

**GREEN SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL
EVALUATION OF ZINC AND COPPER NANOPARTICLES FROM
AQUEOUS LEAVES EXTRACT OF *ERIOBOTRYA JAPONICA* AND
*FICUS CARICA***



Aamir Aziz

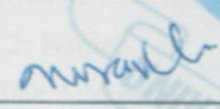
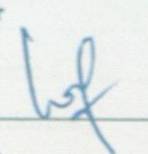
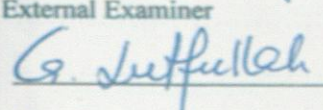
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UNIVERSITY OF PESHAWAR
(Session 2011-2012)**

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EVALUATION OF ZINC AND COPPER NANOPARTICLES FROM
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*FICUS CARICA***

This dissertation is submitted by Aamir Aziz as partial Fulfilment of the
requirements for the Degree of Doctor of Philosophy in Biotechnology.

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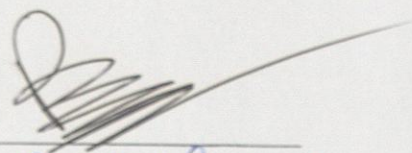
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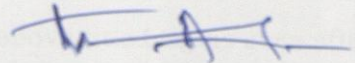
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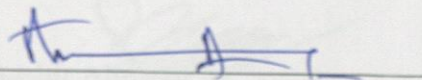
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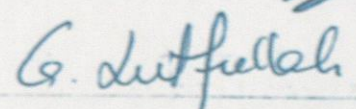


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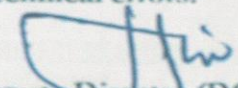
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PIA = Protein Interaction Assay

SEM = Scanning Electron Microscopy

SLA = Surface Layer Analysis

TGA = Thermogravimetric Analysis

FTIR = Fourier Transform Infrared Spectroscopy

EDX = Energy Dispersive X-ray

TGA = Thermogravimetric Analysis

TGA = Thermogravimetric Analysis

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TGA = Thermogravimetric Analysis

LIST OF ABBREVIATION

PCSIR = Pakistan Council of Scientific and Industrial Research

KPK = Khyber Pakhtunkhwa

ZnNPs = Zinc nanoparticles

CuNPs = Copper nanoparticles

NPs = Nanoparticles

AuNPs = Gold Nanoparticles

Ag NPs= Silver nanoparticles

UV-Vis spectroscopy = Ultraviolet-visible spectroscopy

SPR = Surface Plasmon Resonance

FTIR = Fourier transform infrared spectroscopy

SEM = Scanning Electron Microscope

EDX = Energy Dispersive X-ray

TEM = Transmission Electron Microscope

HTEM = High resolution transmission microscopy

XRD = X-ray Diffraction

TG/DTA = Thermogravimetric-differential Thermal Analysis

DMSO = Dimethyl Sulfoxide

ZI = Zone of inhibition

Meth = Methanol

Eth = Ethanol

EtOAc= Ethyl

CH₃Cl = Chloroform

DPPH = 1,1'-diphenyl- 2-picrylhydrazyl

BHT = *tert*-butyl-1- hydroxytoluene

SDA = Sabouraud Dextrose Agar

MS Basal Medium = Murashige and Skoog Basal Medium

ng/ml = Nanograms per milliliter

hrs = Hours

ml = milliliter

L = litter

C⁺ = Positive control

C⁻ = Negative control

MRSA= *Methicillin Resistant S. aureus*

M. morganii= *Morgella morganii*,

A. baumannii=*Acinetobacter baumannii*

P. vulgaris= *Proteus vulgaris*

S. aureus = *Staphylococcus aureus*

E. coli = *Escherichia coli*

E. faecalis = *Enterococcus faecalis*

K. pneumoniae = *Klebsiella pneumoniae*

P. aeruginosa = *Pseudomonas aeruginosa*

V. cholerae = *Vibrio cholera*

S. typhi = *Salmonella typhi*
B. cereus = *Bacillus cereus*
B. subtilis = *Bacillus subtilis*
P. notatum, = *Penicillium notatum*
H. pseudocrispula = *Hemimycena pseudocrispula*
V. longisporum, = *Verticillium longisporum*
P. chrysogenum = *Penicillium chrysogenum*
A. niger = *Aspergillus niger*
A. parasiticus = *Aspergillus parasiticus*
A. flavus = *Aspergillus flavus*
A. fumigatus = *Aspergillus fumigatus*
A. oryzae = *Aspergillus oryzae*
F. oxysporum = *Fusarium oxysporum*
R. arrhizus = *Rhizopus arrhizus*
A. alternata = *Alternaria alternata*
C. albicans = *Candida albicans*
S. cerevisiae = *Saccharomyces cerevisiae*
P. digitatum = *Penicillium digitatum*
A. aegypti = *Aedes aegypti*
A. stephensi = *Anopheles stephensi*
Cx. Quinquefasciatus = *Culex Quinquefasciatus*
T. confusum = *Tribolium confusum*
C. maculatus = *Callosobruchus maculatus*

T. castaneum = *Tribolium castaneum*

ROS = Reactive Oxygen Species

GR = Growth regulation

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ABSTRACT

The main aim of the study was to screen the methanolic, ethanolic and ethyl acetate (EtOAc) fractions of *Eriobotrya japonica* and *Ficus carica* leaves and their Zinc (ZnNPs) and Copper nanoparticles (CuNPs), respectively, for various pharmacological activities such as, antibacterial, antifungal, phytotoxic, insecticidal, anti-termite, hemagglutination and their comparison. The synthesis of ZnNPs and CuNPs was carried out using aqueous leaf extracts of *E. japonica* and *F. carica*, standardizing conditions for their synthesis like temperature, pH and incubation. The characterization of these nanoparticles was done using UV-Vis spectrophotometry, Fourier Transform Infrared spectroscopy, Scanning Electron Microscope (SEM), Energy Dispersive X-ray (EDX), X-ray diffraction (XRD) and Thermogravimetric/Differential Thermal Analysis (TGA/DTA). The UV-Visible absorption spectra of ZnNPs and CuNPs showed peaks at 455 and 426 nm, respectively. The FTIR spectra of ZnNPs and CuNPs confirmed the presence of various functional groups such as, -hydroxyl, amide, carbonyl, aldehyde, and alkynes. The SEM analysis showed that the particle size was between 99 and 96 nm with spherical shape. The presence of zinc and copper along with other elements, such as C, O, S, Si, and K, was confirmed by EDX analysis. The plant extract contributed the last four elements that stabilize and cap ZnNPs and CuNPs. The mean size for ZnNPs and CuNPs in XRD spectra according to Debye-Scherrer's equation was 33 and 32 nm, respectively. A clear antibacterial activity of the methanolic, ethanolic and EtOAc fractions of *E. japonica* was observed in terms of percent inhibition: *E. coli* (62, 59.2 and 44.4%), *S. aureus* (69.2, 46.1 and 50%), *P. aeruginosa* (44.4, 40.7 and 37%), *M. morganii* (59, 40.9 and 68 %), *A. baumannii* (52.1, 43.4 and 0 %), *P. vulgaris* (60, 52 and 64%) and Methicillin Resistant Staphylococcus Aureus (MRSA) (73.6, 52.6 and 0 %). Similar results were also observed for methanolic, ethanolic and EtOAc fractions of *F. carica* against *E. coli* (51.8, 40.7 and 48.8), *S. aureus* (65.3, 46.1 and 65.3), *P.*

aeruginosa (70.3, 25.9 and 48.1), *M. morganii* (54.5, 81.8 and 63.6), *A. baumannii* (43.4, 56.5 and 65.2), *P. vulgaris* (60, 48 and 52) and MRSA (52.6, 68.4 and 78.9). The antibacterial activity of ZnNPs and CuNPs against various bacteria was: *E. coli* (48.1 and 40.7 %), *S. aureus* (73 and 72%), *P. aeruginosa* (37 and 44.4%), *M. morganii* (64 and 54.5%), *A. baumannii* (43 and 56%), *P. vulgaris* (92 and 44%) and MRSA (94 and 89%). The methanolic, ethanolic and EtOAc fractions of *F. carica* and *E. japonica* showed low (<24%) and no antifungal activity, respectively, against the test fungal pathogens: *A. niger*, *H. pseudocrispula*, *P. notatum*, *A. parasiticus*, *V. longisporum* and *P. chrysogenum*. The antifungal activity of ZnNPs and CuNPs was found against all the test fungal strains: *A. niger* (40 and 50%), *H. pseudocrispula* (60 and 50%), *P. notatum* (40 and 40%), *A. parasiticus* (30 and 50%), *V. longisporum* (0 and 10) and *P. chrysogenum* (55 and 10%). The phytotoxic activity (%), as tested against *Lemna minor*, of methanolic, ethanolic and EtOAc extract of *E. japonica* was respectively 50, 50 and 60 at 1000 µg/mL and 50, 40 and 60 at 100 µg/mL, and 40, 20 and 50 at 10 µg/mL. Phytotoxic activity (%) as tested against *L. minor* of ZnNPs and CuNPs at 1000, 100 and 10 µg/mL was 70, 50 and 40 and 70, 60 and 50, respectively. The insecticidal activity of methanol, ethanol and EtOAc fractions was determined using *T. confusum*, *C. maculatus* and *T. castaneum*. The insecticidal effect of methanolic, ethanolic, and EtOAc fractions of *E. japonica* was determined against: *T. confusum* (40, 50 and 50%), *C. maculatus* (0, 50 and 40%) and *T. castaneum* (0, 20 and 10%). The insecticidal activity of methanolic, ethanolic and EtOAc fractions of *F. carica* was in following order: *T. confusum* (50, 40 and 50%), *C. maculatus* (0, 20 and 20%) and *T. castaneum* (20, 10 and 0%). The insecticidal activities of ZnNPs and CuNPs against *T. confusum* was (50 and 50%), *C. maculatus* (60 and 50%) and *T. castaneum* (30 and 60%), respectively. The anti-termite activity (against *H. indicola*) observed for both the plant extracts was moderate unlike the good response for ZnNPs and CuNPs. No

hemagglutination was observed for any of the extracts and nanoparticles, which suggests the lack phyto-lectins in studied plants.

INTRODUCTION

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Nanotechnology is research at molecular or atomic level using a scale of 1–100 nm in any dimension. It uses structures and systems that have novel characteristics and functions owing to their small size, their ability to manipulate and control matter at an atomic scale [1, 2, 3]. Nanotechnology is an emerging science dealing with synthesis, characterization, and multifunctional applications of nanoparticles. Nanoparticles have a number of different applications in both physical and biological worlds such as diagnosis, medicine, communication, drug delivery, and electronics [4, 5].

1.2 History of Nanoparticles

M. C. Lea in 1889 first reported the synthesis of a citrate stabilized silver colloid [6]. Particles synthesized by this method had an average size ranging 7–9 nm [7], similar to recently reported silver nanoparticles using silver nitrate and citrate. The commercial scale synthesis of nanosilver, a medical preparation known as “Collargol”, was reported in 1897. The mean diameter size of Collargol was determined to be about 10 nm. The trend of similar nanoparticles invention followed the next decade, and led to the synthesis of silver nanoparticles (2–20 nm) by Moudry in 1953 and silver nanoparticle imprinted carbon with silver particles of diameter below 25 nm [9–13].

The proposal of nanotechnology was presented for the first time by eminent Laureate, Richard Feynman, at California institute of technology on December 29, 1959, in his lecture, “There is plenty of room at the bottom”. He hypothesized strongly that only 100 atoms, could be enough to store all the books in a cube with sides of 0.02 inch. Norio Taniguchi in 1970 defined Nanotechnology as a science that deals with the partition,

solidification and twisting of materials by one atom or molecule [14]. A glimpse of history of nanobiotechnology is presented in Table 1.1.

Table 1.1 A glimpse of history of nanotechnology

Year	Remarks	Country / People
1200–1300 BC	Discovery of soluble gold	Egypt and China
290–325 AD	Lycurgus cup	Alexandria or Rome
1618	First book on colloidal gold	F. Antonii
1676	Book published on drinkable gold that contains metallic gold in neutral media	J. von Lowenstern-Kunckel (Germany)
1718	Publication of a complete treatise on colloidal gold	Hans Heinrich Helcher
1857	Synthesis of colloidal gold	M. Faraday (The Royal Institution of Great Britain)
1902	Surface Plasmon Resonance (SPR)	R. W. Wood (Johns Hopkins University, USA)
1908	Scattering and absorption of electromagnetic fields by a nanosphere	G. Mie (University of Gottingen, Germany)
1931	Transmission electron microscope (TEM)	M. Knoll and E. Ruska (Technical University of Berlin, Germany)
1937	Scanning electron microscope (SEM)	M. von Ardenne (Forschungslaboratorium für Elektronenphysik, Germany)
1959	Feynman ' s Lecture on "There ' s Plenty of Room at the Bottom"	R. P. Feynman (California Institute of Technology, Pasadena, CA,USA)
1960	Microelectromechanical systems (MEMS)	I. Igarashi (Toyota Central R&D Labs, Japan)
1960	Successful oscillation of a laser	T. H. Maiman (Hughes Research Laboratories, USA)
1962	The Kubo effect	R. Kubo (University of Tokyo, Japan)
1965	Moore' s Law	G. Moore (Fairchild Semiconductor Inc., USA)
1969	The Honda–Fujishima effect	A. Fujishima and K. Honda

		(University of Tokyo, Japan)
1972	Amorphous heterostructure photodiode created with bottom-up process	E. Maruyama (Hitachi Co. Ltd., Japan)
1974	Concept of nanotechnology proposed	N. Taniguchi (Tokyo University of Science, Japan)
1976	Carbon nanofiber	M. Endo (Shinshu University, Japan)
1980	Quantum hall effect (Nobel Prize)	K. von Klitzing (University of Würzburg, Germany)
1982	Scanning tunneling microscope (STM) (Nobel Prize)	G. Binnig and H. Rohrer (IBM Zurich Research Lab., Switzerland)
1986	Atomic force microscope (AFM)	G. Binnig (IBM Zurich Research Lab., Switzerland)
1986	Three-dimensional space manipulation of atoms demonstrated (Nobel Prize)	S. Chu (Bell Lab., USA)
1987	Gold nanoparticle catalysis	M. Haruta (Industrial Research Institute of Osaka, Japan)
1990	Atoms controlled with scanning tunneling microscope (STM)	D. M. Eigler (IBM, USA)
1991	Carbon nanotubes discovered	S. Iijima (NEC Co., Japan)
1995	Nano-imprinting	S. Y. Chou (University of Minnesota, USA)
1996	Nano sheets	T. Sasaki (National Institute for Research in Inorganic Materials, Japan)
2000	National Nanotechnology Initiative (NNI), USA	
2003	21 st Century Nanotechnology Research and Development Act, USA	
2005	Nanosciences and Nanotechnologies: An action plan, Europe	

1.3 Synthesis of Nanoparticles

In the field of material science, metal nanoparticles are of significant interest owing to their unique physico-chemical properties such as size and shape, which appropriate their use in several fields. Various methods of nanoparticles synthesis are discussed in the following paragraphs along with their advantages and limitations [15].

1.3.1 Physical Methods

One of the most common methods of physical synthesis, also known as top down approach, is the thermal decomposition. This method is utilized for making mono-scattered nanoparticles. In this method, we use a large starting structure or natural units that can be controlled in making of nanostructures. The other important techniques in this category include diffusion, dispersion, irradiations, and arc discharge. The physical methods of synthesis are very costly and slow; this makes them inappropriate for scale-up.

In the diffusion method, fatty acids are dissolved in sodium hydroxide solution, mixed with metal salt solution that subsequently gives nanoparticles in precipitate form. The glass ampoules are encircled by short wires of copper, sealed at low pressure followed by annealing at 5000 °C for 24 h. The crystals are then removed from the ampoules and are chilled on a metallic plate. Another important method is UV irradiation where the polycarbonate thin layers are put on microscopic glass and then exposed to UV radiations [16].

1.3.2 Chemical Methods

Another strategy for the synthesis of metal nanoparticles is the chemical methods of synthesis, also known as bottom up approaches. The convenient synthetic manipulations and moderate reaction conditions have made chemical methods, a good option for metal nanoparticles synthesis. The chemical method includes miniaturization of material components with further self-assembly resulting in the synthesis of nanoparticles.

