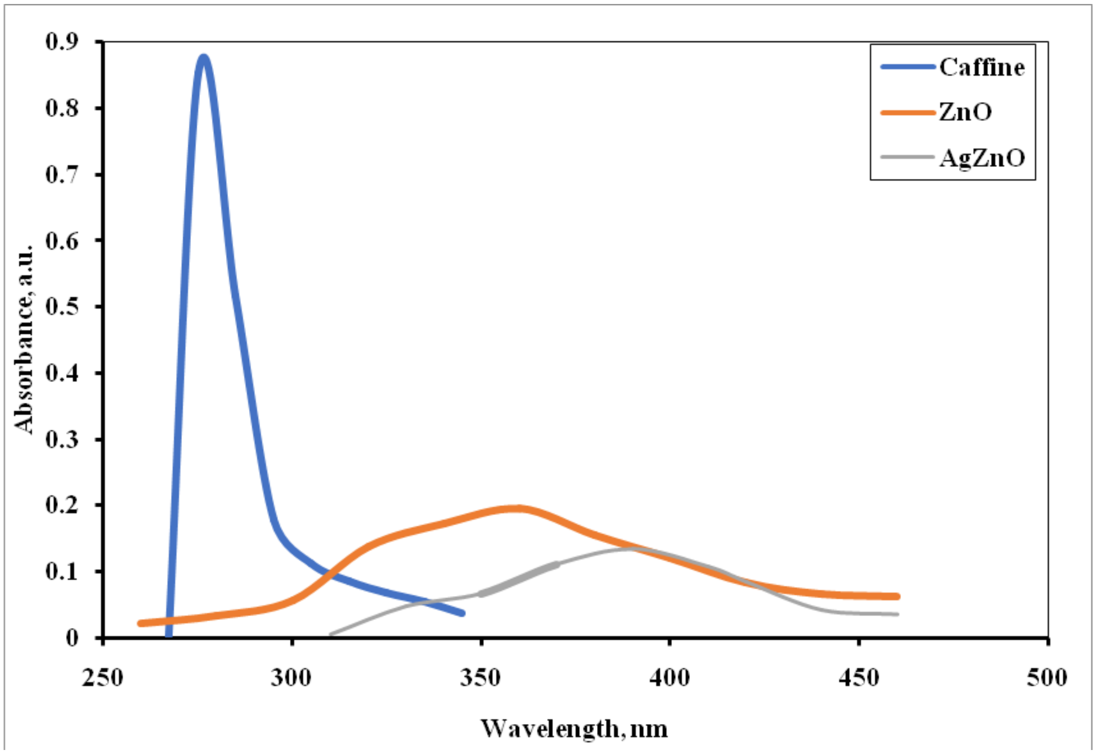


Figure 14. UV-vis spectrum of caffeine, and caffeine mediated ZnO and Ag/ZnO nanocomposite

Figure 14 shows the UV-vis absorption spectrum of caffeine, ZnO and Ag/ZnO nanocomposite in the spectral range of 250-500 nm at room temperature. The figure shows the formation of sharp absorbance peak at 275 nm due to presence of caffeine. The result mentioned is similar to that published in the report of Ashen Hurst, (2021), Belay et al (2008).

UV-vis absorbance peak observed at about 0.854 is due to the electronic transition of the solution at the maximum wavelength of 275 nm. Absorption at 275 nm indicates the longer wavelength and hence less energy photons are needed for this transition (Ashen Hurst, 2021). The maximum absorbance observed in this experiment is quite similar to the result reported by

Belay et al. (2008).

The UV-vis absorption spectrum of ZnO in ethanol synthesized using caffeine extract is indicated by orange colored line in figure 16. The maximum wavelength of ZnO appears at 360 nm which is around the characteristic UV-vis absorbance at about 0.195 of the ZnO nanoparticles. The result agrees with the results obtained by Muhammad. (2019). Thus, the presence of strong peak at 360 nm indicates the formation of ZnO Nps. The UV-vis absorption peak of caffeine mediated ZnO Nps are of broad nature indicating the presence of Nps of varied size distribution (Kavitha et al., 2017).

In the similar way, UV-vis absorption spectrum of Ag/ZnO nanocomposite synthesized from caffeine extract is shown by greyish colored line in above figure 16. The figure shows that the peak of Ag/ZnO is slightly shifted from 360 to 390 nm compared to that of ZnO Nps indicating the formation of nanocomposite. Literature reported that Ag/ZnO composites with molar ratios of 5 mol.% Ag has UV absorption peak at 390 nm. The UV absorbance band of composites appears to be shifted slightly when compared to that of pure_ZnO (Al-Ariki et al., 2021). The result of UV-vis spectroscopy supports well for the formation of Ag/ZnO.

Furthermore, from the UV-vis spectrum, band energy of as-fabricated nanomaterials was calculated. The minimum energy required to excite an electron from the valence band to the conduction band is band gap energy. The energy needed to release an electron increases with the band gap energy. Band gap energy is calculated following the equation based on literature (Malaescu et al., 2016).

$$E_g = 1240/\lambda(\text{nm})\ \text{eV} \ldots\ldots\ldots (2)$$

Where, E_g = band gap energy, λ = maximum wavelength in nm

The band gap energy of ZnO and Ag/ZnO nanocomposite was found to be 3.44 eV and 3.17 eV respectively. The lower band gap of Ag/ZnO nanocomposite confirm for the formation of nanocomposite. Literature reveals that the band gap is reduced as Ag concentration increased. The different factors responsible for the decrease in the band gap energy are grain size, carrier concentration, structural parameters and lattice strain and existence of defects or impurities (Ariki et al., 2021). The result reported about the decrease in band gap energy of nanocomposite is in agreement with the result obtained by Ariki et al. (2021), Rokesh et al. (2016). Therefore, the UV-vis spectra of prepared samples indicate for the formation of ZnO and Ag/ZnO nanocomposite.

4.3 Fourier Transform Infrared Spectroscopy

FTIR is one of the simple, and convenient method for structural characterization of materials. Biomolecules used as a capping agent in green synthesis are predicted using Fourier transform infrared spectroscopy (FTIR) (Zahrani et al., 2021). Figure 15 represents the FTIR spectrum of ZnO and Ag/ZnO nanocomposite which are synthesized from caffeine extract of *camellia sinensis* leaves.

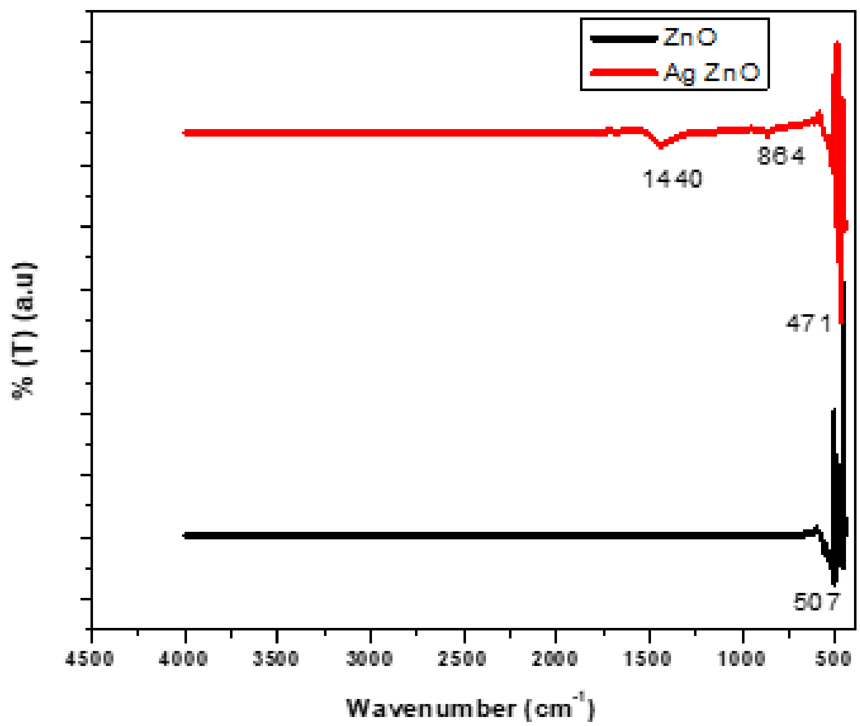


Figure 15. Fourier transform infrared spectroscopy of ZnO Nps and Ag/ZnO nanocomposite synthesized from caffeine extract of tea leaves

Small absorption band observed at 507 cm^{-1} is the characteristic IR peak for Zn-O bond. It is supported by the literature (Xiong et al., 2006). The intensity decreased from 507 to 471 cm^{-1} due to formation of Ag nanoparticles on ZnO surface (Nagaraju et al., 2017). Weak band at 864 cm^{-1} is due to the deformation of the aromatic C-H bonds in the caffeine molecule. The weak spectrum in the region of 1305 to 1507 cm^{-1} is responsible for formation of Ag/ZnO nanocomposite.

A wide peak in the range of 3408 and 3106 to 2952 cm^{-1} corresponds to the stretching vibration of the N-H group in the pyrimidine ring of caffeine and aromatic C-H stretch in the caffeine molecule respectively. The peak at 1661 cm^{-1} is responsible to C-N ring stretching (Rajam et al., 2013). The FT-IR spectrum lack other peaks of biomolecules which may be due to effect of high temperature given during calcinations process. The peak for phenolic compound is not visible;

this might be because the phenolic compound vanishes after being calcined at high temperatures. Similar result was obtained by (Georgekutty et al., 2008). At 400 °C, the peaks associated with the organic precursor are not visible, only nanosized ZnO at 490 cm $^{-1}$ is visible (Georgekutty et al., 2008).

4.4 X-Ray Diffraction

A non-destructive technique commonly used to analyze the phase structure of materials is X-Ray Diffraction. When an X-ray beam is pointed at a sample, the scattered intensity is calculated in relation to the outgoing direction. The sample's crystalline structure is revealed by the diffraction pattern when the beam is splitted.

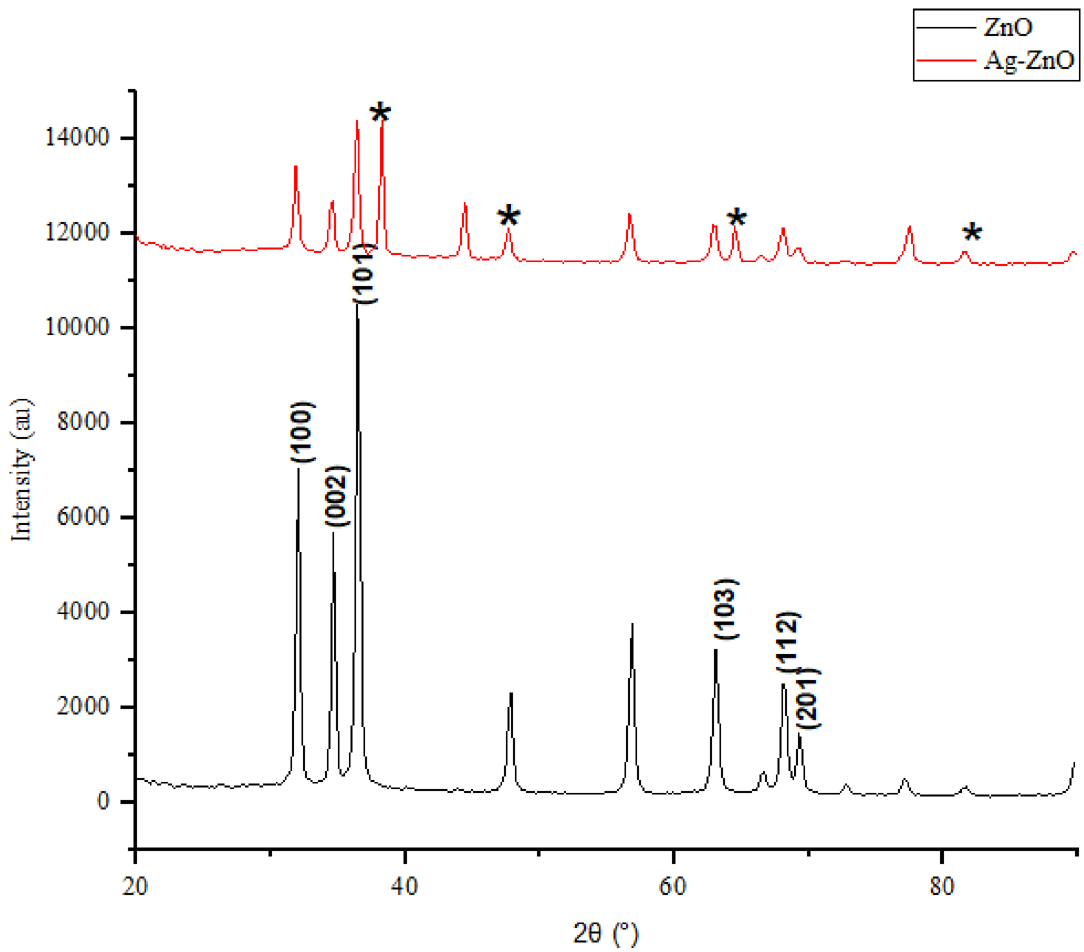


Figure 16. XRD pattern of ZnO and Ag/ZnO synthesized from caffeine extract of tea leaves

The XRD pattern of ZnO nanoparticle synthesized through caffeine extract of tea leaves is shown in figure 16. The diffraction pattern of ZnO Nps shows sharp peaks at 31.96, 34.71, 36.29, 47.94, 57, 63, 66, 68.4, 72, 76 and 78. The sharp peaks at 31.96, 34.71 and 36.29 are responsible to the crystal planes (100), (002) and (101) respectively. It represents the zincite (ZnO) hexagonal crystal plane. Zincite may also be responsible for the subsequent strong peaks in the XRD pattern with Miller indices (103), (112), and (201) at 63, 66, and 68.4. The XRD result is justified from the JCPD file no. JCPDS: 36-1451 based on reference (Kumar et al., 2019). The presented results are consistent with the XRD results of ZnO nanoparticles published in previous research (Chaudhuri and Malodia, 2017, Kumar et al., 2019). These XRD results agreed with previous studies that verified the produced ZnO sample has a hexagonal wurtzite crystalline structure (Agarwal et al., 2017).