Chemical precipitation and colloidal dispersion are some of the commonly used methods. The chemical precipitation procedure enables a controlled nucleation, growth and aging of metal nanoparticles from organic or inorganic solutions. The driving forces behind this strategy are advanced coordination and solution chemistry enabling the synthesis of required precursor particles resulting in agglomeration of metal nanoparticles in the solution.

The typical example of the colloidal dispersion includes quantum dot formation during epitaxial growth. The reducing agents used in these procedures include ethyl alcohol, sodium borohydrate, sodium citrate, tetra-n-tetra-fluoroborate (TFATEB), N, N-dimethyl formamide (DMF) and poly (N-vinyl pyrrolidine (PVP). The homogenous chemical precipitation is another important and economical method for the synthesis of monodisperse metal nanoparticles of different sizes and shapes. This method offers a better control on chemical and morphological properties [17–19].

1.3.1 Nanoparticle synthesis by bacteria

An attractive route for nanoparticle synthesis is bacteria due to their small size, low breeding and maintenance requirements, low cost. Bacteria have gained an important position due to their multitude of applications in biological systems. They have the capability to reduce silver and make nanoparticles in the range of 10–20 nm. Several species have been used for synthesizing silver nanoparticles [20]. The silver nanoparticles (Ag-NPs) produced by bacteria have been reported for silver usage and have shown that Ag-NPs are deposited in the periplasmic space of *Pseudomonas aeruginosa* [21]. *Escherichia coli* has been reported for the synthesis of silver

1.3.3 Biological Methods

Although the chemical and physical methods have a lot of advantages, use of harmful chemicals and radiations are of great concerns for the personnel and to the environment. For examples, the UV radiations are mutagenic while sodium borohydrate can have serious toxic effects. The limitations of physical and chemical methods give an impetus for a biocompatible, renewable, and ecofriendly method for the synthesis of nanoparticles [20]. Biological methods for the synthesis of metal nanoparticles, popularly known as green synthesis, utilizes living organisms like bacteria, fungi and plant extracts and thus interconnect nanotechnology and biotechnology. These methods are biocompatible and ecofriendly as they avoid use of harmful irradiations, high pressure, and chemicals. The factors that have been observed to influence the morphologies of NPs are molar ratio of reducing agent to metallic salt, concentration, time, availability of protective agents, temperature, order of addition of reagents, type and degree of agitation, and type of protective agents [21]. Monodispersed metal NPs can be obtained by quick nucleation [22]. The following examples highlight the use of these organisms for the synthesis of metal nanoparticles.

1.3.3.1 Nano-particle synthesis by bacteria:

An attractive host for nanoparticle synthesis is Bacteria, due to their small size, easy handling and minimum requirements for growth. Bacteria have gained an important position due to their multitude of applications in biological sciences. They have the capabilities to reduce silver and make nanoparticles in the range of 10–20 nm. *Bacillus* species have generally been used for synthesizing metal nanoparticles [23]. The Silver nanoparticle (AgNPs) producing bacteria have been isolated from the silver mines and have shown that AgNPs are deposited in the periplasmic space of *Pseudomonas stutzeri* (AG259) [24]. *Marinobacter Pelagius* has been reported for the synthesis of stable,

monodispersed gold nanoparticles [25]. *Lactobacillus* strains have been reported in the synthesis of titanium nanoparticle. Understanding the natural processes of bacteria is a major factor that can lead to novel and unexplored strategy of metal nanoparticles synthesis [26].

However, there are few disadvantages of biological methods in culturing of microorganisms, such as these methods are time consuming and laborious. These issues have been overcome by strain improvement and optimization of growth conditions like incubation temperature and time, pH and concentration of metal particles, making large scale synthesis possible [27].

1.3.3.2 Nano-particle synthesis by fungi:

Fungi are getting more attention for nanoparticle production because of higher accumulation of biological materials, easy synthesis method, and very simple downstream processing. The fungi reported in the literature for the synthesis of AgNPs are *Aspergillus oryzae*, *Aspergillus niger*, *Fusarium solani*, *Pleurotus sajor-caju* and *Inoderma viridea* [28–31]. AgNPs produced using *Phoma glomerata* have been evaluated for their antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* [32]. The extracellular synthesis of AgNPs in *Penicillium* makes it an excellent fungal system for the synthesis of AgNPs [33].

1.3.3.3 Biosynthesis of metal nano-particles by plants

The plants are the most prevalent photosynthetic autotrophs producing glucose and high biomass in terrestrial environment. As plants are sustainable, renewable and produce high biomass; they are more suitable for the synthesis of nanoparticles than bacteria and fungi. Various plant extracts have been utilized for the synthesis of different metal nanoparticles. A study has reported the use of leaf extracts of *Dodonaea viscosa* for biosynthesis of copper nanoparticles (CuNPs) and AgNPs. The morphology of the synthesized

nanoparticles was round and size was 29 and 16 nm for CuNPs and AgNPs, respectively. These nanoparticles demonstrated good antibacterial activity against *Pseudomonas fluorescens*, *Bacillus subtilis*, *Klebsiella pneumonia*, *E. coli* and *S. aureus* [34]. *Manihot esculenta* leaf extract was utilized for the synthesis of CuNPs where the sugars present in the leaves to act as reducing agent. The characterization of these CuNPs was carried out using Scanning Electron Microscope (SEM), X-Ray Diffraction (XRD), UV-Visible spectroscopy and Fourier Transform Infrared Spectroscopy (FTIR). A lot of plants have been reported in the literature for the synthesis of gold, nickel, and other nanoparticles. The leaves extract of *Medicago sativa* has been used for the synthesis of gold nanoparticles (AuNPs) ranging from 2 to 20 nm [35]. The synthesis of nickel, silver, zinc, cobalt and CuNPs has been reported from *Brassica juncea*, *M. sativa* and *Helianthus annuus*; and *B. juncea* was found to be a hyper accumulator [36]. The main phytochemicals required for the synthesis of nanoparticles are flavones, aldehydes, terpenoids, ketones, amides, carboxylic acids, and quinones. Xerophytic plants contain emodin (anthraquinone), which could undergo redial tautomerization producing AgNPs [37]. The flower extract of *Cassia auriculata* has been utilized for the synthesis of ZnO nanoparticles. These ZnO nanoparticles were characterized using UV-Vis spectroscopy, SEM, XRD and FTIR [38]. The modern techniques of plant tissue culture and downstream processing open up the possibilities where plants can be used as factories for various metal nanoparticles after addressing the issues like metabolic status of the plant [39].

1.4 Zinc (Zn) Nanoparticles

Zinc oxide (ZnO) is nearly insoluble in water and appears as a white powder. ZnO is found as "zincite" in the earth crust; however, most of the ZnO that is used commercially is synthetic in nature. ZnO is a suitable additive for textile as it is non-hazardous and inert to

human skin. The increased surface area at nanoscale ZnO imparts great potential to increase material function [40].

1.5 Importance of Zinc oxide Nanoparticles

1. ZnONPs can be used in cosmetics, paints, plastic, electronics and pharmaceutical products.
2. It has the potential to treat carcinomas and leukemia [41].
3. They possess antibacterial properties and can be used in drug delivery [42, 43].

The surface area and concentration of ZnONPs have been found to influence their antibacterial activity while the particle shape and crystalline structure have little role. Some researchers suggest that the generation of hydrogen peroxide may also have antibacterial activity while others suggest that it is due to the electrostatic interactions leading to the binding of the particles to the surface of bacteria [44].

1.6 Copper Nanoparticles

The excellent electrical conductivity of copper makes it relatively cost effective with broad range of applications in the nanodevices. CuNPs play important role in various industries; optoelectronics, catalysts, and metallurgy [45–48]. Copper is more economical than gold and silver, and also it does not create the problem of ion migration at high humidity and temperatures [49].

1.7 Significance of Cu Nano-particles

1. CuNPs are used in plastics, coatings, and textiles as an antimicrobial agent.
2. Due to their high strength, they can be used in different products.
3. In many chemical reactions, they are used as a catalyst, e.g., in the synthesis of glycol and methanol.
4. They are used as sintering excipient and capacitor material

5. CuNPs can be used in place of expensive noble metals for conductive pastes and inks expanding its applications in displays, printed electronics and transmissive conductive thin films [50–55].

6. Copper has been enlisted by EPA (Environmental Protection Agency of USA) as a potent antimicrobial agent against potentially dangerous microbes [56]. Unlike antibiotics, no resistance of pathogenic microorganisms to copper has been reported yet, giving it a unique position in pharmaceuticals. The large surface area and small size of CuNPs enables a direct interaction with bacterial cell walls that lead to membranes damage and subsequent killing of bacteria [57].

1.8 Rosaceae (Family)

Description

Rosaceae is a medium-sized family of deciduous or evergreen trees, sarmentous shrubs or perennial herbs. The leaves are mostly simple, alternate, interruptedly imparipinnate, trifoliolate, ternate or palmately to pinnately compound, stipules persistent or deciduous (absent in *Spiraea* and *Aruncus*). In genus *Prunus*, glands are often present on the leaves. The stem bears prickles or spines. The flowers are actinomorphic, bisexual or rarely unisexual, (4-)5-merous, solitary, in racemes or compound racemes. Sepals (4-)5, mostly borne on hypanthium, imbricate, epicalyx and are often present outside calyx. Petals (4-)5, are free, imbricate, rarely zero. Stamens are four to numerous, anthers two or one-locular, dehiscing longitudinally, intrastaminal. The ovary is superior to inferior, carpels one to many, free to variously connate, with mostly two ovules per carpel or locule, stylodia one per carpel. Fruits are diverse, drupetum, drupe, pome, nuculanium, pometum, achenetum, follicetum or glandetum. Seeds range from one to several in each carpel or locule, usually without endosperm [58].

Distribution

Rosaceae family consists of 85 genera and about 3000 species and is represented by 27 genera and about 160 species in Pakistan [58].

Importance

Different species of Rosaceae family are used traditionally for the treatment of various diseases. The decoction of ariel parts of *Fragaria nubicola* is consumed twice a day for a week to treat fever. The root extract of *Prinsepia utilis* is used as an antidote for poisoning. The paste of roots, heated at low temperature, is applied to wounds. The stem bark decoction of *Prunus cerasoides* is used to cure joint pains. The paste of the fresh root tips of *Rubus niveus* is made in butter and buffalo milk is used in excessive bleeding during menstrual cycle [59].



Figure 1. *Eriobotrya japonica* leaves

1.9 *Eriobotrya japonica* (Plant)

Local name	Loquat
Specie	<i>E. japonica</i>
Genus	<i>Eriobotrya</i>
Family:	<i>Rosaceae</i>
Order:	Rosales
Kingdom:	Plantae

Description

Eriobotrya japonica is an evergreen shrub in the family Rosaceae, with a short trunk, rounded crown and woolly new twigs that grows from 3–10 meters. The leaves are simple, alternate, dark green, 10–25 cm long, tough with densely velvety-hairy and a serrated margin. The white flowers are in stiff panicles of 3–10 flowers that consist of five petals each. A strong aroma of the flowers can be smelled from a distance. The yellow, orange, sometimes red-blushed fruits grow 3–5 cm in length and are pear or oval shaped. The ovules are 1–10, with 3–5 being most common [60].

Distribution

Eriobotrya japonica is cultivated worldwide with different names in more than 30 countries including Pakistan, Japan, India, China, Australia, United States (largely California, and Florida), Venezuela, Brazil, Spain, Italy and Greece [61, 62]. *E. japonica* has industrial importance in many countries like China, Pakistan, Spain, Japan and Turkey. Worldwide 549,220 tonnes fruit of *E. japonica* is produced and the main producers are Spain (43,300 tonnes) and China (460,000 tonnes) [63–65]. In different provinces of Pakistan, *E. japonica* is cultivated on commercial scale. In Punjab it is cultivated at Hasan Abdal, Choa Saiden Shah, Kalar Kahar, Wahand and Tret while in Khyber Pakhtunkhwa in Haripur, Peshawar and Mardan region [66].

Importance

The leaves and fruits are the main parts of medicinal values [67]. 'Feitai' is a compound formulation, which contains a number of herbs along with *E. japonica* leaves. It is a folk medication in China for the treatment of pulmonary tuberculosis [68]. *E. japonica* leaves are used traditionally not only as anti-inflammatory agent and expectorant but also for pain and skin diseases. *E. japonica* leaves have oleanolic and ursolic acid, which have anti-hyperlipidemic and hypoglycemic properties. *E. japonica* seeds may be used to improve liver functions, as they contain unsaturated fatty acids linolenic, linoleic acids and the β -sitosterol [69–71]. The fruits of *E. japonica* are sweet and juicy with a high nutritional value, containing minerals (calcium and phosphorous), sugars and vitamins (A, B and C) [72].

1.10 Moraceae (Family)

Description

Deciduous or evergreen, dioecious or monoecious, unarmed or armed, shrubs or trees, rarely climbers. Leaves are serrate, entire, simple or petiolate lobed. Stipules are 2 and in lateral form. The inflorescence in this family are axillary cymes, mostly in the form of racemes, catkins, often spikes, hypanthodium or globose heads. The flowers are mostly unisexual, minute, actinomorphic, epigynous or hypogynous. The sepals are occasionally absent or (2–3-) 4 (–6), free, valvate, biseriate or united and envelop the carpel in female flower. Stamens in this family can be as many as sepals, often less, erect or filaments in bud. The ovary is bicarpellate with one carpel frequently abortive, superior or inferior and unilocular. The fruit are in simple drupe or nut form and sometimes syconium and sorosis. The seeds may be endospermous or exalbuminous and are surrounded by endocarp and the embryo is mostly curved.

Distribution

This family consists of 40 genera and 1400 species that are preferentially distributed in tropical and subtropical regions with a very limited number of species found in the temperate zones. In Pakistan, this family is represented by 5 genera and 16 species.

Importance

Fruits of *Morus alba* are eaten both as fresh and in dry forms and are known to exert laxative, purgative, antiseptic and antidiabetic effects. It is also well known that they promote sweating and can be used for lowering blood pressure. The leaves of this plant are used for the treatment of eye infections, cold, influenza and nose bleeds. The bark of this plant is traditionally used for lowering blood pressure [73]. The alcoholic extract of the leaves of *Artocarpus Lacucha* Buch-Ham is known to possess exceptional antibacterial properties. The seeds of this plant, when mixed with mother's milk, are observed to cure constipation. The bark of this plant is applied to cracked skin and small pimples [74]. Another traditionally important species of this family is *Ficus benghalensis* whose latex has aphrodisiac, vulnerary, tonic, and maturant properties and is used in the treatment of toothache, diarrhea, dysentery, piles, and diabetes. The hanging tips of the roots of this plant are used as a cure for vomiting. An infusion of the bark is regarded as a tonic and is used for the treatment of diabetes. Previous work has shown that in rabbits the bark extract has anti-diabetic effects whereas in human (both men and women) this decoction exhibits strong anti-fertility activity. An infusion of the leaves of this plant is useful in the management of both diarrhea and dysentery. A decoction made up of the aerial rootlets of *Ficus elastica* is used for treatment of wounds, cuts as well as sores [75]. The entire *Ficus hispida* plant body is used for treatment of ulcers, piles, jaundice, hemorrhage of the nose and mouth as well as an astringent to the bowels and ant dysenteric. The pulverized

dried fruits of *Ficus religiosa*, when taken with water, are useful in managing fortnight asthma [74–76].

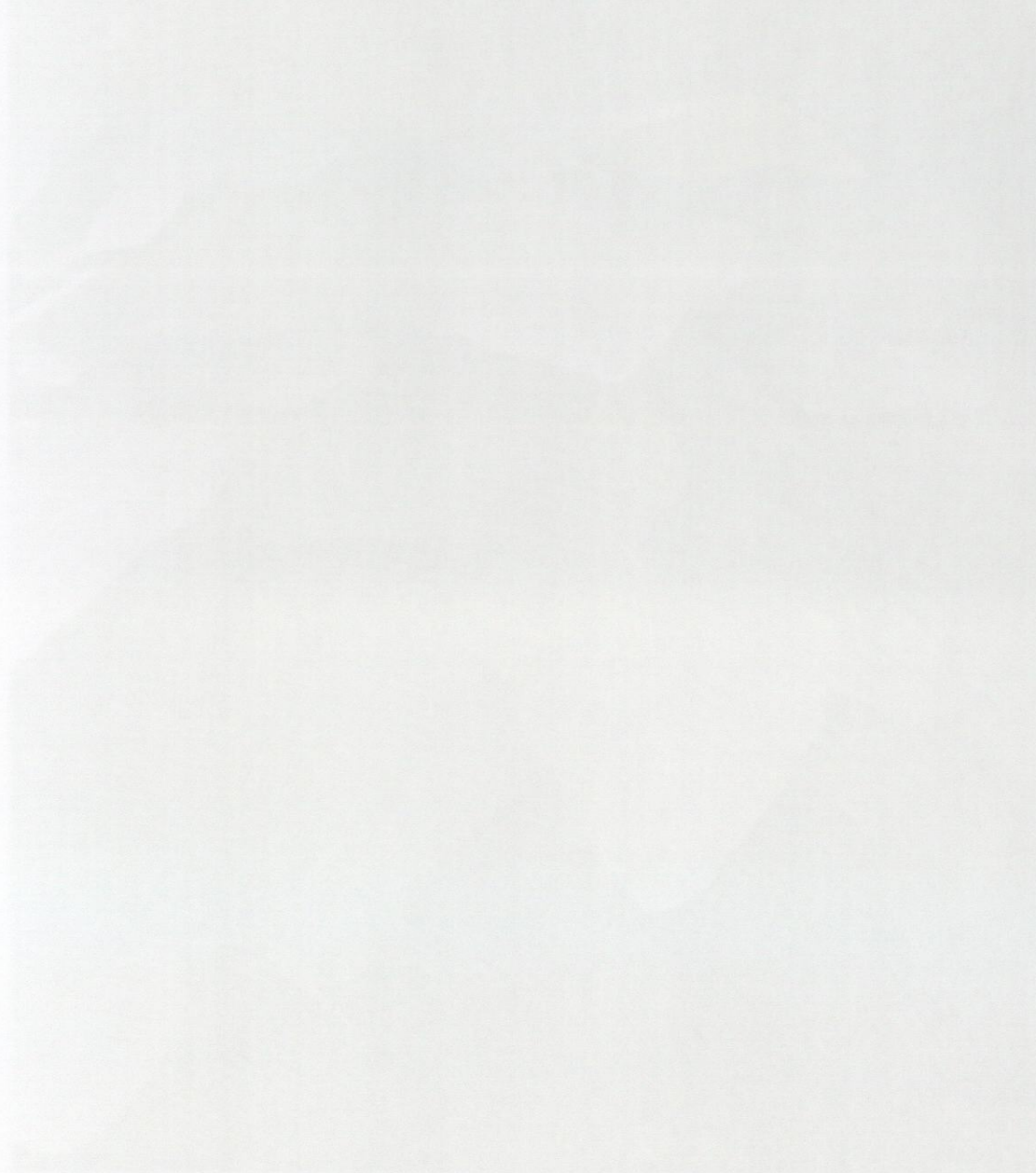


Figure 1.1. Ficus religiosa



Figure 2. Aerial parts of *Ficus carica*

1.11 *Ficus carica*

Local names: Enzer, fig

Species: *F. carica*

Genus: *Ficus*

Family: *Moraceae*

Order: *Rosales*

Kingdom: *Plantae*

Description

Size wise the variation can span between that of a large shrub to a small deciduous tree around 5–9 m tall with several branches. The bark of the plant is smooth, grey or dull white in color and young twigs are softly hairy or glabrous. Leaves are glabrous to tomentose. In case of male flowers sepals are usually four in number and united with lobes that are lanceolate. Stamens are four in number and filaments are oval long with exerted anthers. Female flowers of this plant have four sepals and are pedicellate with lobes that are lanceolate-oblong. This plant have lateral ovary with style and the stigma is entire. Figs are usually 2–5 (–8) cm in diameter, pyriform-obovoid, shortly hispid or glabrous and are yellowish to brownish violet in color.

Distribution

F. carica is cosmopolitan in nature. It is abundant in Portugal, USA, and Chile and, to a lesser extent in India, China, Arabia, Japan and Persia [77].

Medicinal importance

The fruit of *F. carica*, a superior source of vitamins and minerals, is consumed in both dry and fresh form [78]. More than 28% of the total fiber content of the fruit is of the soluble variety which has been shown to aid in weight loss as well as help control blood sugar and cholesterol. The consumption of the fruit, which is an important component of the

Mediterranean diet, has been reported to prevent the development of patho-physiological conditions related to both cancer and coronary heart diseases [79]. *F. carica* has been used in traditional Middle Eastern and European medicine to treat irregular swellings which are commonly but mistakenly reported as a tumors [80]. Its pharmacological actions include effects that can be classified as antitumor, antibacterial, antioxidant, anti-inflammatory, antidiarrheal, anticancer, immune balancing, and immune harmonizing in nature [81]. Extracts derived from different parts of the plant exhibit a variety of biological activities. The fruits, root and leaves of the plant are used in native medicine for the treatment of disorders afflicting the metabolic, cardiovascular, respiratory, inflammatory and gastrointestinal systems. *Ficus carica* has been reported to show hypo-lipidemic, anti-HSV (anti-herpes simplex virus), hypoglycemic, hemostatic and antioxidant activities [82].

The current expansion and execution of novel path-breaking technologies have given rise to a new era in nano-revolution with increased focus being directed towards the green synthesis of nanoparticles. Green synthesis has many advantages over other methods with biocompatibility, environment friendly, and cost effectiveness being just a few. Published literature includes several reports that detail the synthesis, characterization and pharmacological investigation of nanoparticles made through green synthesis. The principle underlying green synthesis is the utilization of bacteria, fungi, plants, along with the various enzymes and biomolecules present inside these organisms, as capping and reducing agents for effecting the synthesis of nanoparticles [83].

Chandan *et al.* utilized extracts from the leaves of *Dalbergia sissoo* for the biosynthesis of AgNPs and AuNPs. Reduction of silver nitrate and chloroauric acid after treatment with broth made from *D. sissoo* leaves resulted in the formation of AgNps and AuNPs. An intense Surface Plasmon Resonance (SPR) peak was observed at 425 and 545 nm for AgNps and AuNPs, respectively. The nanoparticles were characterized using XRD (confirming the crystalline nature of NPs), FTIR (confirming the role of biomolecules involved in capping and synthesis) and TEM (analyzing the shape and size). The size of AgNps was 5–55 nm while that of AuNPs was in the range of 50–80 nm [84].

On a similar note, Asmathunisha *et al.* used *Lycopersicon esculentum* derived *in vitro* callus culture and leaf broth for the preparation of AgNPs and AuNPs. Dynamic light scattering (DLS) and SEM analyses revealed spheroid AgNPs (33.6 nm) and round shaped AuNPs (36.7 nm). FTIR analysis was used to confirm the presence of carbonyl and hydroxyl groups in the synthesized NPs. The AuNPs of this study were found to have superior antimicrobial properties as compared to AgNPs. They were observed to be more active against bacterial strains as compared to fungal infections and exhibited maximal activity against *S. aureus* [85].

Kalpana *et al.* synthesized AuNPs by using leaf extracts of *Cinnamomum japonicum*, *Nerium indicum* and *Torreya nucifera*. The nanoparticles were characterized by UV spectroscopy, TEM and FTIR. The size of the nanoparticles was found to be approximately 4–35 nm for *C. japonicum*, 8–42 nm for *T. nucifera* and 4–38 nm for *N. indicum*. These AuNPs were tested against 3T3-L1 cell line; and it was found that even at higher concentrations these AuNPs exhibit minimal toxicity. This finding underlines the safety of these AuNPs in biomedicine [86].

One of the published studies reported the synthesis of ZnO nano-particles using aqueous root extract of *Zingiber officinale* (ginger). The root extract of this plant is rich in flavonoids, which act in the capacity of reducing agents. For characterization, different techniques including UV-Vis, SEM, FTIR and XRD were utilized and the size of the nanoparticles was determined to be between 6–20 nm. Keeping in view the size, the authors have indicated that the nanoparticles have the potential to be used for a multitude of functions such as vectors for gene delivery, for drug delivery or as biosensors, or at interphases wherein there is a direct contact with blood [87].

In another study, leaf extract from *Capparis zeylanica* was used to synthesize CuNPs. Change of color subsequent to the accumulation of leaf extract in the copper sulfate solution was used as an indicator to confirm the synthesis of CuNPs. The characterization of biosynthesized CuNPs was done via UV-Vis, FTIR, XRD, SEM, EDX and TEM analysis. The synthesized CuNPs were cubical in arrangement with sizes ranging from 50–100 nm. The synthesized CuNPs showed concentration dependent antibacterial activity against *P. aeruginosa*, *E. coli* and *S. aureus* [88].

In keeping with the increased interest of researchers in biological biosynthesis, one study reported the leaves extract of the *Ocimum sanctum* for the synthesis of CuNPs. The biosynthesized CuNPs were characterized with the help of XRD and FTIR and the size was

recorded as 77 nm. Thus, it can be concluded that this methodology can be used as a rapid and ecofriendly method for the biosynthesis of stable CuNPs [89].

Another report discussed the feasibility of using leaf extract of *Tabernaemontana divaricate* for the synthesis of ZnNPs. The formation of ZnNPs was confirmed and characterized through a combination of XRD, TEM, UV-Vis, and FTIR with a size of 36 ± 5 nm. The synthesized ZnNPs were found to be effective against MCF-7 breast cancer cell (IC_{50} : $30.65 \mu\text{g/mL}/24$ h). These results demonstrated that biologically synthesized ZnNPs can be a potential source for cancer treatment [90].

Another study has reported the synthesis of stable ZnONPs utilizing the leaves extract of *Aloe Vera*. The synthesized ZnONPs were characterized using Photoluminescence, FTIR, UV-Vis, TEM, SEM and XRD. After characterization, it was revealed that the nanoparticles were spherical in shape and varied size (25-40 nm) [91].

A study was conducted to compare the possible effects of synthesized metal nanostructures and their bulk counterparts on plants and a variety of Silver (Ag), Gold (Au), Copper (Cu) and Silicon (Si) NPs were considered. The NPs analyzed in this study were synthesized by utilizing the biomass of the *zucchini* plant; their effects were studied at two different concentrations namely 250 and 750 mg/mL. It was concluded that AuNPs do not exert any significant effect on the plants. Approximately 80% of the bulk Au as a metal was deposited in the plants than did *AuNPs*. This implies that the choice of solution influences the NPs and that the AuNPs possess distinct phytotoxic effects [92].

In another study into the synthesis of CuNPs, leaf extracts from *Vitis vinifera* were used to reduce copper sulfate to CuNPs. In UV-Visible spectrum, an integration peak at 384 nm corresponding to the SPR of CuNPs was observed. The highest inhibitory zone was observed when the CuNPs were applied against *S. aureus* [93]. In another study, the reduction of copper sulfate was carried out using the aqueous leaves extract of *Nerium*

oleander for forming CuNPs. Characterization of synthesized nano-particles was performed through UV-Vis and FTIR analysis. The synthesized CuNPs exhibited impressive antibacterial activity against *K. pneumonia*, *E. coli* and *S. aureus* [94]. The synthesis of ZnONPs was carried out using leaf extract of *Hibiscus rosa-sinensis* and was characterized through XRD and SEM [95].

ZnONPs obtained using extract from the leaves of *M. oleifera* were characterized using different techniques; Field Discharge Examining Electron Microscopy (FE-SEM), UV-Vis spectroscopy, XRD, EDX, FTIR and Photoluminescence Spectroscopy (PL). The synthesized ZnONPs (200 µg/mL) exhibited maximum antibacterial activity against Gram-positive, followed by Gram-negative bacteria and fungi [96].

Another study showed that small ZnONPs, obtained from *Hibiscus sabdariffa*, had better effect on diabetic mice as compared to large ZnONPs. Analysis by both Enzyme Linked Immuno Sorbent Assay (ELISA) as well as Real Time PCR (RT-PCR) has shown that ZnO can induce the expression of insulin receptors, Th1, Th2 cells and other diabetes-associated genes of the pancreas [97].

One researcher utilized the leaves extract of *Ocimum tenuiflorum* for the synthesis of ZnONP (13.86 nm). The characterization of the resultant ZnONP was carried out by SEM, XRD and FTIR [98].

One study reported using of the fruit extracts of *F. carica* as a reducing agent for the synthesis of AgNPs. The synthesized AgNPs were characterized using UV-Vis spectroscopy, FTIR, FE-SEM and XRD. The particle size was determined to be in the range of 20–25 nm. The antimicrobial action of AgNPs was evaluated against both Gram negative and positive bacteria and it was determined that AgNPs synthesized using the fruit extract exhibited superior antimicrobial activity as compared to AgNPs synthesized by the EDTA method [99].

Bashir *et al.* conducted a study that used aqueous leaves extracts from *Mentha spicata*, *Agave americana* and *Mangifera indica* for the synthesis of AgNPs. The AgNPs were characterized using UV-Vis spectroscopy, XRD, EDX, TEM and SEM revealing the size of synthesized nanoparticle in the range of 30-150 nm. The AgNPs were screened for biological activity and results revealed significant phytotoxic effect with no hemagglutination activity [100].

In another study the researchers focused on making AuNPs and AgNPs by utilizing leaves and bark extracts of the *F. carica*. The leaves and bark were observed to act as the reducing and capping agents capable of quickly reducing to auric particles and silver particles. The synthesized nanoparticles were characterized using UV-VIS spectroscopy and Scanning Tunneling Microscopy (STM) [101].

A rapid and eco-friendly method for synthesis of AgNPs utilizing *E. japonica* leaves extract has also been described. The impact of the leaf concentrate, silver nitrate concentration, response time and temperature on the synthesis of AgNPs were studied. Biosynthesized AgNPs were described by UV-Vis spectroscopy, XRD, and FTIR. The antibacterial action of biosynthesized AgNPs indicated a good inhibitory effect against water borne pathogens such as *Shigella* and *Listeria* [102].

AuNPs were also synthesized by using leaves and fruits pulp of *Artocarpus lakoocha* and *E. japonica*. The characterization was done through the TEM, FTIR and UV-Visible spectroscopy and revealed size of 15.06, 36.8, and 25.08 nm, respectively [103].

AgNPs synthesized using methanolic leaf extract of *Litsea reticulata* were characterized by UV-visible spectroscopy, TEM, and XRD. The synthesized AgNPs were evaluated for their antimicrobial and antioxidant activity and were observed to exhibit promisingly robust activity. Furthermore, the *in vitro* cytotoxic impact of the AgNPs was evaluated

against the HCT15 cell line and tumor cells viability was affirmed using the MTT assay [104].

Bark of *Boswellia ovalifoliolata* was used to synthesize AgNPs, which were then characterized by UV-Vis, SEM, and FTIR [105]. These AgNPs were screened for their effect on germination and seed growth. Seeds were placed in Murashige and Skoog (MS) medium in presence of the AgNPs and it was observed that germination efficiency was approximately 90% as compared to the control, where only 70% germination was observed. This can be attributed to the fact that AgNPs can enter the seed and cause activation of the embryo. It was found that seed sprouted on 7th day in test tubes containing AgNPs, whereas those in the control tubes germinated on 15th day. Seed germination completed between the 7th and 10th day in case of medium that contained AgNPs. The length of seedling was 10.6 cm in experiments wherein the seeds were treated with 30 mg/mL of AgNPs. This technique has the potential to prove invaluable in augmenting the population of the trees, which are on the verge of extinction. Almost 11 wetland plants species were exposed to Gum Arabic coated silver nanoparticles (GA-AgNPs) and polyvinyl pyrrolidone-coated silver nanoparticles (PVP-AgNPs) in an attempt to investigate their germination and growth profile. It was found that GA-AgNPs had a significant impact on the germination of various plant species while PVP-AgNPs had no measurable effect. From the findings of the study, it was concluded that GA-AgNPs had superior effect on growth of seedlings when compared to AgNO₃ which proves that the positive effect of the AgNPs was not purely due to ionic silver content [106].