Figure 16 above displays the XRD pattern of an Ag/ZnO nanocomposite that was made using caffeine extract of tea leaf. Ag/ZnO nanocomposite's XRD pattern shows three diffraction peaks that can be correlated to cubic phase silver (JCPDS No. 01-087-0597), at 38.1, 44.3, and 64.4. This indicates that ZnO surface has been successfully loaded with silver nanoparticles (Dou at al., 2015). Similar additional peaks are observed at 38.31, 44.4, 64.5 and 81.4 in fig 16 along with characteristic peaks of ZnO Nps. The presence of additional peaks at 38.31, 44.4, 64.5 and 81.4 in the XRD and several secondary phases confirms formation of Ag/ZnO nanocomposite. The precursor $AgNO_3$ produced these phases as a result of its reaction product (used for synthesis) and oxides of Ag [Ag-Zn, Ag_2O_3 and Ag_2O] (Kumar et al., 2019).

Using the Debye-Scherrer formula, the average particle size (D) of synthesized nanoparticles was calculated as,

$$D = K\lambda/\beta \cos\theta \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where,

K = Scherrer constant (0.9)

λ = wavelength of X-ray source (CuK_α line = 0.1541nm)

β = full width at half maximum (FWHM) in radians

θ = Bragg`s diffraction angle

The average crystallite size of ZnO Nps is 27.98 nm and Ag/ZnO nanocomposite is 33.4 nm as calculated from the nonlinear fit.

4.5 Phytochemical Screening

The standard procedure was followed while screening the phytochemical composition of the aqueous extract of tea leaves. The results are shown in Table 5.

Table 5. Phytochemical screening of leaf extract of *Camelia sinesis*

Phytoconstituents	**Test Used**	**Water extract**
Alkaloids	Maeyer's Test Dragendroff's Test	+
Flavonoid	NH_4OH test	+
Glycosides	Molisch's Test	+
Polyphenols	$FeCl_3$ Test	+
Terpenoids	Salkowski Test	+
Quinones	HCl test	+

+ indicates presence

The result shows that the aqueous extract of *Camelia sinesis* contains the phytochemicals like alkaloids, flavonoid, glycosides, polyphenols, terpenoids, quinones which is in agreement with the result obtained by Jain et al., (2011) except for terpenoids. The result obtained by Anita et al., (2015) showed the presence of glycosides, tannin, flavonoid, terpenoid, quinone, alkaloid, in aqueous extracts of fresh tea leaves.

4.6 Antimicrobial Assay

Nanomaterials as antibacterial supplements to drugs are extremely promising and receiving widespread interest because they may cover gaps where medicines commonly fail (Beyth et al., 2015). Furthermore, as an effective transporter, nanoparticles can replace and enhance standard antibiotics (Wamg et al., 2017).

The agar well diffusion method was used to evaluate the nanoparticles' antimicrobial activity against the Gram-negative bacteria *Escherichia coli*, Gram positive bacteria *Bacillus subtilis* and fungus *Candida albicans*. Each 6 mm well was filled with ZnO and Ag/ZnO. During the antibacterial test, equivalent volume of 50% DMSO, and conventional antibiotic, *kanamycin* (5 mg/mL) were used as negative and positive control respectively. The clear inhibition zones of microbial growth around the wells were observed in the presence of different nanoparticles at the end of the incubation period. The results are depicted in figure 17.

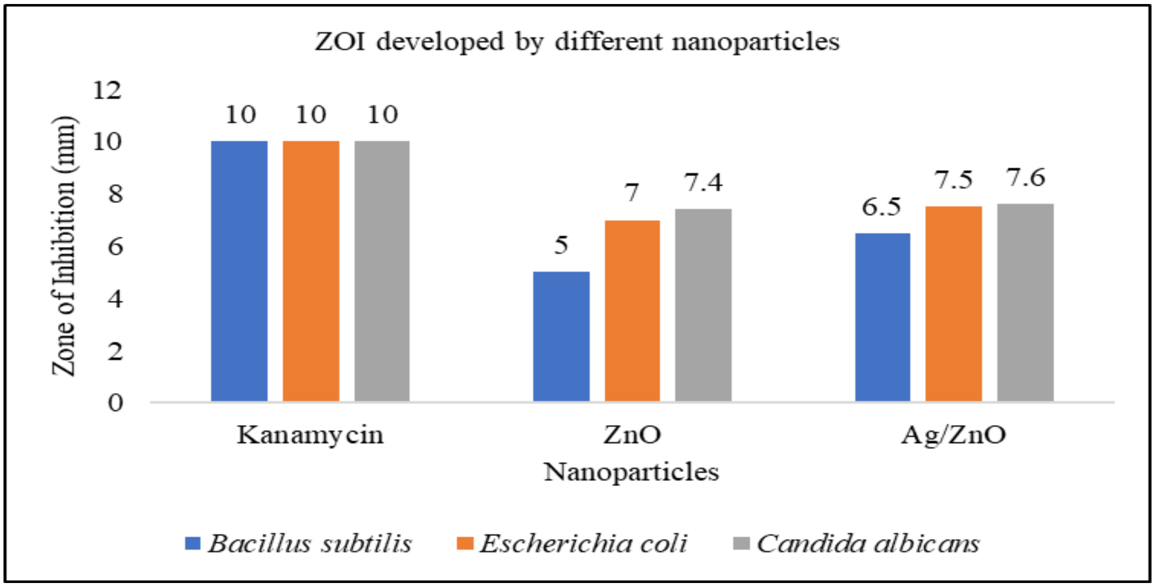


Figure 17. Zone of inhibition developed by different nanoparticles for different test microorganisms

The zone of inhibition developed by different nanoparticles for different test microorganisms is shown in figure 17. The picture shows that the effect of ZnO nanoparticles on *B. subtilis* is weak, with a zone of inhibition of 5.0 mm as compared to the positive control Kanamycin (10.0 mm), and that it improves when Ag is added (ZOI = 6.5 mm). The antibacterial activity of ZnO (7 mm) against *E. coli* improved with the addition of silver (ZOI = 7.5 mm). The ZOI of 7.4 mm for ZnO and 7.6 mm for Ag/ZnO shows an improvement against *Candida albicans*. The microbial susceptibility increased significantly as the silver content of the nanocomposite increased (Franci et al., 2015). Zinc oxide nanoparticles have a biocidal effect on *B. subtilis*, *E. coli* and *C. albicans* due to their ability to generate oxygen species on their surface, which penetrates bacterial cell membranes and kills the bacteria (Siddiqi et al., 2018). The result is supported by Senthilkumar and Shivakumar, (2014), who found that Gram negative bacteria had a high sensitivity to green produced ZnO nanoparticles at a concentration of 20 µg/mL.