We are using plant based products against economically important insects and vectors. It has been found in many studies that phytochemicals have the ability to regulate insect growth, repel insects, kill larvae, and works as ovipositional attractants and thereby effect preventive actions [107]. Plants are constantly attacked by insects that feed on them as a

main source of diet. For example, *Tribolium castaneum* (adult and larvae) is an insect that primarily attacks stored grains [108]. It discards its exoskeleton during the growth phase and pollutes the flour by feces deposition thereby causing both qualitative as well as quantitative losses of stored grains. An infestation by this insect changes the flour quality by making it moldy, grey in color with an odor that is unsuitable for human consumption [109]. In China, the bark of roots and leaves of many plants are used for protection against the insects that can damage stored grains; examples of such insects include *Pieris rapae* (Lepidoptera: Pieridae), and *Platyedra gossypiella* (Lepidoptera: Gelechiidae) [110]. Different extracts of *Ailanthus altissima* were evaluated for their insecticidal activity against *T. castaneum* [111] and *Acalymma vittata* (striped cucumber beetle) [112], and promising results were obtained. Tsao *et al.* 2002 [113] evaluated the aqueous extract of *A. altissima* against the larvae of *Aedes aegypti* and noted that the extract demonstrated a low activity.

A novel area in the field of metal nanotechnology is surface modification wherein ligands are added to NPs. A large variety of functional groups are used for this purpose. These functional groups change the chemical properties of NPs by forming either covalent, non-covalent or both type of bonds between NPs and surface ligands [114,115].

Saponins have been found to have an anti-feedant ability and kill insects by interfering with their digestion process [116,117]. The anti-feedant potential of saponins is by virtue of their ability to reduce absorption of sterols. Insects obtain sterols from external sources as they are unable to synthesize it. Saponins exert their effect by decreasing sterol absorption by insects which affects the formation of ecdysteroids and hydroxyecdysteroids needed for molting. Saponins have been found to naturally occur in various plant families such as Alliaceae, Leguminosae, Solanaceae, Theaceae and Chenopodiaceae [118–119].

Heterotermes indicola (termites) are classified under the group Isoptera. They are pale in

color, delicate in description and their primary diet is composed of cellulosic materials [120]. They colonize both dry and moist wood and are responsible for its decay. The colony consists of soldiers, workers, reproductive forms and immature individuals. Reproductive forms are of two types: primary and supplementary. The primary forms include both the king and queen; they are completely developed and have wings as well as pigmented bodies. The life span of the queen is 25 years and she is capable of laying about 3000 eggs per day [121]. As a consequence of the above, within 4–5 years, almost 60,000 to 200,000 workers are produced and the size of colony greatly increases. Each colony has one pair of primary reproductive forms, which are replaced by supplementary forms upon the death of the former. The supplementary reproductive forms have reduced pigmentation on their bodies and while they may have wings, their size is greater than the workers present in colony. Both workers as well as soldiers are without eyes and wings [122]. Keeping in view their economic importance, a large number of studies have been conducted to investigate and identify medicinal plants that could have a potentially damaging effect on termites. Ravi *et al.* 2010[123] screened various organic extracts of the *Capparis decidua* against the Indian white termite (*Odontotermes obesus*). The results revealed that the extracts were highly toxic to *O. obesus*.

Aims and Objectives

The aims and objectives of the current study are as follows:

1. Preparation of Copper and Zinc nano-particles using aqueous extract from the leaves of *E. japonica* and *F. carica*, respectively.
2. Characterization of the bioinspired nano-particles using techniques such as: Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope (SEM), Energy Dispersive X-ray, (EDX), X-Ray Diffraction (XRD) and Thermo gravimetric (TG) and Differential Thermal Analysis (DTA) TG/DTA.
3. Optimization of the biosynthesis process by analyzing effect of variable parameters such as temperature, pH and time.
4. Determination of the biological impact (antibacterial, antifungal, hemagglutination, antioxidant, cytotoxic, phytotoxic, anti-termite and larvicidal) of the nano-particles and a comparison between the usages of different leaf fractions.

2.1 General

2.1.1 Instrumental Methods

2.1.1.1 Instrumental Methods

Amidate, Lanthan Flow Model 6.200, Oven, Temperature 110-125 °C, and other

Chemicals A.R. (200), UV spectrophotometer (Spectra 1.5-1000), and other

books, papers, and other materials for the purpose of the study.

Some chemical and other materials were used at different stages of the study.

2.1.2 Chemicals and reagents

All the chemicals used in the study were of analytical grade.

Water, acid, sodium hydroxide, and other chemicals were used as received.

2.1.3 Collection and Identification of Plant Leaves

2.1.3.1 Collection and Identification of Plant Leaves

2.1.3.2 Collection and Identification of Plant Leaves

2.1.3.3 Collection and Identification of Plant Leaves

2.1.3.4 Collection and Identification of Plant Leaves

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2.1.3.12 Collection and Identification of Plant Leaves

2.1.3.13 Collection and Identification of Plant Leaves

2.1.3.14 Collection and Identification of Plant Leaves

2.1.3.15 Collection and Identification of Plant Leaves

2.1.3.16 Collection and Identification of Plant Leaves

2.1.3.17 Collection and Identification of Plant Leaves

Experimental

2.0 Materials and Methods

2.1 Instruments and Glass wares

Autoclave, Laminar Flow Hood (LFH), Oven, Incubator (15–60 °C), analytical balance (Shimadzu AX-200), UV spectrophotometer (Shimadzu UV-1800), test tubes, volumetric flasks, beakers, Petri plates, aluminum foil, para-film, graduated cylinders, inoculation loop, cotton and culture swabs were used at different stages of the experimental work.

2.2 Chemicals and reagents

All the chemicals and reagents (Merck and Oxoid) were used in the current experimental work were of analytical grade. The main chemicals and media used were: Nutrient broth, Nutrient agar, Sabouraud Dextrose Agar (Oxoid), methanol, ethanol, ethyl acetate (EtOAc), $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{ZnSO}_4 \cdot 5\text{H}_2\text{O}$ (Merck).

2.3 Collection and Identification of Plants Leaves

Fresh leaves of *F. carica* (Voucher No.Botany/16789) were collected from Pakistan Council of Scientific and Industrial Research (PCSIR), Peshawar, Pakistan and were identified by Prof. Dr. Abd-ur-Rashid, Department of Biodiversity, University of Peshawar. The collected leaves were washed with water prior to being dried in shade. The dried leaves were ground to a fine powder using an electric grinder. Same procedure was followed for *E. japonica* (Voucher No.Botany/17899).

2.4 Extraction

The powdered leaves of *E. japonica* were soaked in methanol, ethanol and EtOAc in separate flasks for 48 hours. After soaking, all the filtrates were concentrated using a rotary evaporator under reduced pressure to get crude methanolic (Cr. Met. Ext), ethanolic and EtOAc extracts. The crude extracts, so obtained, were kept at 4°C until required. The same

protocol was followed to obtain Cr. Met. Ext, ethanolic and EtOAc extracts of *F. carica*.

2.5 Phytochemical Screening

The selected plants were screened for various classes of secondary metabolites; anthraquinone, alkaloids, phenols, glycosides, tannins, flavonoids, terpenoids, steroids and saponins.

2.5.1. Alkaloids

For the selected plant sample in question, a test solution was prepared by dissolving 0.2 g in 3 mL of *n*-hexane. The mixture was shaken well prior to being filtered. Five milliliters of 2% HCl were added to the filtrate and then addition of few drops of picric acid was carried out. The appearance of a yellow colored precipitate is indicative of the presence of alkaloids in the test sample [124].

2.5.2. Anthraquinone

The test sample (0.5g in 10% HCl solution) was boiled for a few minutes in a water bath. After boiling, the mixture was cooled to room temperature followed by filtration and CHCl_3 addition to the filtrate. The appearance of a rose-pink color upon heating was taken as indicative of the presence of anthraquinone after addition of few drops of 10% ammonia [125].

2.5.3. Glycosides

The test sample was mixed with few drops of ferric chloride and glacial acetic acid solution. This was followed by the addition of concentrated sulfuric acid and the sample was observed two layers' formation. The lower layer acquired a reddish brown shade while the upper acetic acid layer turned bluish green is indicative of glycosides [125].

2.5.4. Saponins

One milliliter of the test sample was added to 20 mL of distilled water; the mixture was shaken well for about 15 min. Formation of a layer of foam on the top is indicative of a positive result [125].

2.5.5. Flavonoids

0.2 g of the crude extract was dissolved in diluted Sodium hydroxide followed by the addition of a few drops of HCl. The transient appearance of a red-yellow color is confirmatory for the presence of flavonoids [125].

2.5.6. Steroids

Crude plant extract (1 mg) was dissolved in 10 mL of CHCl_3 followed by the addition of equal volume of concentrated H_2SO_4 to the test tube from the side. If the test sample is positive for the presence of steroids then the upper layer in the test tube will turn reddish while the lower H_2SO_4 layer appears yellow with a green fluorescence [124].

2.5.7. Terpenoids

In CHCl_3 (2mL) and concentrated H_2SO_4 (3mL), the crude extract (2g) was added. The sample was rested until the appearance of a layer. A reddish-brown color is a positive indicator for the presence of terpenoids [125].

2.5.8. Carbohydrates

Molisch's test was used to confirm the presence of carbohydrates. The Cr. Ext was dissolved in distilled water followed by addition of Molisch's reagent and concentrated H_2SO_4 (1 mL). Before dilution by distilled water (5 mL), the mixture was allowed for 2 minutes. A dull violet or red color formation is confirmatory for the presence of carbohydrates [126].

2.6 Synthesis of Nanoparticles

2.6.1 Synthesis of ZnNPs

Leaves of *E. japonica* (25 g of dried powder) were extracted in distilled water (500 mL) by heating for 30 minutes. 5 mL of the extract was added to 95 mL of 1Mm $\text{ZnSO}_4 \cdot 5\text{H}_2\text{O}$. This solution was kept in a 75°C water bath for 4 h with constant stirring. The change in color from dark to light brown is indicative of Zinc nanoparticles (ZnNPs) formation. The characterizations of synthesized ZnNPs were carried out by Scanning Electron Microscope (SEM), Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), Energy Dispersive X-ray, (EDX) and Thermo gravimetric (TG) and Differential Thermal Analysis (DTA) TG/DTA.

2.6.2 Synthesis of CuNPs

Leaves of *F. carica* (25 g dried powder) were extracted in distilled water (500 mL) by heating for 30 minutes. Five mL of the extract was added to 95 mL of 1Mm $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution. This solution was kept in a 75 °C water bath for 4 h with constant stirring. Change in color from dark green to light brown is indicative of the formation of CuNPs. The synthesized nano-particles were characterized using FTIR, SEM, EDX, XRD and TG/DTA

2.7 Conditions Optimization for Bio-inspired Synthesis of ZnNPs and CuNPs

To refine and streamline the synthesis of the nanoparticles as well as to augment the reproducibility of the methods, parameters such as temperature, time and pH were optimized.

2.7.1. Temperature

The optimization of temperature for the synthesis of ZnNPs and CuNPs was attempted at the following temperatures: 25, 35, 45, 55, 65, 75 and 85°C. UV-Visible spectrophotometers was used to monitor the formation of nano-particles by measuring absorbance at 455 and 426 nm for ZnNPs and CuNPs, respectively.

2.7.2. pH

The optimization of pH for the synthesis of ZnNPs and CuNPs was accomplished at various pH values; 4, 5, 6, 7 and 8. HCl (0.1 N) and NaOH (0.1 N) were used for adjustment of pH. UV-Visible spectrophotometry was used for monitoring synthesis by measuring the absorbance of ZnNPs and CuNPs solutions.

2.7.3. Time

For time optimization, the synthesis of ZnNPs and CuNPs was conducted in the time range of 1, 2, 3, 4 and 5 h. Absorbance was used to monitor the formation of ZnNPs and CuNPs as described previously.

2.8. Scanning Electron Microscope (SEM)

For sample preparation, a certain quantity of ZnNPs and CuNPs powder was dispersed in distilled water and coated on a copper grid surface with the help of carbon (30–40 °C) electrode. This leads to the formation of a thin film coating. Using gold sputting through a sputter coater (SPL 1-5A) for 120 s at 10 mA, the samples were observed by Scanning Electron Microscope (SEM JSM-5610, JEOL, Japan).

2.8.1. Energy Dispersive X-Ray (EDX) Analysis

EDX coated with SEM JSM-5610 JEOL, Japan. Model No. JET A200 was used to study the elemental composition of ZnNPs and CuNPs. From this data, we can predict that the synthesized particles were spherical and their particle size is nanoscale and made of zinc/copper (Zn).

2.8 Characterization of Synthesized ZnNPs and CuNPs

2.8.1. UV-Vis Spectrophotometer

A dual beam UV-1100 Shimadzu spectrophotometer was used to confirm the formation of ZnNPs and CuNPs. Aqueous plant extracts of *E. japonica* and *F. carica* were diluted with distilled water for the purpose of scanning in the spectrophotometer. The extracts were scanned in the wavelength range of 300–600 nm to observe for the peaks characteristic for ZnNPs and CuNPs.

2.8.2. Fourier Transform Infrared Spectroscopy

The FTIR spectrum of ZnNPs and CuNPs from *E. japonica* and *F. carica* were obtained by FTIR Prestige-21 Shimadzu Japan. The scanning was carried out from 3900–500 cm^{-1} at a resolution of 4 cm^{-1} for 10 scans and the obtained data was analyzed by IR solution software.

2.8.3. Scanning Electron Microscope (SEM)

For sample preparation, a minute quantity of ZnNPs and CuNPs powder was re suspended in distilled water and dried on a copper grid carbon with the help of hot air (50–60 °C; 5 minutes). This leads to the formation of a thin film coating. Using gold coating through a sputter coater (SPI, USA) for 120 s at 30 mA, the images were obtained by Scanning Electron Microscope (SEM JSM-5910 Japan).

2.8.4. Energy Dispersive X-Ray (EDX) Analysis

EDX paired with SEM JSM-5910 JEOL, Japan, Model No. INCA200 was used to study the elemental composition of ZnNPs and CuNPs. Onto dual sided gummed tape, the synthesized particles were spread and then placed over a microscopic end made of aluminum (Al).

2.8.5. X-ray Diffraction (XRD) Analysis

X-ray diffraction is a technique useful for measuring size and shape. In this study, an X-Ray Diffractometer (JDX-3532, JEOL Japan) was used for analyzing the ZnNPs and CuNPs. A layer of ZnNPs and CuNPs was prepared by dipping a glass plate into the respective solution. The diffracted intensities were figured at 2θ angles at $0.02^\circ/\text{min}$ with a time constant of 2s from 10° to 80° . Measurement condition were; 30 mA current, wavelength of $1.5418 \text{ \AA}$ in θ - 2θ configuration and voltage (V) ranging 20–40 kV in the presence of Cu K α radiation. For determining the crystalline domain, XRD peak width was used. For application of the Scherrer's formula, it was assumed that the ZnNPs and CuNPs are free commencing non-uniform strains

$$D = 0.94 \lambda / \beta \cos \theta$$

β = full width at half maximum (FWHM)

D = crystalline domain size (mean) at right angles to the reflecting planes

θ = Diffraction angle.

λ = X-ray wavelength

2.8.6. Thermogravimetric/Differential Thermal Analysis (TGA/DTA)

For determining the physical and chemical properties of the synthesized ZnNPs and CuNPs, Simultaneous Thermo-gravimetric and Differential Thermal Analysis (Shimadzu DTG-60/DTG-60A) was used. By increasing the temperature, mass gain and loss of synthesized nanoparticles was analyzed.

2.9 Biological activities

2.9.1 Antibacterial activity

The crude methanolic, ethanolic and EtOAc extracts as well as the ZnNPs and CuNPs were screened for antibacterial activity. The test pathogens include *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Morganella morganii*, *Acinetobacter baumannii*, *Proteus vulgaris* and Methicillin Resistant *S. aureus* (MRSA)].

Experimental Procedure [127]

Nutrient broth and nutrient agar media were prepared, autoclaved and checked for sterility by incubation at 37°C for 24 h. The test bacterial cultures were refreshed in sterile nutrient broth and these cultures were then used to make a uniform bacterial lawn on sterile nutrient agar plates. A sterile borer was used for making wells (6 mm) in the plates after introduction of the test cultures. Then, 100 µL each of the test samples (3 mg/mL) stock solution prepared in DMSO) was added to their respective well using a micropipette followed by incubation for 24 h at 37°C. Amoxicillin served as positive control while DMSO was used as a negative control. The percent zone of inhibition of the test samples was measured in millimeters with reference to the positive control.