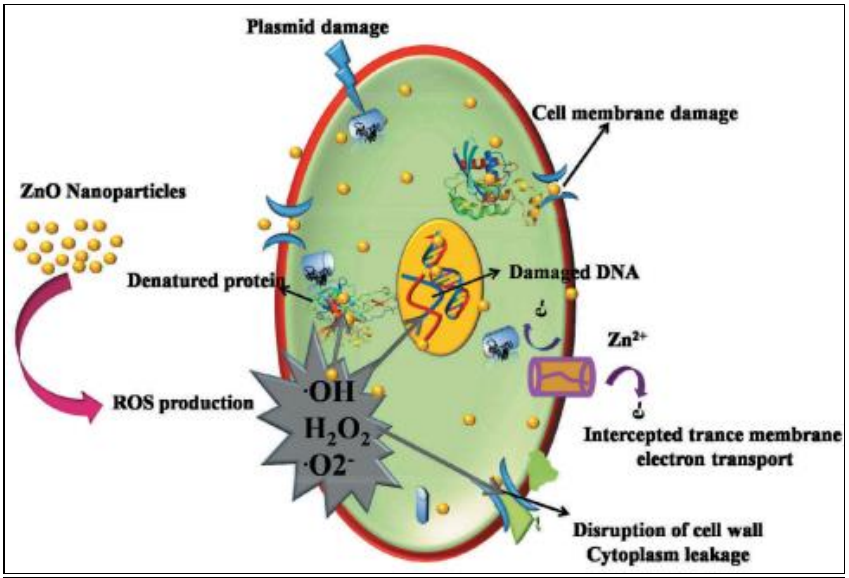


Figure 18 Schematic diagram showing the ZnO nanoparticles are poisonous (Kim et al., 2020)

Figure 18 shows the ZnO Nps are poisonous to bacteria. Due to their interfacial potential, ZnO Nps have antibacterial action against both Gram-positive and Gram-negative bacteria. Mechanical stress increases the production of reactive oxygen when bacteria with negative surface potentials contact with the positively charged ZnO surface resulting in membrane depolarization. The *S. aureus* cell membrane was less negatively charged than *E. coli*. This could possibly be the reason for the enhanced resistance against ZnO Nps activity in *E. coli* (Sonohara et al., 1995).

CHAPTER V: CONCLUSION

5.1 Conclusion

ZnO nanoparticles and Ag/ZnO nanocomposite was successfully prepared using caffeine extract of tea leaves. As-synthesized ZnO nanoparticles and Ag/ZnO nanocomposite were characterized by UV-vis, FT-IR and XRD. At first, UV-vis absorption peak at 360 nm and 390 nm reveals formation of ZnO nanoparticles and Ag/ZnO nanocomposite, respectively. The band gap energy calculated to be 3.44eV and 3.17eV respectively for nanoparticles and nanocomposite. The synthesized nanoparticles were found to consist of crystalline nature possessing hexagonal wurtzite crystals. The absorption band appeared at 507 cm^{-1} and the weak bands in the region of 1305 to 1507 cm^{-1} in FT-IR spectrum confirmed for formation of ZnO and Ag/ZnO nanocomposite. Due to the substitution of ZnO lattice with Ag, it exhibits the slight change in the position of bands in the composite. The average size of ZnO and Ag/ZnO was calculated to be 27.98 nm and 33.4 nm as revealed from XRD data.

Moreover, the nanomaterials were further studied for its antimicrobial activity. The antimicrobial activity was studied for gram-positive, gram-negative bacteria and anti-fungus. When compared to ZnO nanoparticles, the nanocomposite demonstrated superior antibacterial activities against a broad spectrum of bacteria and fungus, which may be indicated by their lower size. Both had a greater zone of inhibition for *E. coli* and a lower zone for *Bacillus subtilis*. Comparatively, the large zone of inhibition of the fungi (*Candida albicans)* was observed. This approach produces stable ZnO and Ag/ZnO nanocomposite that can be stored for several months in an inert environment.

5.2 Suggestions for Further Work

The several parts of the tea, such as the root, stem, leaves, and tea itself, may be utilized to make numerous nanoparticles and nanocomposites. Aqueous extract of caffeine may be changed to solvents such as ethanol, methanol, or dichloromethane for getting better yield than that in aqueous extract. The existence of several medically active compounds is shown by a phytochemical screening of the leaf extract in water. It may be extracted in large quantities using various solvents such as methanol, ethanol, ethyl acetate, and so on. Many other biological and catalytic properties of the ZnO Nps and Ag/ZnO nanocomposites could be studied in future.

CHAPTER VI: REFERENCES

Aboulwafa, M, M., Youssef, S, F., Gad, A, H., Altyar, E, A., Al-Azizi, M, M., & Ashour, L, M. (2019). A comprehensive insight on the health benefits and phytoconstituents of *camellia sinensis* and recent approaches for its quality control. *Antioxidants*, *8*(10), 455-487. https://doi.org/10.3390/antiox8100455

Agarwal, H., Venkat Kumar, S., & Rajeshkumar, S. (2017). A review on green synthesis of zinc oxide nanoparticles – an eco-friendly approach. *Resource-Efficient Technologies*, *3*(4), 406–413. https://doi.org/10.1016/j.reffit.2017.03.002

Ai, T., Fan, Y., Wang, H., Zou, X., Bao, W., Deng, Z., Zhao, Z., Li, M., Kou, L., Feng, X., & Li, M. (2021). Microstructure and properties of Ag-doped ZnO grown hydrothermally on a graphene-coated polyethylene terephthalate bilayer flexible substrate. *Frontiers in Chemistry, 9*. https://doi.org/10.3389/fchem.2021.661127

Al-Ariki, S., Yahya, N. A. A., Al-A'nsi, S. A., Jumali, M. H. H., Jannah, A. N., & Abd-Shukor, R. (2021). Synthesis and comparative study on the structural and optical properties of ZnO doped with Ni and Ag nanopowders fabricated by sol gel technique. *Scientific reports*, *11*(1), 11948-11959. https://doi.org/10.1038/s41598-021-91439-1

Alharthi, F. A., Alghamdi, A. A., Al-Zaqri, N., Alanazi, H. S., Alsyahi, A. A., Marghany, A. E., & Ahmad, N. (2020). Facile one-pot green synthesis of Ag-ZnO Nanocomposites using potato peeland their Ag concentration dependent photocatalytic properties. *Scientific reports*, *10*(1), 20229-20243. https://doi.org/10.1038/s41598-020-77426-y

Alhujaily, M., Albukhaty, S., Yusuf, M., Mohammed, M. K., Sulaiman, G. M., Al-Karagoly, H., Alyamani, A. A., Albaqami, J., & AlMalki, F. A. (2022). Recent advances in plant-mediated zinc oxide nanoparticles with their significant biomedical properties. *Bioengineering*, *9*(10), 541-560. https://doi.org/10.3390/bioengineering9100541

Ali, A., Chiang, Y. W., & Santos, R. M. (2022). X-ray diffraction techniques for mineral characterization: A review for engineers of the fundamentals, applications, and Research Directions. *Minerals*, *12*(2), 205-230. https://doi.org/10.3390/min12020205

Al-Zahrani, S., Astudillo-Calderón, S., Pintos, B., Pérez-Urria, E., Manzanera, J. A., Martín, L., & Gomez-Garay, A. (2021). Role of synthetic plant extracts on the production of silver-derived nanoparticles. *Plants*, *10*(8), 1671-1691. https://doi.org/10.3390/plants10081671

Ambu, G., Chaudhary, R. P., Mariotti, M., &Cornara, L. (2020). Traditional uses of medicinal plants by ethnic people in the Kavrepalanchok District, central Nepal. *Plants*, *9*(6), 759-793. https://doi.org/10.3390/plants9060759

Andrew, E, I., G. Sunday, H., A. Ukamaka, I., & O. Bamidele, T. (2022). Bioactive components and antioxidant properties of aqueous leaf extract of fresh and processed *camellia sinensis* in alloxan-induced Albino Rats. *Asian Journal of Biotechnology and Bioresource Technology*, 8 (3) 62–74. https://doi.org/10.9734/ajb2t/2022/v8i330130

Anita, P., Balan, I. N., Ethiraj, S., Madan Kumar, P. D., & Sivasamy, S. (2015). In vitro antibacterial activity of *camellia sinensis* extract against cariogenic microorganisms. *Journal of Basic and Clinical Pharmacy*, *6*(1), 35-39. https://doi.org/10.4103/0976-0105.145777

Anjum, S., Hashim, M., Malik, S. A., Khan, M., Lorenzo, J. M., Abbasi, B. H., & Hano, C. (2021). Recent advances in zinc oxide nanoparticles (ZnO Nps) for cancer diagnosis, target drug delivery, and treatment. *Cancers*, *13*(18), 4570-4601. https://doi.org/10.3390/cancers13184570

Ashenhurst, J. (2021, May 6). *UV-vis spectroscopy: Absorbance of carbonyls*. Master Organic Chemistry. Retrieved September 22, 2022, from https://www.masterorganicchemistry.com/2016/09/26/uv-vis-spectroscopy-absorbance-of-carbonyls/

Aziz, M. A., Khan, A. H., Adnan, M., & Ullah, H. (2018). Traditional uses of medicinal plants used by indigenous communities for veterinary practices at Bajaur Agency, Pakistan. *Journal of Ethnobiology and Ethnomedicine*, *14*(1). https://doi.org/10.1186/s13002-018-0212-0

Baig, N., Kammakakam, I., & Falath, W. (2021). Nanomaterials: A review of synthesis methods, properties, recent progress, and challenges. *Materials Advances*, *2*(6), 1821–1871. https://doi.org/10.1039/d0ma00807a

Barber, D. J., & Freestone, I. C. (1990). An investigation of the origin of the colour of the Lycurgus Cup by Analytical Transmission Electron Microscopy. *Archaeometry*, *32*(1), 33–45. https://doi.org/10.1111/j.1475-4754.1990.tb01079.x

Bayda, S., Adeel, M., Tuccinardi, T., Cordani, M., &Rizzolio, F. (2019). The history of nanoscience and nanotechnology: From chemical–physical applications to nanomedicine. *Molecules*, *25*(1), 112-127. https://doi.org/10.3390/molecules25010112

Belay, A., Ture, K., Redi, M., & Asfaw, A. (2008). Measurement of caffeine in coffee beans with UV/vis spectrometer. *Food Chemistry*, *108*(1), 310–315. https://doi.org/10.1016/j.foodchem.2007.10.024

Beyth, N., Houri-Haddad, Y., Domb, A., Khan, W., & Hazan, R. (2015). Alternative antimicrobial approach: Nano-Antimicrobial Materials. *Evidence-Based Complementary and Alternative Medicine*, *2015*, 1–16. https://doi.org/10.1155/2015/246012

Bharat, T. C., Shubham, Mondal, S., S.Gupta, H., Singh, P. K., & Das, A. K. (2019). Synthesis of doped zinc oxide nanoparticles: A Review. *Materials Today: Proceedings*, *11*, 767–775. https://doi.org/10.1016/j.matpr.2019.03.041

Boehm, K., Horneber, M., Borrelli, F., & Ernst, E. (2004). Green tea (*camellia sinensis*) for the prevention of cancer. *Cochrane Database of Systematic Reviews*. https://doi.org/10.1002/14651858.cd005004

Chandrasekaran, J., Brumin, M., Wolf, D., Leibman, D., Klap, C., Pearlsman, M., Sherman, A., Arazi, T., & Gal-On, A. (2016). Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology. *Molecular plant pathology*, *17*(7), 1140–1153. https://doi.org/10.1111/mpp.12375

Chang, W.-C., Cheng, Y.-Y., Yu, W.-C., Yao, Y.-C., Lee, C.-H., & Ko, H.-H. (2012). Enhancing performance of ZnO dye-sensitized solar cells by incorporation of multiwalled carbon nanotubes. *Nanoscale Research Letters*, *7*(1). https://doi.org/10.1186/1556-276x-7-166

Chaudhuri, S. K., & Malodia, L. (2017). Biosynthesis of zinc oxide nanoparticles using leaf extract of *Calotropis gigantea*: Characterization and its evaluation on tree seedling growth in nursery stage. *Applied Nanoscience*, *7*(8), 501–512. https://doi.org/10.1007/s13204-017-0586-7

Cuadra, J. G., Scalschi, L., Vicedo, B., Guc, M., Izquierdo-Roca, V., Porcar, S., Fraga, D., & Carda, J. B. (2022). ZnO/Ag nanocomposites with enhanced antimicrobial activity. *Applied Sciences*, *12*(10), 5023-5036. https://doi.org/10.3390/app12105023

Dhandapani, P., Siddarth, A. S., Kamalasekaran, S., Maruthamuthu, S., & Rajagopal, G. (2014). Bio-approach: Ureolytic bacteria mediated synthesis of ZnO nanocrystals on cotton fabric and evaluation of their antibacterial properties. *Carbohydrate polymers*, *103*, 448–455. https://doi.org/10.1016/j.carbpol.2013.12.074

Dhanemozhi, A. C., Rajeswari, V., & Sathyajothi, S. (2017). Green synthesis of zinc oxide nanoparticle using green tea leaf extract for supercapacitor application. *Materials Today: Proceedings*, *4*(2), 660–667. https://doi.org/10.1016/j.matpr.2017.01.070

Dou, P., Tan, F., Wang, W., Sarreshteh, A., Qiao, X., Qiu, X., & Chen, J. (2015). One-step microwave-assisted synthesis of Ag/ZnO/graphene nanocomposites with enhanced photocatalytic activity. *Journal of Photochemistry and Photobiology A: Chemistry*, *302*, 17–22. https://doi.org/10.1016/j.jphotochem.2014.12.012

Espitia, P. J. P., Otoni, C. G., & Soares, N. F. F. (2016). Zinc oxide nanoparticles for food packaging applications. *Antimicrobial Food Packaging*, 425–431. https://doi.org/10.1016/b978-0-12-800723-5.00034-6

Faraji, M., Yamini, Y., & Salehi, N. (2021). *Characterization of magnetic nanomaterials*. Magnetic Nanomaterials in Analytical Chemistry. Retrieved July 25, 2022, from https://www.sciencedirect.com/science/article/pii/B9780128221310000145

Franci, G., Falanga, A., Galdiero, S., Palomba, L., Rai, M., Morelli, G., & Galdiero, M. (2015). Silver nanoparticles as potential antibacterial agents. *Molecules (Basel, Switzerland)*, *20*(5), 8856–8874. https://doi.org/10.3390/molecules20058856

Georgekutty, R., Seery, M. K., & Pillai, S. C. (2008). A highly efficient Ag/ ZnO photocatalyst: Synthesis, properties, and mechanism. *The Journal of Physical Chemistry C*, *112*(35), 13563–13570. https://doi.org/10.1021/jp802729a