Percent zone of inhibition: $\frac{\text{Zone of inhibition of test sample}}{\text{Zone of inhibition of the standard}} \times 100$

2.9.2 Antifungal Bioassay

The antifungal activity of all crude extracts along with the synthesized ZnNPs and CuNPs were evaluated against the following selected fungal strains: *Penicillium notatum*, *Aspergillus niger*, *Aspergillus parasiticus*, *Hemimycena pseudocrispula*, *Verticillium longisporum* and *Penicillium chrysogenum*.

Stock solutions of the test samples were prepared in DMSO (24 mg/mL). All the selected fungal pathogens were refreshed on SDA. The SDA media (4 mL) was introduced into test tubes and autoclaved. When the temperature reached approximately 50 °C, 67 µL of each stock solution was introduced into their respective tubes. The test tubes were kept in slanted position and were checked for sterility by incubating it at 37°C for 24 h. The test tubes were inoculated with fungal strains, the next day and incubated at 28 ±1 °C for 7 days. The percent linear non-mycelial growth (mm) was measured in comparison with standards.

$$\text{Percent linear growth inhibition} = \frac{\text{Linear growth in test sample}}{\text{Linear growth in control}} \times 100$$

2.9.3 Insecticidal Activity

The contact toxicity assay was used to evaluate the insecticidal activity of crude methanolic, ethanolic, EtOAc, ZnNPs and CuNPs of the selected plants against *Tribolium confusum*, *Callosobruchus maculatus* and *Tribolium castaneum* [128].

The selected insects were reared under controlled conditions using breeding media in plastic bottles. Insects of a uniform size were selected for the experimental studies. Two hundred mg of the test samples was prepared in methanol to make stock solution. Filter paper of the size of the Petri plate was cut and placed on the plate prior to the introduction of the test sample. The solvent was allowed to evaporate and 10 healthy insects of uniform size were introduced into the respective plates. The plates were incubated in growth chambers at 27°C with 50% relative humidity for 24 hours. The results of this activity were recorded by counting the number of survivors in each plate to determine percent inhibition or mortality with Permethrin (235.9 $\mu\text{g}/\text{cm}^2$) served as positive control.

2.9.4 Anti-Termites Bioassay

Materials

Heterotermes indicola, test samples (Cr. Met. Ext, ethanolic and EtOAc extracts, ZnNPs and CuNPs), blotting paper, Petri plates, desiccators and magnifying glass.

Procedure

Anti-termite bioassay was performed as per the procedure described by Salihah *et al.* [129], by cutting the blotting paper of the size of Petri dish. The blotting paper was immersed in the stock solution (2 mg in methanol) of the test samples. The solvent was allowed to evaporate from the blotting paper. *H. indicola* (10 in number) was then transferred to the Petri dish using a brush. The Petri plates were then incubated in a desiccator till all the termites were dead. The experiments were conducted in triplicate and results were measured by counting number of survivors.

2.9.5 Phytotoxic Assay

The test samples (plant extracts, ZnNPs and CuNPs) were screened against *Lemna minor* for their phytotoxic potential as per previously published procedure [130]. Stock solutions of the test samples were prepared in methanol (20 mg). From the stock solution (20mg in methanol), 1000, 100 and 10 μ L were introduced into their respective volumetric flasks. After the solvent was evaporated, E-media (20 mL) with sixteen healthy plants were introduced into respective flask and incubated for in a growth chamber at $28 \pm 1^\circ\text{C}$ for seven days. The number of damaged plants was counted to determine the phytotoxic potential of the test samples.

2.9.6 Haemagglutination Activity

Materials

Blood samples of all ABO groups, Dipotassium Hydrogen Phosphate (K_2HPO_4), Disodium Hydrogen Phosphate (Na_2HPO_4), test tubes, centrifuge and incubator. The human blood samples were taken after ethics committee approval.

Procedure

The phosphate buffer was prepared by separately dissolving 0.453g of K_2HPO_4 and 0.47g of Na_2HPO_4 in 50 mL of distilled. The solutions were then mixed in a 3:7 ratios. Stock solution (1 mg test sample in 1 mL of DMSO) was prepared and different dilutions were then prepared; 1:2, 1:4, 1:8 and 1:16. RBC's were obtained by centrifuging fresh blood collected from the healthy individuals. Each of the test sample (1mL) and 2% RBC suspension (in phosphate buffer) was mixed and incubated for 30 minutes at 37°C. After incubation, centrifugation was performed and checked for smooth and rough button formation which indicates negative and positive results [130].

DISCUSSION

RESULTS AND DISCUSSION

The first part of the study was devoted to the study of the effect of the concentration of the monomer on the rate of polymerization. It was found that the rate of polymerization increases with the concentration of the monomer. The effect of the temperature on the rate of polymerization was also studied. It was found that the rate of polymerization increases with the temperature. The effect of the catalyst on the rate of polymerization was also studied. It was found that the rate of polymerization increases with the concentration of the catalyst.

RESULTS

1.1. Effect of monomer concentration on the rate of polymerization

The effect of the concentration of the monomer on the rate of polymerization was studied. It was found that the rate of polymerization increases with the concentration of the monomer. The effect of the temperature on the rate of polymerization was also studied. It was found that the rate of polymerization increases with the temperature. The effect of the catalyst on the rate of polymerization was also studied. It was found that the rate of polymerization increases with the concentration of the catalyst.

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DISCUSSION

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Results and Discussion

E. japonica and *F. carica* belongs to the families Rosaceae and Moraceae, respectively and are important medicinal plants. Ethnobotanical studies and previous literature have documented an array of biological uses of these plants, including antimicrobial, antidiuretic, anticancer and many more. Since potential therapeutic applications of these plants have been developed, we attempted to synthesize CuNPs and ZnNPs from these plants, using standard procedures. The synthesized NPs and other organic solvent extracts were also screened for various pharmacological activities including antimicrobial, phytotoxic, insecticidal, anti-termite and haemagglutination.

3.1 Extraction and phytochemical screening

The leaves of *E. japonica* and *F. carica* were collected from the Pakistan Council of Scientific and Industrial Research (PCSIR) Labs, Peshawar. They were further dried and ground into a fine powder (500 gm). The powdered leaves (200gm) of the selected plant were extracted in methanol, ethanol and EtOAc, yielding crude methanolic (15 gm), ethanolic (11 gm) and EtOAc (11 gm) fractions, respectively.

Both the *F. carica* and *E. japonica* were qualitatively screened for the existence of secondary metabolites. Both the plants were found to contain terpenoids alkaloids, glycosides, anthraquinone, saponins, flavonoids and steroids (Table 3.1).

Different reducing agents and phytochemicals in the plant extracts are responsible for the synthesis of NPs. The main constituents that are responsible for reduction of ions are terpenoids, carboxylic acids, amides, aldehydes, carboxylic acids, flavones, organic acids, and quinones. A previous study has revealed that in xerophytes, anthraquinones such as emodin, are responsible for the synthesis of AgNPs [131].

3.1 Synthesis and qualitative optimization of 2a-2f

Phenylpropanoid metabolites of 2a-2f were synthesized using the synthetic scheme of 2. japonica. In accordance with standard procedure, a color change was observed from a color light brown reflecting the formation of 2a-2f. This color change was used for the optimization of reaction conditions for maximum production of 2a-2f.

Table 3.1: Qualitative Phytochemicals profile <i>E. japonica</i> and <i>F. carica</i> Constituents	<i>E. japonica</i>	<i>F. carica</i>
Terpenoids	+	+
Alkaloids	+	+
Glycosides	+	+
Anthraquinone	+	+
Saponins	+	+
Flavonoids	+	+
Steroids	+	+
Carbohydrates	+	+

Present: +, Absent: -

3.2 Synthesis and condition optimization of ZnNPs

Bio-inspired synthesis of ZnNPs was achieved using the aqueous extract of *E. japonica*. In accordance with standard procedures, a color change was observed from dark to light brown, indicating the formation of ZnNPs. The following paragraphs describe the optimization of various conditions, for maximum production of ZnNPs.

At a fixed concentration of 0.2 mg/mL ZnCl₂, the highest absorbance value recorded was 0.43 at 25 °C (Figure 3.1). The absorbance values recorded at other temperatures were 0.34 at 20 °C, 0.44 at 35 °C, 0.48 at 45 °C, 0.40 at 55 °C, 0.32 at 65 °C and 0.22 at 85 °C (Table 3.1). A similar study has been reported for the synthesis of AgNPs, where an increase in the incubation temperature to a particular value, yields an increased absorbance, making it in line with our study [12]. NPs prepared at different temperatures exhibit different physicochemical properties [13]. At various incubation stages, following incubation time of 30 min, during the temperature from 25 °C to 85 °C, indicate the synthesis of AgNPs, but any further increase in temperature resulted in a decrease in absorbance [14].

3.2.1 Temperature

Temperature plays a key role in the synthesis of NPs; therefore, determination of the optimum temperature for effective synthesis of ZnNPs is essential. To achieve this, the synthesis was carried out at various temperatures ranging from 25 °C to 85 °C. The formation of NPs was monitored using UV-visible spectrophotometry at a maximum wavelength of 455 nm (λ max). For ZnNPs, the highest absorbance value recorded was 0.65 at 75 °C (Figure 3.1). The absorbance values recorded at other temperatures were 0.3 at 25 °C, 0.43 at 35 °C, 0.44 at 45 °C, 0.46 at 55 °C, 0.52 at 65 °C and 0.58 at 85 °C (Figure 3.1). A similar study has been reported for the synthesis of AgNPs, where an increase in the incubation temperature to a particular value, results in increased synthesis, making it in line with our study [132]. NPs prepared at elevated temperature exhibit surface plasmon resonance (SPR) at narrow absorption range, indicating monodispersity. In another study, raising the temperature from 25 °C to 45 °C enhance the synthesis of AgNPs, but any further increase in temperature resulted in decreased absorbance [133].

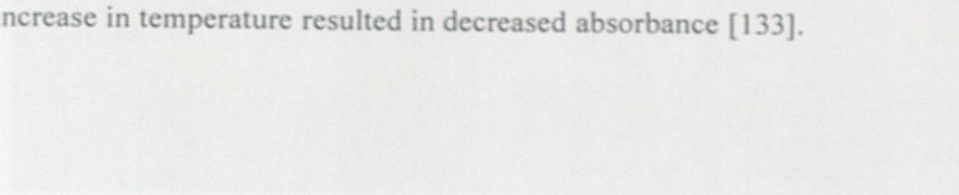


Figure 3.1 Effect of temperature on the absorbance of ZnNPs.

3.3.2 pH

To determine the optimum pH for effective synthesis of ZnNPs, synthesis was carried out at various pH levels ranging from 4–9. The best results were obtained for ZnNPs synthesized at pH 7, at which a highest absorbance value of 0.65 was recorded (Figure 3.1). The absorbance values recorded at other pH levels were 0.3 at pH 4, 0.43 at pH 5, 0.45 at pH 6 and 0.57.

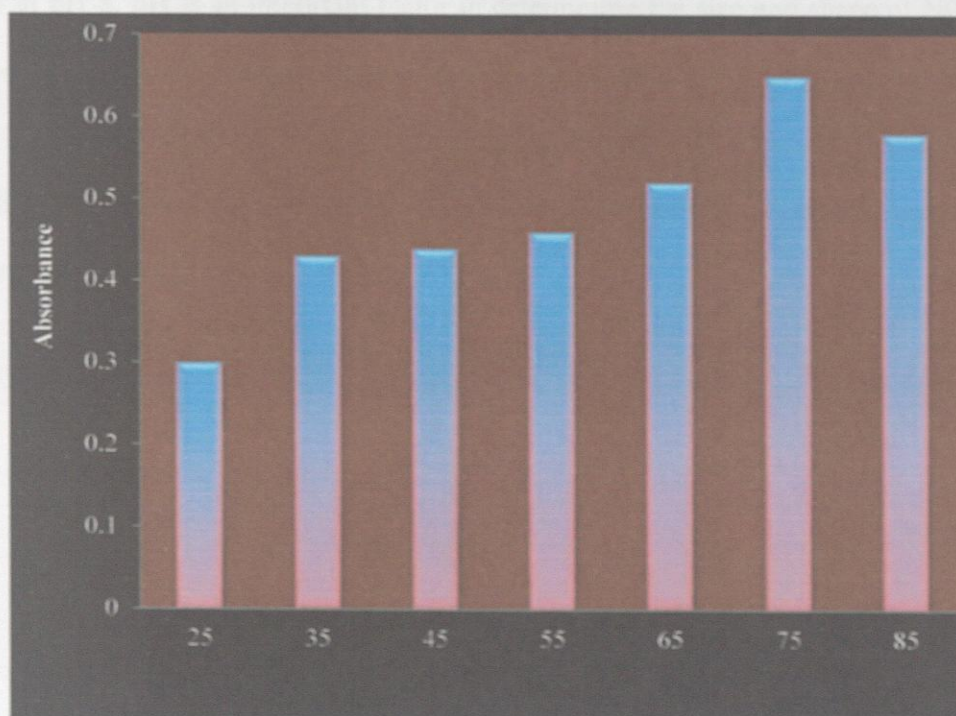


Figure 3.1: Optimization of temperature for the synthesis of ZnNPs

3.2.2. pH

To determine the optimum pH for effective synthesis of ZnNPs, synthesis was carried out at various pH levels ranging from 4–8. The best results were obtained for ZnNPs synthesized at pH 7, at which a highest absorbance value of 0.6 was recorded (Figure 3.2). The absorbance values recorded at other pH levels were 0.3 at pH 4, 0.42 at pH 5, 0.52 at pH 6 and 0.43 at pH 8. pH is an important factor in determining the size and shape of NPs. Low pH is required for the synthesis of larger NPs, while high pH results in the formation of smaller NPs [128, 133]. At low pH, nucleation process takes place, resulting in the aggregation of ZnNPs to form larger NPs; while at high pH there is no aggregation, resulting in smaller sized NPs [134].

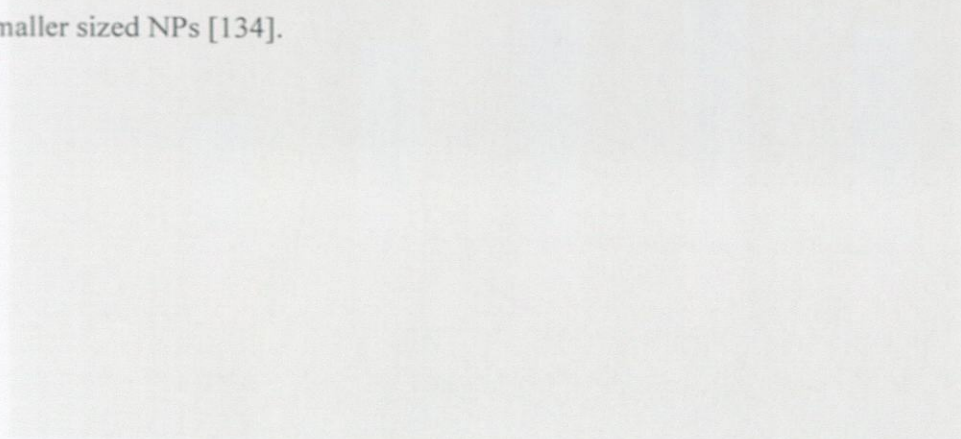


Figure 3.2: Optimization of pH for the synthesis of ZnNPs.