Gonçalves Bortolini, D., Windson Isidoro Haminiuk, C., Cristina Pedro, A., de Andrade Arruda Fernandes, I., & Maria Maciel, G. (2021). Processing, chemical signature and food industry applications of *Camellia sinensis* teas: An overview. *Food Chemistry: X*, *12*, 100160. https://doi.org/10.1016/j.fochx.2021.100160

Government of Nepal national tea and Coffee Development Board 2019/020. National Tea and Coffee Development Board: Government of Nepal. (n.d.). Retrieved July 6, 2022, from https://teacoffee.gov.np/

Hoseinnejad, M., Jafari, S. M., & Katouzian, I. (2017). Inorganic and metal nanoparticles and their antimicrobial activity in food packaging applications. *Critical Reviews in Microbiology*, *44*(2), 161–181. https://doi.org/10.1080/1040841x.2017.1332001

Hosseini, S. M., Sarsari, I. A., Kameli, P., & Salamati, H. (2015). Effect of Ag doping on structural, optical, and photocatalytic properties of ZnO nanoparticles. *Journal of Alloys and Compounds*, *640*, 408–415. https://doi.org/10.1016/j.jallcom.2015.03.136

Iravani, S. (2011). Green synthesis of metal nanoparticles using plants. *Green Chemistry*, *13*(10), 2638-2650. https://doi.org/10.1039/c1gc15386b

Irshad, S., Salamat, A., Anjum, A. A., Sana, S., Saleem, R. S. Z., Naheed, A., & Iqbal, A. (2018). Green tea leaves mediated ZnO nanoparticles and its antimicrobial activity. *Cogent Chemistry*, *4*(1), 1469207. https://doi.org/10.1080/23312009.2018.1469207

Jain, D. P., Pancholi, S. S., & Patel, R. (2011). Synergistic antioxidant activity of green tea with some herbs. *Journal of Advanced Pharmaceutical Technology & Research*, *2*(3), 177. https://doi.org/10.4103/2231-4040.85538

Jan, T., Iqbal, J., Ismail, M., Zakaullah, Badshah, & Haider, S. (2013). Sn doping induced enhancement in the activity of ZnO nanostructures against antibiotic resistant *S. aureus bacteria*. *International Journal of Nanomedicine,* 3679-3687. https://doi.org/10.2147/ijn.s45439

Jin, S.-E., & Jin, H.-E. (2021). Antimicrobial activity of zinc oxide nano/microparticles and their combinations against pathogenic microorganisms for biomedical applications: From physicochemical characteristics to pharmacological aspects. *Nanomaterials, 11*(2), 263-298. https://doi.org/10.3390/nano11020263

Joshi, L. P., Poudel, Y., Nakarmi, M. L., Niraula, P. R., & Shrestha, S. P. (2017). Preparation and characterization of zinc oxide based photoanode for dye-sensitized solar cell using delonix regia natural dye extract. *Journal of Nepal Physical Society*, *4*(1), 1-6. https://doi.org/10.3126/jnphyssoc.v4i1.17329

Kalauni, D., Joshi, B., & Joshi, A. (2020). Production, marketing, and future prospects of Nepali Orthodox Tea. *Cogent Food & Agriculture*, *6*(1), 1757227. https://doi.org/10.1080/23311932.2020.1757227

Kavitha, S., Dhamodaran, M., Prasad, R., & Ganesan, M. (2017). Synthesis and characterization of zinc oxide nanoparticles using terpenoid fractions of *andrographis paniculata* leaves. *International Nano Letters*, *7*(2), 141–147. https://doi.org/10.1007/s40089-017-0207-

KC, Y., Parajuli, A., Khatri, B. B., & Shiwakoti, L. D. (2020). Phytochemicals and quality of green and black teas from different clones of Tea Plant. *Journal of Food Quality*, *2020*, 1–13. https://doi.org/10.1155/2020/8874271

Khan, G. R., & Khan, R. A. (2015). Ergonomic synthesis suitable for industrial production of silver-festooned zinc oxide nanorods. *International Journal of Nanoscience*, *14*(4), 1550018-1550026. https://doi.org/10.1142/s0219581x15500180

Khatami, M., Varma, R. S., Zafarnia, N., Yaghoobi, H., Sarani, M., & Kumar, V. G. (2018). Applications of green synthesized Ag, ZnO and Ag/ZnO nanoparticles for making clinical antimicrobial wound-healing bandages. *Sustainable Chemistry and Pharmacy*, *10*, 9–15. https://doi.org/10.1016/j.scp.2018.08.001

Kim, I., Viswanathan, K., Kasi, G., Thanakkasaranee, S., Sadeghi, K., & Seo, J. (2020). ZnO nanostructures in active antibacterial food packaging: Preparation methods, antimicrobial mechanisms, safety issues, future prospects, and challenges. *Food Reviews International*, *38*(4), 537–565. https://doi.org/10.1080/87559129.2020.1737709

Koca, F. D., Halici, M. G., Işik, Y., & Ünal, G. (2022). Green synthesis of Ag-ZnO nanocomposites by using *usnea florida* and *pseudevernia furfuracea* lichen extracts and evaluation of their neurotoxic effects. *Inorganic and Nano-Metal Chemistry*, 1–8. https://doi.org/10.1080/24701556.2022.2078351

Kumar, K., Dinesh, N. D., & Murari, S. K. (2019). Microwave assisted green synthesis of zno and Ag doped ZnO nanoparticles as antifungal and antibacterial agents using *colocasia esculenta* leaf extract. *International Journal of Nanoparticles, 11*(3), 239. https://doi.org/10.1504/ijnp.2019.102624

Kuponiyi, A., Kassama, L., & Kukhtareva, T. (2014). Physicochemical characterization of silver nanoparticles synthesizes using Aloe Vera (*Aloe barbadensis*). *SPIE Proceedings*. https://doi.org/10.1117/12.2062170

Kyomuhimbo, H. D., Michira, I. N., Mwaura, F. B., Derese, S., Feleni, U., & Iwuoha, E. I. (2019). Silver–zinc oxide nanocomposite antiseptic from the extract of Bidens pilosa. *SN Applied Sciences*, *1*(7). https://doi.org/10.1007/s42452-019-0722-y

Makarov, V. V., Love, A. J., Sinitsyna, O. V., Makarova, S. S., Yaminsky, I. V., Taliansky, M. E., & Kalinina, N. O. (2014). “Green” nanotechnologies: Synthesis of metal nanoparticles using plants. *Acta Naturae*, *6*(1), 35–44. https://doi.org/10.32607/20758251-2014-6-1-35-44

Malaescu, D., Grozescu, I., Sfirloaga, P., Vlazan, P., & Marin, C. N. (2016). The electrical properties of some composite materials based on sodium and tantalum oxides. *Acta Physica Polonica A*, *129*(1), 133–137. https://doi.org/10.12693/aphyspola.129.133

Matai, I., Sachdev, A., Dubey, P., Uday Kumar, S., Bhushan, B., & Gopinath, P. (2014). Antibacterial activity and mechanism of Ag– ZnO nanocomposite on *S. aureus* and GFP-expressing antibiotic resistant *E. coli*. *Colloids and Surfaces B: Biointerfaces*, *115*, 359–367. https://doi.org/10.1016/j.colsurfb.2013.12.005

McClements, D. J., DeLoid, G., Pyrgiotakis, G., Shatkin, J. A., Xiao, H., & Demokritou, P. (2016). The role of the food matrix and gastrointestinal tract in the assessment of biological properties of ingested engineered nanomaterials (ienms): State of the science and knowledge gaps. *NanoImpact*, *3-4*, 47–57. https://doi.org/10.1016/j.impact.2016.10.002

Mittal, A. K., Chisti, Y., & Banerjee, U. C. (2013). Synthesis of metallic nanoparticles using plant extracts. *Biotechnology Advances*, *31*(2), 346–356. https://doi.org/10.1016/j.biotechadv.2013.01.003

Muhammad, W., Ullah, N., Haroon, M., & Abbasi, B. H. (2019). Optical, morphological and biological analysis of zinc oxide nanoparticles (ZnO Nps) using *Papaver somniferum* L. *RSC Advances*, *9*(51), 29541–29548. https://doi.org/10.1039/c9ra04424h

Muñoz-Fernandez, L., Sierra-Fernandez, A., Milošević, O., & Rabanal, M. E. (2016). Solvothermal synthesis of Ag/ZnO and Pt/ZnO nanocomposites and comparison of their photocatalytic behaviors on dyes degradation. *Advanced Powder Technology*, *27*(3), 983–993. https://doi.org/10.1016/j.apt.2016.03.021

Nagaraju, G., Udayabhanu, Shivaraj, Prashanth, S. A., Shastri, M., Yathish, K. V., Anupama, C., & Rangappa, D. (2017). Electrochemical heavy metal detection, photocatalytic, photoluminescence, biodiesel production and antibacterial activities of Ag– ZnO nanomaterial. *Materials Research Bulletin*, *94*, 54–63. https://doi.org/10.1016/j.materresbull.2017.05.043

Noohpisheh, Z., Amiri, H., Farhadi, S., & Mohammadi-gholami, A. (2020). Green synthesis of Ag-ZnO nanocomposites using *Trigonella foenum-graecum* leaf extract and their antibacterial, antifungal, antioxidant and photocatalytic properties. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *240*, 118595-118616. https://doi.org/10.1016/j.saa.2020.118595

Oran, D., Rodriques, S. G., Gao, R., Asano, S., Skylar-Scott, M. A., Chen, F., Tillberg, P. W., Marblestone, A. H., & Boyden, E. S. (2018). 3D nanofabrication by volumetric deposition and controlled shrinkage of patterned scaffolds. *Science*, *362* (6420), 1281–1285. https://doi.org/10.1126/science.aau5119

Oudhia, A., & Choudhary, A. (2014). Microwave pulse-assisted wet chemical synthesis of ZnO nanoparticles with excellent UV emission. *2014 IEEE 2nd International Conference on Emerging Electronics (ICEE)*. https://doi.org/10.1109/icemelec.2014.7151181

Pentassuglia, S., Agostino, V., & Tommasi, T. (2018). EAB—electroactive biofilm: A biotechnological resource. *Encyclopedia of Interfacial Chemistry*, 110–123. https://doi.org/10.1016/b978-0-12-409547-2.13461-4

Poudel, K. (2014). Orthodox tea production and its contribution in Nepal. *The Third Pole: Journal of Geography Education*, 34–42. https://doi.org/10.3126/ttp.v8i0.11510

Qian, Y., Zhang, X., Qi, D., Xie, L., Chandran, B. K., Chen, X., & Huang, W. (2016). Thin-film organic semiconductor devices: From flexibility to ultraflexibility. *Science China Materials*, *59*(7), 589–608. https://doi.org/10.1007/s40843-016-5091-1

Rajam, K., Rajendran, S., & Banu, N. N. (2013). Effect of caffeine-Zn system in preventing corrosion of carbon steel in well water. *Journal of Chemistry*, *2013*, 1–11. https://doi.org/10.1155/2013/521951

Reto, M., Figueira, M. E., Filipe, H. M., & Almeida, C. M. (2007). Chemical composition of green tea (*camellia sinensis*) infusions commercialized in Portugal. *Plant Foods for Human Nutrition*, *62*(4), 139–144. https://doi.org/10.1007/s11130-007-0054-8

Rokesh, K., Pandikumar, A., Chandra Mohan, S., & Jothivenkatachalam, K. (2016). Aminosilicate sol-gel supported zinc oxide-silver nanocomposite material for photoelectrocatalytic oxidation of methanol. *Journal of Alloys and Compounds*, *680*, 633–641. https://doi.org/10.1016/j.jallcom.2016.04.089

Rothemund, P. W. (2006). Folding DNA to create nanoscale shapes and patterns. *Nature*, *440*(7082), 297–302. https://doi.org/10.1038/nature04586

Sarfraz, J., Gulin-Sarfraz, T., Nilsen-Nygaard, J., & Pettersen, M. K. (2020). Nanocomposites for food packaging applications: An overview. *Nanomaterials*, *11*(1), 10-37. https://doi.org/10.3390/nano11010010

Senthilkumar, S. R., & Sivakumar, T. (2014). Green tea (*Camellia sinensis*) mediated synthesis of zinc oxide (ZnO) nanoparticles and studies on their antimicrobial activities. *Int. J. Pharm. Pharm. Sci*, *6*(6), 461-465.

Shaikshavali, P., Madhusudana Reddy, T., Venu Gopal, T., Venkataprasad, G., Kotakadi, V. S., Palakollu, V. N., &Karpoormath, R. (2020). A simple sonochemical assisted synthesis of nanocomposite (zno/mwcnts) for electrochemical sensing of epinephrine in human serum and pharmaceutical formulation. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *584*, 124038. https://doi.org/10.1016/j.colsurfa.2019.124038

Sharma, K., Sharma, V., & Sharma, S. S. (2018). Dye-sensitized solar cells: Fundamentals and current status. *Nanoscale Research Letters*, *13*(1). https://doi.org/10.1186/s11671-018-2760-6

Siddiqi, K. S., ur Rahman, A., Tajuddin, & Husen, A. (2018). Properties of zinc oxide nanoparticles and their activity against microbes. *Nanoscale Research Letters*, *13*(1), 141-154. https://doi.org/10.1186/s11671-018-2532-3

Singhal, A. K., Fernández-Ibáñez, P., & Byrne, J. A. (2023). Photocatalytic and photoelectrocatalytic treatment of water and wastewater. *Industrial Applications of Nanoparticles*, 104–117. https://doi.org/10.1201/9781003183525-7

Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N. H., Ann, L. C., Bakhori, S. K., Hasan, H., & Mohamad, D. (2015). Review on zinc oxide nanoparticles: Antibacterial activity and toxicity mechanism. *Nano-Micro Letters*, *7*(3), 219–242. https://doi.org/10.1007/s40820-015-0040-x

Slathia, S., Gupta, T., & Chauhan, R. P. (2021). Green synthesis of Ag–ZnO nanocomposite using *Azadirachta indica* leaf extract exhibiting excellent optical and electrical properties. *Physica B: Condensed Matter*, *621*, 413287-95. https://doi.org/10.1016/j.physb.2021.413287