3.1.3 Time

To optimize the reaction time for maximum yield of NPs, the synthesis of ZnNPs was accomplished in a time range of 1, 2, 3, 4 and 5 h. Highest absorbance value of 0.6 was obtained at the reaction time of 4 h (Figure 3.2), indicating the reaction time was 4 h needed for maximal absorbance. The absorbance values at other time duration, were 0.3 at 1 h, 0.4 at 2 h, 0.45 at 3 h and 0.42 at 5 h. Other authors have reported that the NPs

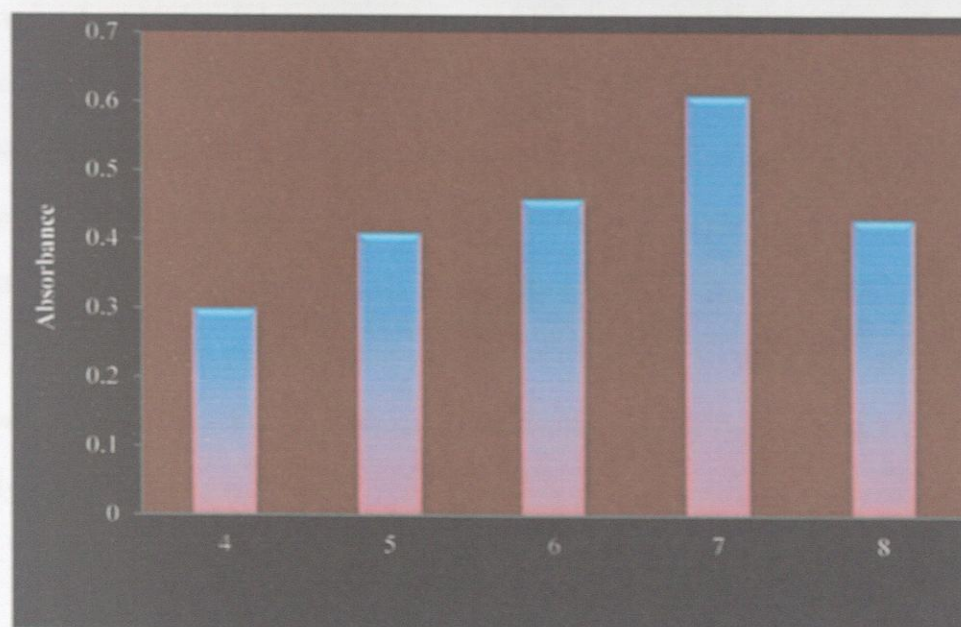


Figure 3.2: Optimization of pH for the synthesis of ZnNPs

3.2.3. Time

To optimize the reaction time for maximum yield of NPs, the synthesis of ZnNPs was accomplished in a time range of 1, 2, 3, 4 and 5 h. Highest absorbance value of 0.6 was obtained at the reaction time of 4 h (Figure 3.3). Increasing the reaction time over 4 h resulted in decreased absorbance. The absorbance values at other time durations were 0.49 at 1 h, 0.1 at 2 h, 0.18 at 3 h and 0.49 at 5 h. Other studies have reported that the NPs formed after 36 h of incubation are unstable in nature [133]. These findings were consistent with the conclusions drawn earlier [135]. With the passage of time, more AgNPs are formed. An optimum incubation period is required for the synthesis of AgNPs, which results in the aggregation of AgNPs to form large sized NPs. Contrary to our results, another study reported the optimum time period needed for the successful synthesis of NPs as 1 h [136]. It was observed that till 30 min there was no formation of NPs, however, formation started quickly after 60 min and completed within 5 h, making it in line with our study [137].

Figure 3.3: Effect of time on the synthesis of ZnNPs.

3.3 Characterization of synthesized ZnNPs

3.3.1 UV-Visible Spectroscopy

The aqueous solutions were characterized by scanning the wavelength between 200 nm to 400 nm, to observe the absorbance peaks for ZnNPs. The presence and formation of NPs in aqueous solution was confirmed with the help of UV-Visible spectroscopy. A broad peak was found at 430 nm, clearly indicating the formation of

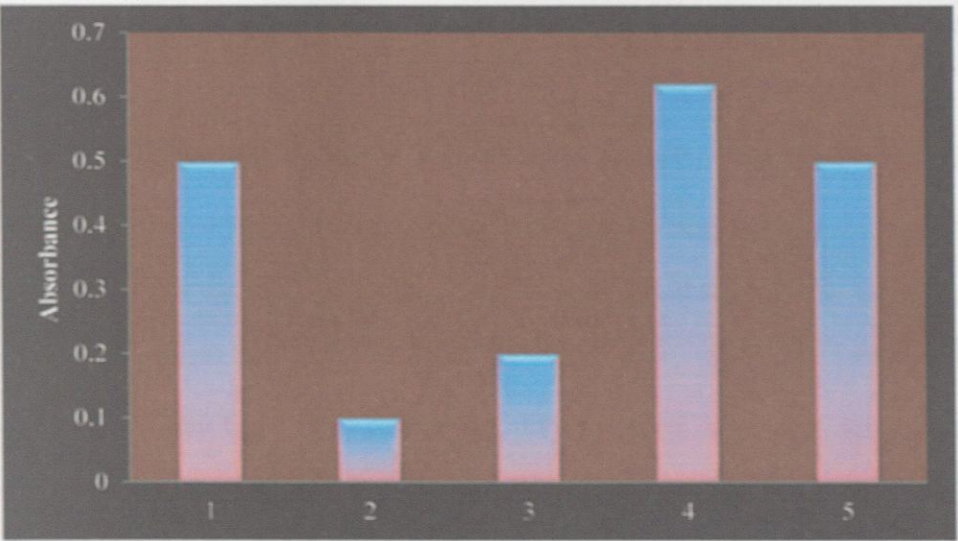


Figure 3.3: Optimization of time for the synthesis of ZnNPs

3.3. Characterization of Synthesized ZnNPs

3.3.1. Ultra Violet-Visible Spectroscopy

The aqueous extracts were characterized by scanning the wavelength emission over the range of 300–600 nm, to observe the absorbance peaks for ZnNPs. The steadiness and formation of NPs in aqueous solution was confirmed with the help UV-visible spectroscopy. A broad peak was found at 455 nm, clearly indicating the formation of ZnNPs (Figure 3.4). In accordance with previous studies, a broad peak was observed for formation of CuNPs with less reducing agent (IG). With a limited quantity of reducing agent, the peak intensity gradually increases; additionally the peak fluctuation was minimum as compared to peaks with more quantity reducing agent, which intensify the requirement of minimum threshold amount of reducing agent for the effective formation of the particular NPs [138].

In an earlier study, the UV-vis spectra of ZnONPs prepared with 0.5% soluble starch was analyzed. The absorption peak was detected at a wavelength of 360 nm. A significant widening in peaks was observed between 350–480 nm, revealing that the NPs were polydispersed [141].

3.3.1 FTIR analysis

The functional group identification in the spectra were done using the help of IR spectral analysis as shown in Figures 3.5-3.8. Strong absorption bands observed at 3295.35 and 3333 cm^{-1} in the IR spectra are due to the stretching vibrations of hydroxyl groups in ZnO. The absorption band appeared at 1593 cm^{-1} is believed to be associated with stretching vibrations of $\text{C}=\text{O}$ bonds. Absorption peaks at 1111.75 and 1286 cm^{-1} are attributed to in-plane $\text{C}-\text{H}$ stretching and out-of-plane $\text{C}-\text{H}$ bending vibrations, respectively.

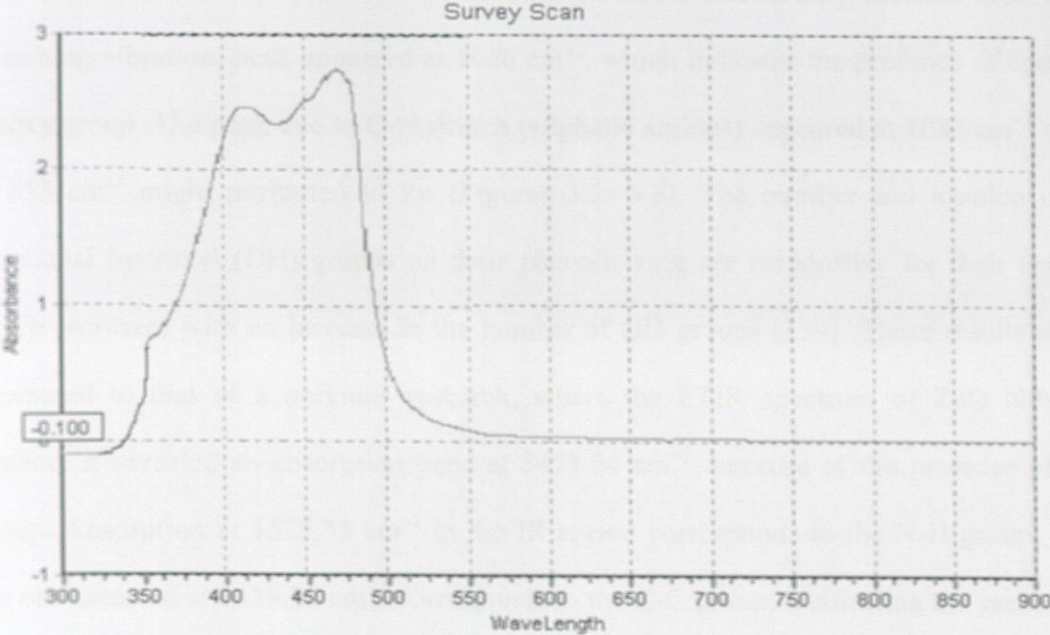


Figure 3.4: UV spectra of ZnNPs

3.3.2. FTIR Analysis

The functional group identification in the active ingredients was done with the help FT-IR spectral analysis as shown in Figures 3.5–3.8. Strong absorption bands observed at 3298.28 and 3338 cm^{-1} in the IR-spectrum are due to the stretching vibrations of hydroxyl (OH) or carboxyl (COOH) groups. The absorption band appeared at 1593 cm^{-1} is believed to be associated with stretching vibration of C=C bonds. Absorption peaks at 1116.78 and 1386 cm^{-1} are attributed to n-plane C—H bending vibration and tertiary alcohol. Due C—O stretching vibration, peak appeared at 1066 cm^{-1} , which indicates the presence of epoxy or alkoxy group. The peak due to C-N stretch (aliphatic amines) appeared at 1083 cm^{-1} while, at 653 cm^{-1} might attributed to Zn (Figures 3.5- 3.8). The number and location of the functional hydroxyl (OH) groups on their phenolic ring are responsible for their toxicity, which increases with an increase in the number of OH groups [139]. These results can be compared to that of a previous research, where the FTIR spectrum of ZnO NPs was studied. It revealed an absorption band at 3458.04 cm^{-1} , because of the presence of O-H group. Absorption at 1625.35 cm^{-1} in the IR region corresponds to the N-H group, while the one detected at 1418.86 cm^{-1} corresponds to the C-C group, confirming the presence of alcohol (R-OH), carboxylic acid (R-COOH), ethers (R-O-R) and esters (R-CO-R). The absorption peak at 875.23 nm confirms the tetrahedral dexterity of Zn [98].

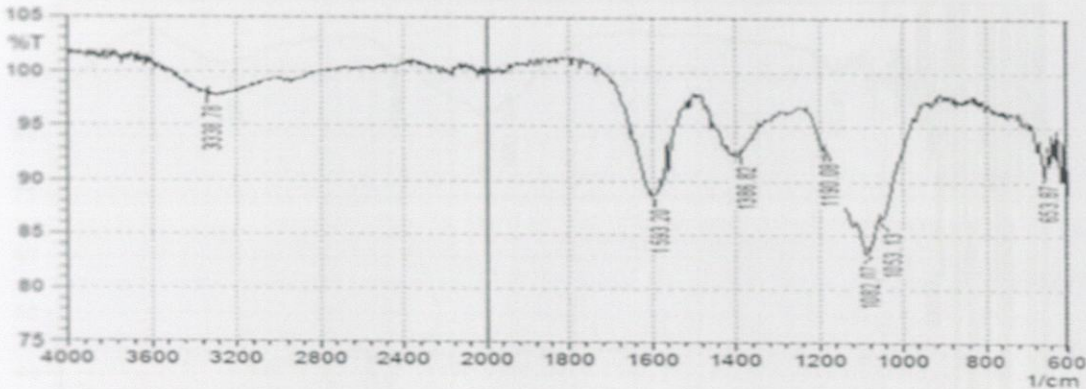


Figure: 3.5: FTIR spectra of ZnNPs

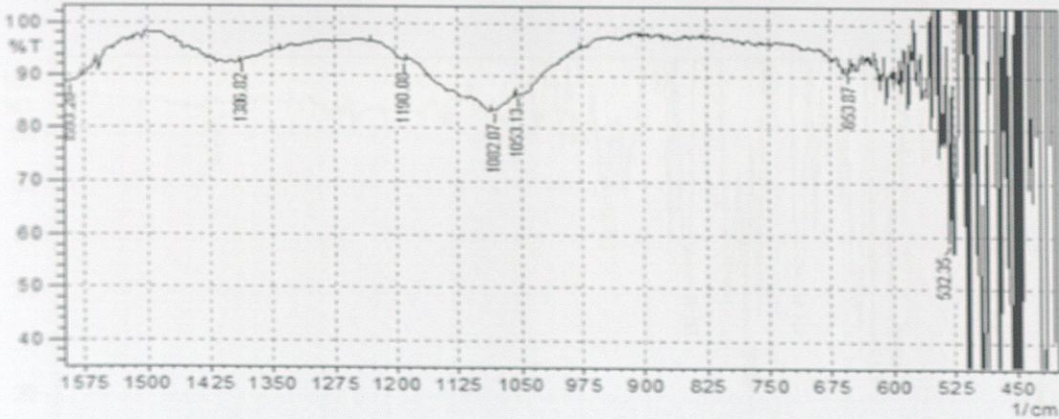


Figure:3.6. FTIR of ZnNPs

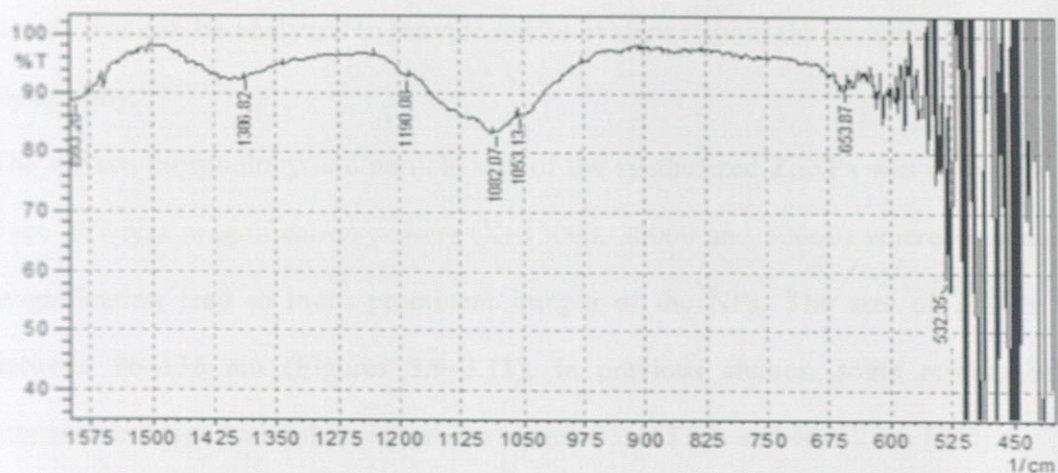


Figure: 3.7: FT-IR spectra of ZnNPs

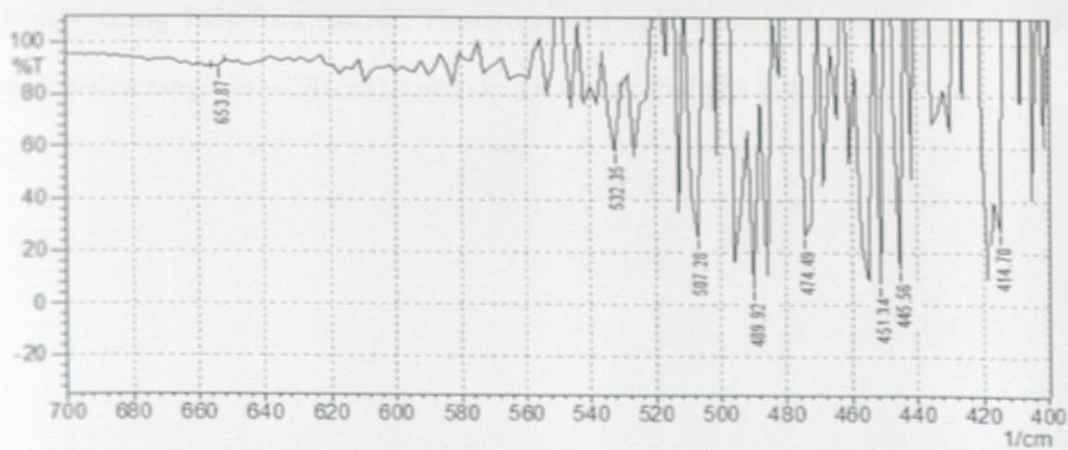


Figure: 3.8: FT-IR spectra of ZnNPs

3.3.3 Scanning Electron Microscope (SEM)

SEM is a type of electron microscope that scans a focused beam of electrons over a surface to form an image. These electrons interact with the sample surface, producing various signals that can be analyzed to provide useful evidence about the composition and surface topography.