Soltys, L., Olkhovyy, O., Tatarchuk, T., & Naushad, M. (2021). Green synthesis of metal and metal oxide nanoparticles: Principles of Green Chemistry and raw materials. *Magnetochemistry*, *7*(11), 145-179. https://doi.org/10.3390/magnetochemistry7110145

Sonohara, R., Muramatsu, N., Ohshima, H., & Kondo, T. (1995). Difference in surface properties between *Escherichia coli* and *Staphylococcus aureus* as revealed by electrophoretic mobility measurements. *Biophysical Chemistry*, *55*(3), 273–277. https://doi.org/10.1016/0301-4622(95)00004-h

Sudha, P. N., Sangeetha, K., Vijayalakshmi, K., & Barhoum, A. (2018). Nanomaterials history, classification, unique properties, production and market. *Emerging Applications of Nanoparticles and Architecture Nanostructures*, 341–384. https://doi.org/10.1016/b978-0-323-51254-1.00012-9

Sun, Y., Shen, Z., Xin, S., Ma, L., Xiao, C., Ding, S., Li, F., & Gao, G. (2017). Ultrafine co-doped ZnO nanoparticles on reduced graphene oxide as an efficient electrocatalyst for oxygen reduction reaction. *Electrochimica Acta*, *224*, 561–570. https://doi.org/10.1016/j.electacta.2016.12.021

Swati, V, R., Chauhan, A., Shandilya, M., Li, X., Kumar, R., & Kulshrestha, S. (2020). Antimicrobial potential of Ag-doped ZnO nanostructure synthesized by the green method using moringa oleifera extract. *Journal of Environmental Chemical Engineering*, *8*(3), 103730-103764. https://doi.org/10.1016/j.jece.2020.103730

Vuong, Q. V., & Roach, P. D. (2013). Caffeine in green tea: Its removal and isolation. *Separation & Purification Reviews*, *43*(2), 155–174. https://doi.org/10.1080/15422119.2013.771127

Wang, W., Ai, T., Li, W., Jing, R., Fei, Y., & Feng, X. (2017). Photoelectric and electrochemical performance of al-doped ZnO thin films hydrothermally grown on graphene-coated polyethylene terephthalate bilayer flexible substrates. *The Journal of Physical Chemistry C*, *121*(50), 28148–28157. https://doi.org/10.1021/acs.jpcc.7b08181

Xiong, G., Pal, U., Serrano, J. G., Ucer, K. B., & Williams, R. T. (2006). Photoluminesence and FTIR study of ZnO nanoparticles: The impurity and defect perspective. *Physica Status Solidi c*, *3*(10), 3577–3581. https://doi.org/10.1002/pssc.200672164

Yeganeh-Faal, A., Bordbar, M., Negahdar, N., & Nasrollahzadeh, M. (2017). Green synthesis of the Ag/ZnO nanocomposite using *Valeriana officinalis l.* root extract: Application as a reusable catalyst for the reduction of organic dyes in a very short time. *IET Nanobiotechnology*, *11*(6), 669–676. https://doi.org/10.1049/iet-nbt.2016.0198

Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The traditional medicine and modern medicine from natural products. *Molecules*, *21*(5), 559. https://doi.org/10.3390/molecules21050559

Zehiroglu, C., & Ozturk Sarikaya, S. B. (2019). The importance of antioxidants and place in today's scientific and Technological Studies. *Journal of Food Science and Technology*, *56*(11), 4757–4774. https://doi.org/10.1007/s13197-019-03952-x

Zhang, Y., Chen, Y., Westerhoff, P., Hristovski, K., & Crittenden, J. C. (2008). Stability of commercial metal oxide nanoparticles in water. *Water Research*, *42*(8-9), 2204–2212. https://doi.org/10.1016/j.watres.2007.11.036

APPENDICES

B) Phytochemical Screening Protocol

For the phytochemical screening, leaf extract was prepared. 10g leaf powder was kept in beaker and 100 mL of water was added in 250 mL Round Bottom Flask and heated for 2 hours at 80 °C.

1.Test for Alkaloids

i) Dargendroff's Test

About 3 drops of Dargendroff's reagent were added to 2 mL extract and shaken well. Formation of yellow precipitate indicates the presence of alkaloids.

ii) Mayer’s Test

About 3 drops of Mayer’s reagent was added to 2 mL extract and shaken well. Formation of Yellow precipitate indicates the presence of alkaloids.

2. Test for Flavonoid

About 5 mL dil. ammonia was added to 2 mL leaf extract and conc. H_2SO_4 was added sidewise. Formation of yellow ppt confirms the presence of flavonoid.

3. Test for Glycosides

i) Molisch's Test

About 3 drops of Molisch's reagent was added to 2 mL extract and shaken well. Few drops of conc. H_2SO_4 was added sidewise. Appearance of a violet ring at the junction of two liquids which on shaking turns the solution into violet colour indicates the presence of glycosides.

4. Test for Polyphenols

To about 2 mL of extract 3 drops of 5% $FeCl_3$ was added and shaken well. The presence of black color indicates the presence of polyphenols.

5. Test for Terpenoids

To about 5 mL extract, 2 mL of chloroform ($CHCl_3$) and then 3mL of concentrated sulphuric acid were added carefully. Formation of reddish-brown coloration at the interface indicates the presence of terpenoids.

6. Test for Quinones

To about 2 mL of extract, conc. HCl was added. The formation of yellow color indicates the presence of quinones.

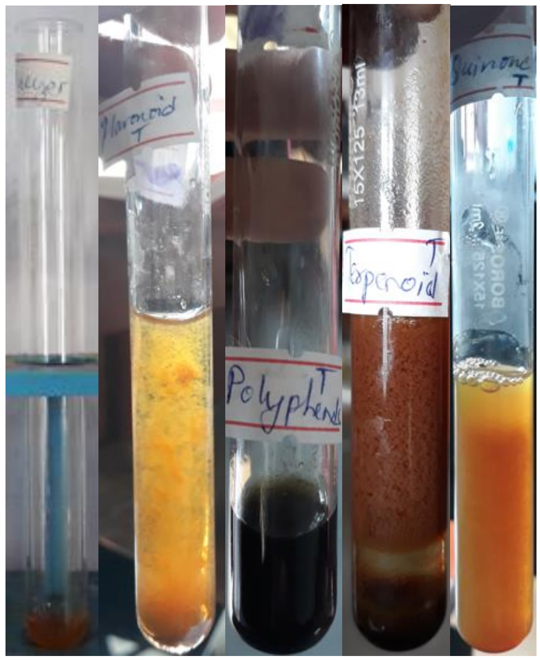


Figure 19. Phytochemical screening of alkaloid, flavonoid, polyphenols, terpenoid and quinone respectively.

B) Antimicrobial Activity

Figure 20. Antimicrobial activity of nanoparticles against *Escherichia coli*

Figure 21. Antimicrobial activity of nanoparticles against *Bacillus subtilis*

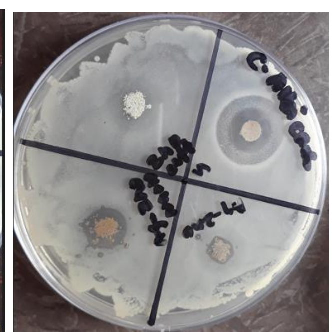


Figure 22. Antimicrobial activity of nanoparticles against *Candida albicans*