The surface morphology and particle size of the synthesized ZnNPs was determined, using three different magnification powers ($X=15000$, 30000 and 50000) where, enhancing the magnification lead to more prominent images of the NPs. The size of ZnNPs ranged between $96\text{--}136\text{ nm}$ (Figures 3.9–3.11). In previous studies, some researchers have prepared and reported ZnNPs in the size range of $53\text{--}65\text{ nm}$ and $44\text{--}62\text{ nm}$ [140].

SEM images of ZnO NPs were prepared through green synthesis and chemical synthesis methods, using 10% fruit extract. These revealed an extremely dense image of ZnO NPs (particle size range: $60\text{--}100\text{ nm}$), consistent with our findings [141].

Figure 3.10: ZnNPs at $15000\times$

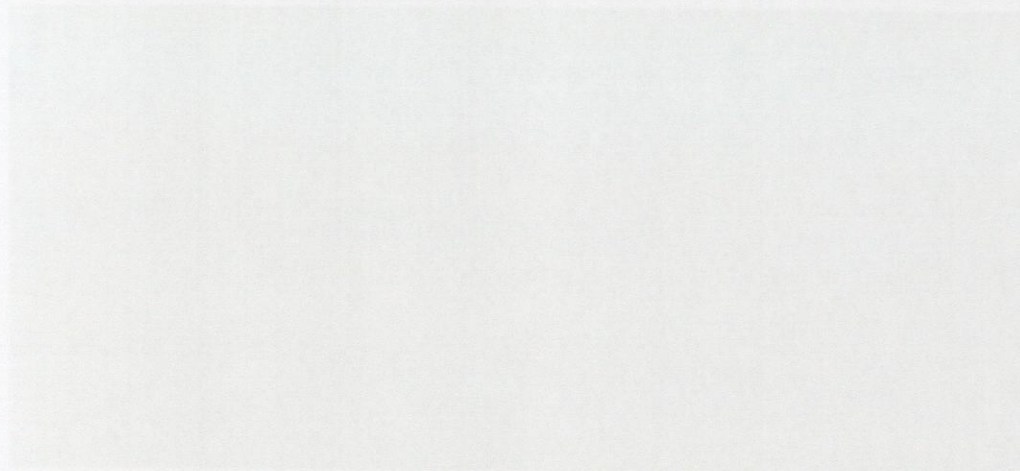


Figure 3.11: ZnNPs at $30000\times$

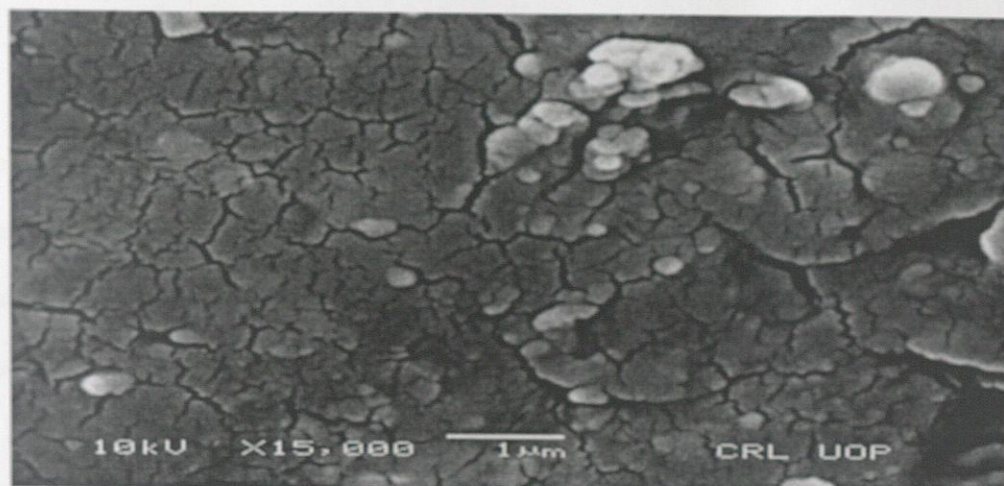


Figure 3.9: ZnNPs at 15000x

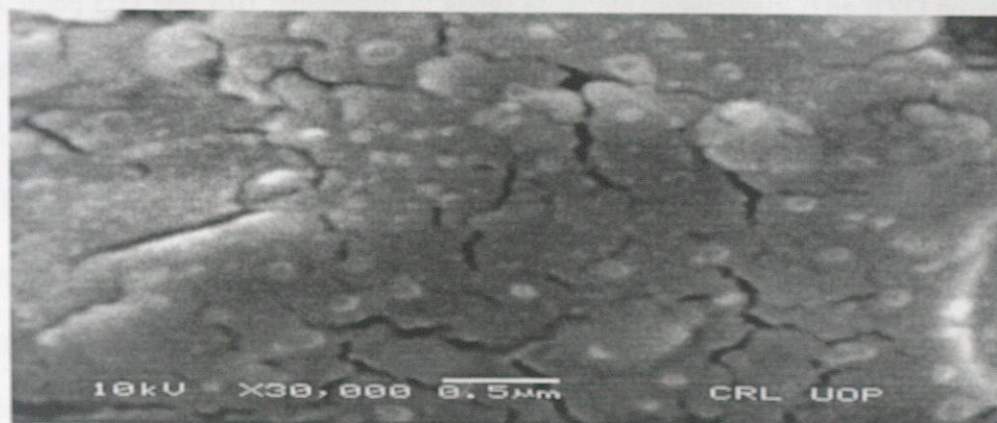


Figure: 3.10: ZnNPs at 30000x

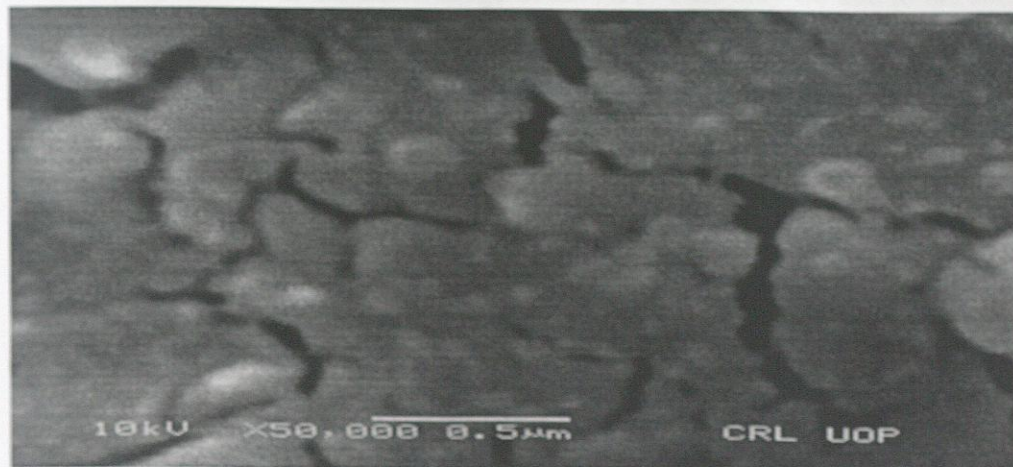


Figure 3.11: ZnNPs at 50000x

3.3.4 Energy Dispersive X-ray Analysis

This technique is commonly used to identify the existence of various elements in a sample. In the present work elemental Zn was confirmed from the EDX profile showed, a signal found at 8.2 keV showing elemental Zn (Figure 3.12). Furthermore, the EDX profile of ZnNPs showed absorption bands at 0.6 keV for Magnesium, 1.2 for Silicon, 1.9 for Sulfur, 2.9 for Potassium, 2.2 for Chlorine and at 3.3 for Calcium (Figure 3.12).

The ZnNPs were found to comprise percent composition of C (41.10), Cu (47.06), Mg (0.79), S (3.66) and O (4.28), respectively by weight, while were found to contain 51.40, 44.18, 0.49, 1.72 and 1.01 percentage of above elements respectively. The EXD provided an intense peak for Zn, followed by C. The ZnNPs were found to be 42.20% by mass and 45% by atomic level, while the other elemental composition of ZnNPs included C, Zn, Mg, S and K as 45.23, 42.20, 1.06, 2.56 and 2.59%, respectively. Their atomic percent composition was found to be 42.36, 45.23, 0.65, 1.20 and 0.98, respectively.

The present study can be compared to a previous study, where EDX plot analysis demonstrated that Zn and O are present in the sample. The graph of EDX detected the presence of C, Si, and Ca in the EDX image of Zn NPs. This may be due to the substrate on which the NP sample was placed during SEM examination [141].

Table 3.2: ZnNPs Mass and Atomic weight percentage

Name of Element	Mass (%)	Atom (%)
C K	45.23	42.36
Zn K	42.20	45.23
Mg K	1.06	0.65
S K	2.56	1.20
K K	2.59	0.98

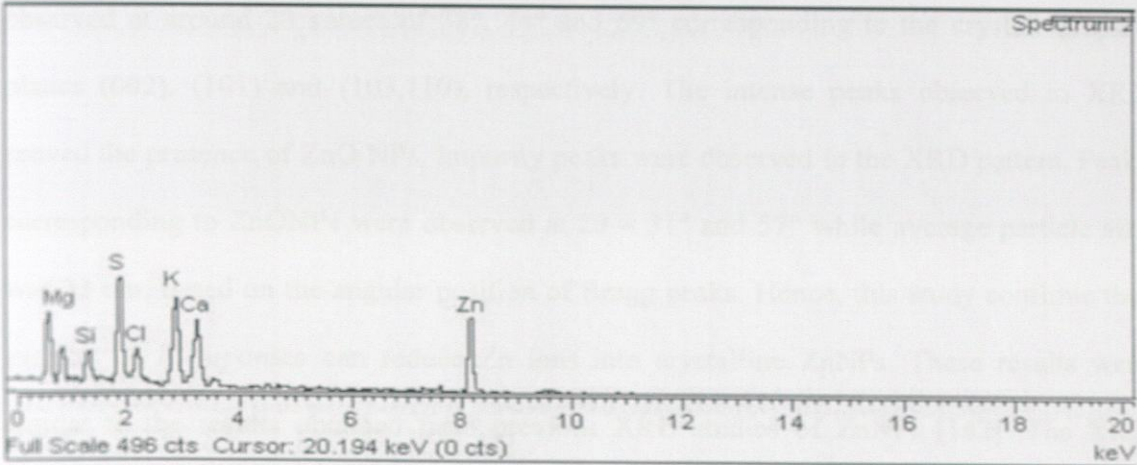


Figure 3.12: EDX spectra of ZnNPs

3.3.5 X-ray Diffraction

The XRD pattern of ZnNPs, using aqueous leaf extracts of *E. japonica* (Figure 3.13). The indexing of the diffraction pattern is done and *Miller Indices* (h, k l) were assigned to each peak, while degree of crystallinity of ZnNPs was determined from the peak intensity. Debye-Scherrer formula was used to estimate size of the ZnNPs and was found to be 33 nm.

E. japonica was found effective to stabilize and reduce the Zn ions to their corresponding ZnNPs, as showed by the XRD pattern (Figure. 3.13). Strongest peaks of ZnNPs were observed at around 2θ values of 38° , 44° and 69° corresponding to the crystallographic planes (002), (101) and (103,110), respectively. The intense peaks observed in XRD proved the presence of ZnO NPs. Impurity peaks were observed in the XRD pattern. Peaks corresponding to ZnONPs were observed at $2\theta = 31^\circ$ and 57° while average particle size was 33 nm, based on the angular position of Bragg peaks. Hence, this study confirms that extracts of *E. japonica* can reduce Zn ions into crystalline ZnNPs. These results were similar to the results obtained from previous XRD studies of ZnNPs [142]. The XRD pattern of ZnNPs was found in close proximity with the published XRD pattern for the ZnONPs [143].

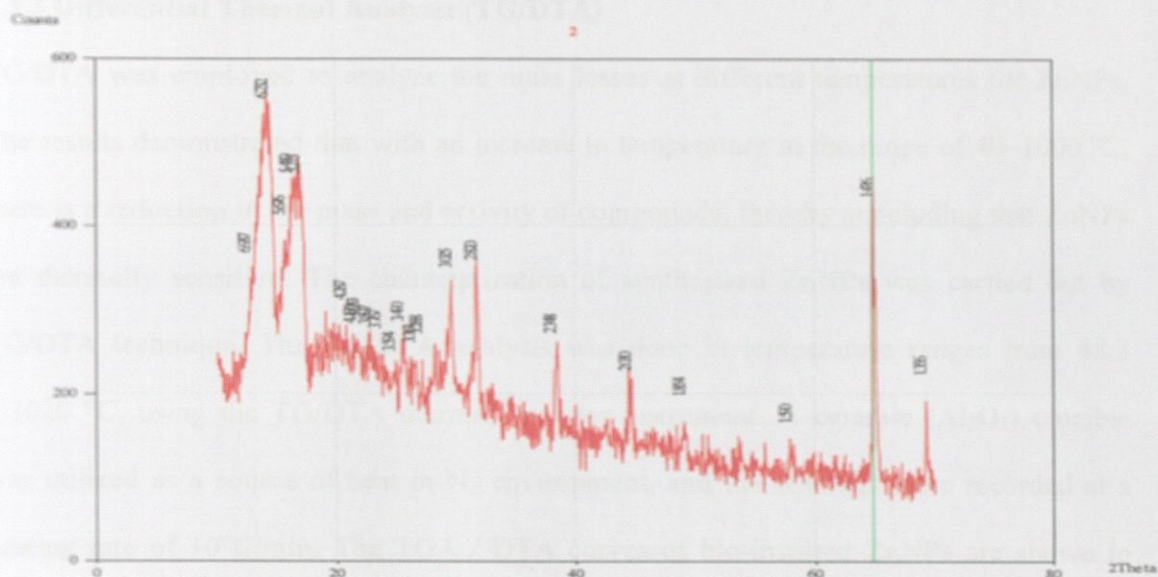


Figure: 3.13: XRD of ZnNPs

3.3.3 Differential Thermal Analysis (TG/DTA)

TG/DTA was employed to analyze the mass losses at different temperatures for ZnNPs. The results demonstrated that with an increase in temperature in the range of 40–1000 °C, there is a reduction in the mass and activity of compounds, thereby concluding that ZnNPs are thermally sensitive. The characterization of synthesized ZnNPs was carried out by TG/DTA technique. The TG/DTA analysis was done in temperature ranges from 48.3 to 1020 °C, using the TG/DTA thermal analyser instrument. A ceramic (Al_2O_3) crucible was utilized as a source of heat in N_2 environment, and the readings were recorded at a heating rate of 10°C/min. The TGA / DTA curves of bio-inspired ZnNPs are shown in Figure 3.14.

The TGA curve in Figure 3.14 shows that at 250.100, 100.034 and 45.134 °C, the weight reduction of ZnNPs was 5.710, 5.890 and 6.100 mg, respectively. It does not show any prominent reduction in weight. The significant weight reduction started from 400 to 560.324 °C and weight reduced from 4.001 to 2.103 mg, but no further weight loss beyond 600°C. This weight reduction was correlated to the organic matrix ignition, which behaved as a capping and stabilizing agent. It was concluded that the heating processes might be connected through the leftover carbon residue and the organic matrices burning.

A previously reported study showed that TG-DTA analysis of ZnNPs have an initial weight reduction in the range of 30–100°C, the highest being 17.87 % at 150–280 °C, showing the presence of ZnONPs [144]. In another study, the combined pattern of TGA and DTA curves discovered the undoped and Zn^{2+} (4%) doped NPs. The DTA analysis showed a strong exothermic peak at 328°C [145].

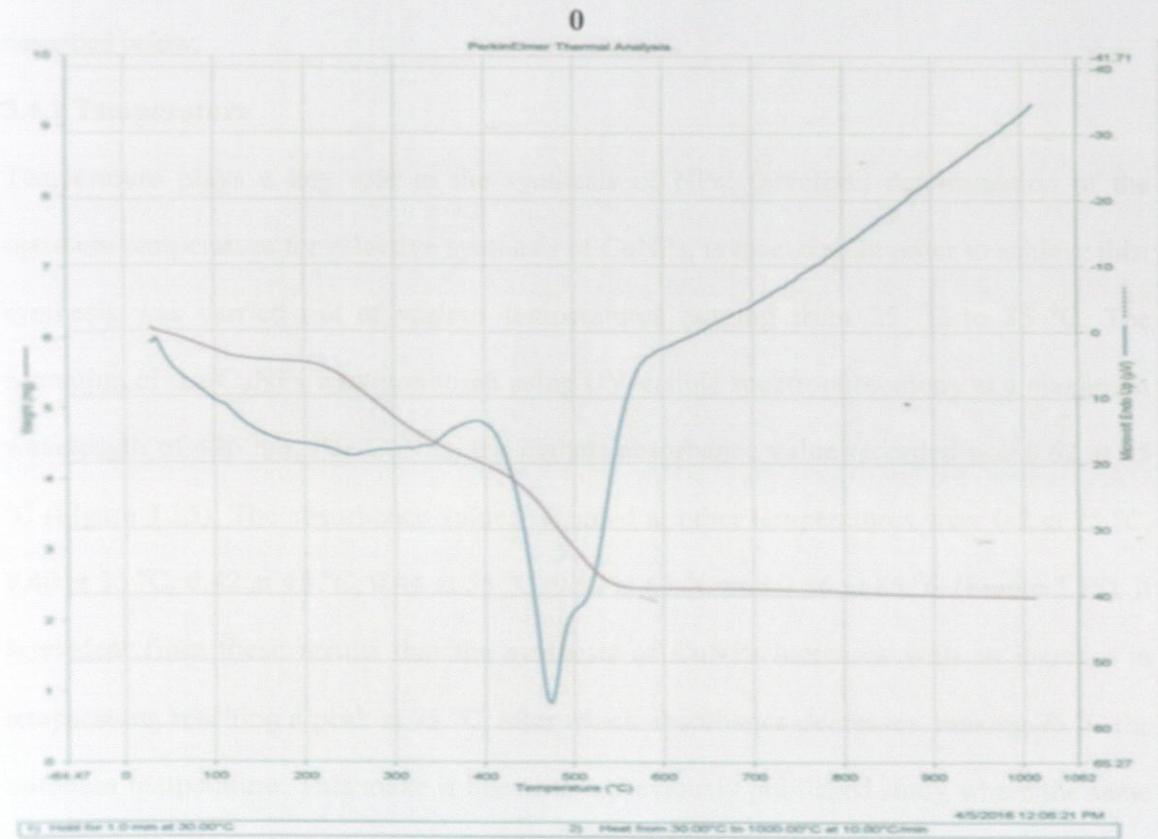


Figure 3.14: Thermal Analysis “TGA/DTA” of ZnNPs

3.4 Synthesis and Conditions Optimization for CuNPs

Aqueous extracts of *F. carica* leaves were utilized for the synthesis of CuNPs using a standard procedure. The color change from deep green to light brown revealed the formation of CuNPs. The different conditions that were optimized for the maximum synthesis of CuNPs have been described below.

3.4.1 Temperature

Temperature plays a key role in the synthesis of NPs; therefore, determination of the optimum temperature for effective synthesis of CuNPs, is essential. In order to achieve this, synthesis was carried out at various temperatures ranging from 25 °C to 85 °C. The formation of the CuNPs was monitored using UV-visible spectrophotometry at a maximum wavelength of 426 nm. For CuNPs, the highest absorbance value recorded was 0.62 at 75 °C (Figure 3.15). The absorbance values recorded at other temperatures were 0.2 at 25 °C, 0.40 at 35 °C, 0.42 at 45 °C, 0.44 at 55 °C, 0.50 at 65 °C and 0.56 at 85 °C (Figure 3.15). It is evident from these results that the synthesis of CuNPs increases with an increase in temperature, reaching a peak at 75 °C, after which absorbance decreases, making 75 °C the optimum temperature. This make it line with a previously published study where the same trend was observed for the synthesis of AgNPs [132]. NPs exhibit SPR at narrow absorption range, when synthesized at a higher temperature range, specifying monodispersity. With increasing temperature, the formation of AgNPs increases from silver ions, and also reduces secondary reduction processes. It was found that with an increase in temperature from 25–45 °C absorbance also increases, but further increase in temperature reduces absorbance [133].

3.4.2. pH

The pH at which efficient synthesis of CuNPs can take place with maximum yield was determined at pH levels ranging from 4–8. A maximum absorbance of 0.36 was recorded at neutral pH (7), indicating optimum synthesis of CuNPs at this pH (Figure 3.14). The absorbance values recorded at other pH levels were 0.23 at pH 4, 0.26 at pH 5, 0.41 at pH 6

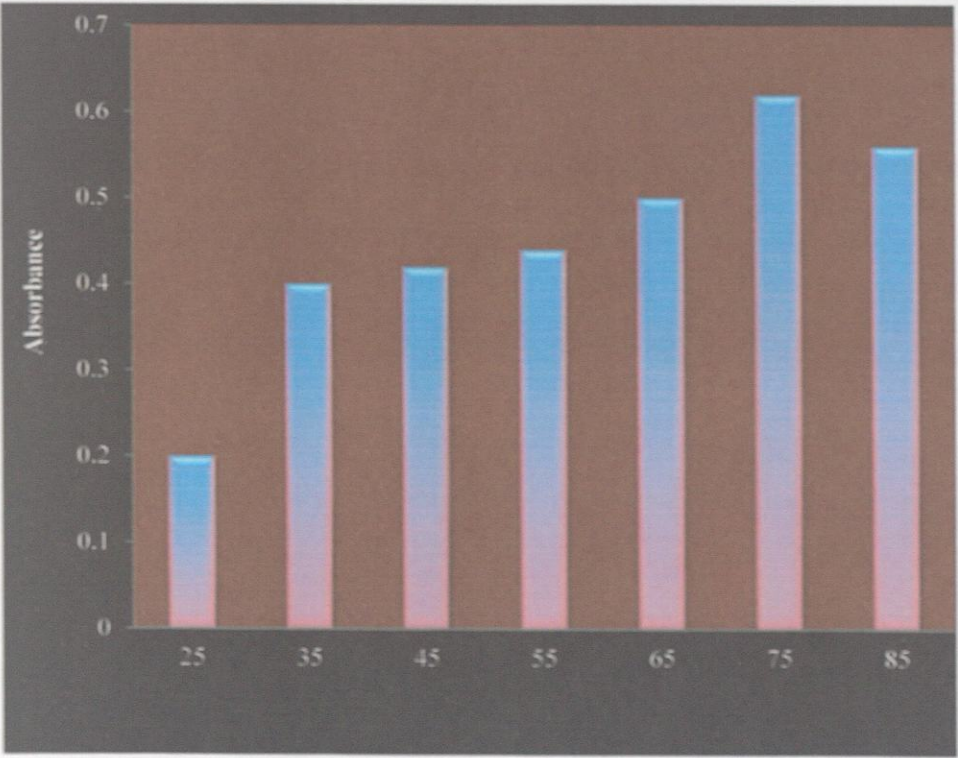


Figure 3.15: Optimization of temperature for the synthesis of CuNPs

3.4.2. pH

The pH at which effective synthesis of CuNPs can take place with maximum yield was determined at pH levels ranging from 4–8. A maximum absorbance of 0.56 was recorded at neutral pH (7), indicating maximum synthesis of CuNPs at this pH (Figure 3.16). The absorbance values recorded at other pH levels were 0.35 at pH 4, 0.38 at pH 5, 0.41 at pH 6 and 0.52 at pH 8. pH affects the size and shape of NPs. Low pH is required for the synthesis of larger NPs, while high pH results in formation of smaller NPs [128]. At low pH, nucleation process takes place, resulting in the aggregation of CuNPs to form larger NPs; while at high pH there is no aggregation, resulting in smaller sized NPs [134].

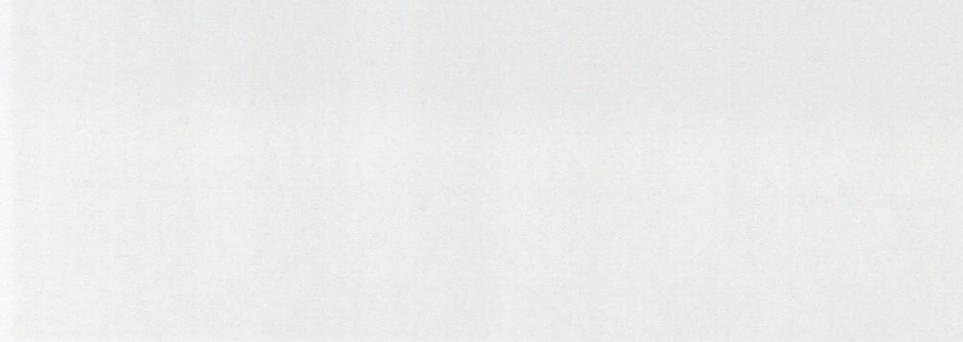


Figure 3.16: Dependence of pH on the synthesis of CuNPs.

3.4.3 Time

To optimize the reaction time, the reaction time of 20% was kept constant and the reaction time was varied from 1 to 5 h. The highest absorbance value of 0.5 was observed at the reaction time of 4 h (Figure 3.17). Increasing the reaction time over 4 h resulted in decreased absorbance. The absorbance values at other time durations were 0.35 at 1 h, 0.37 at 2 h, 0.41 at 3 h and 0.49 at 5 h. Another study has reported that the NPs formed at 4 h of reaction time include no agglomeration. These findings were

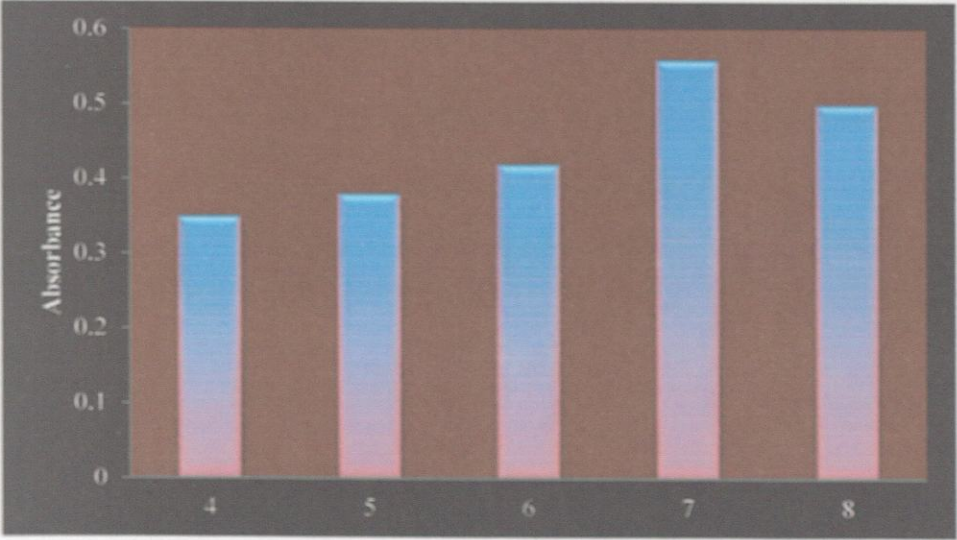


Figure 3.16: Optimization of pH for the synthesis of CuNPs

2.4.3. Time

To optimize the reaction time for maximum yield of NPs, the synthesis of CuNPs was accomplished in a time range of 1, 2, 3, 4 and 5 h. Highest absorbance value of 0.5 was obtained at the reaction time of 4 h (Figure 3.17). Increasing the reaction time over 4 h resulted in decreased absorbance. The absorbance values at other time durations were 0.45 at 1 h, 0.2 at 2 h, 0.14 at 3 h and 0.42 at 5 h. Another study has reported that the NPs formed after 36 h of incubation are unstable in nature [133]. These findings were consistent with the conclusions drawn from an earlier study [135]. Contrary to our results, another study reported that 1 h is the optimum time duration for the maximum synthesis of CuNPs [136]. It was observed that until 30 min, there was no formation of NPs, however, formation started quickly after 60 min and completed within 5 h, making it in line with our study [137].

Figure 3.17 Optimization of time for the synthesis of CuNPs

3.3.1 Characterization of CuNPs

3.3.1.1 UV-Vis Spectroscopy

UV-Vis spectroscopy was used to evaluate the synthesis of CuNPs. The absorbance was measured at 400 nm for the synthesis of CuNPs and was compared to the range of 200–800 nm. The absorbance was measured at 400 nm, which is the characteristic

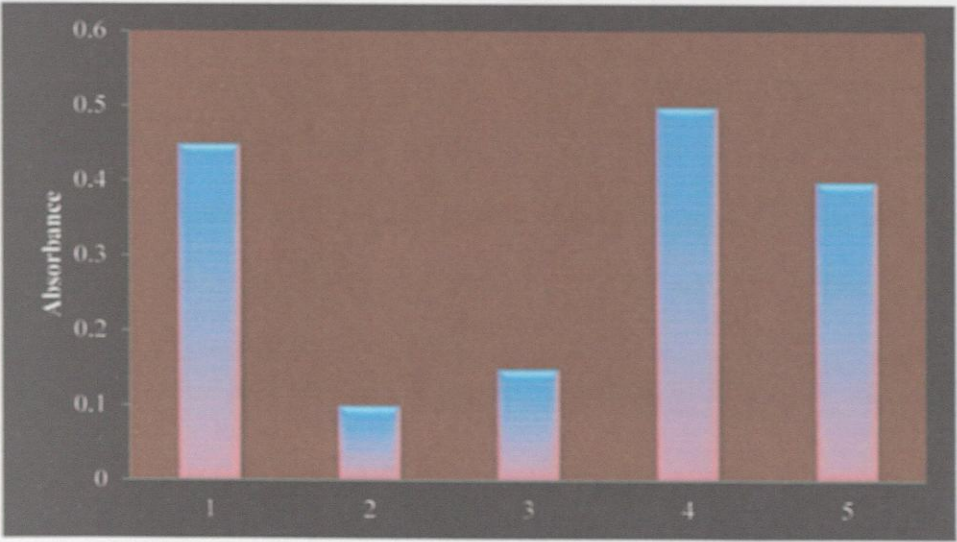


Figure 3.17: Optimization of time for the synthesis of CuNPs

3.5 Characterization of CuNPs

3.3.1 UV-Vis Spectroscopy

UV-visible spectroscopy was used to confirm the steadiness and making of NPs in aqueous solution. The aqueous leaf extract was used for the synthesis of CuNPs and was screened in the range of 300–600 nm. A broad peak was found at 426 nm, clearly indicating the formation of CuNPs (Figure 3.18). A broad peak indicated the formation of CuNPs in the same range of wavelengths and it was concluded that a threshold level of reducing agent is important for the formation of specific NPs [138]. A previous study has mentioned a broad peak at 410 nm as an indication for the formation of CuNPs, using plant extract. One of the reported studies synthesized CuNPs utilizing bacterial cell extract, and revealed a sharp band for CuNPs at 426 nm [146].



Figure 3.18: UV-Vis spectrum of CuNPs.

3.1.1 FTIR Analysis

FTIR spectra and their peaks for CuNPs are shown in Figure 3.19–3.21. The IR spectra all exhibited strong absorption bands at 3436 and 3425 cm^{-1} , corresponding to stretching vibrations of H_2O molecules, which may be due to hydroxyl or carboxyl groups. Different peak assignments at different dimensions: 1577 cm^{-1} (broadband absorption of $\text{C}=\text{O}$), 1544 and 1524 cm^{-1} ($\text{C}=\text{O}$), 1414 cm^{-1} ($\text{C}=\text{O}$), 1384 cm^{-1} ($\text{C}=\text{O}$), 1364 cm^{-1} ($\text{C}=\text{O}$), 1344 cm^{-1} ($\text{C}=\text{O}$), 1324 cm^{-1} ($\text{C}=\text{O}$), 1304 cm^{-1} ($\text{C}=\text{O}$), 1284 cm^{-1} ($\text{C}=\text{O}$), 1264 cm^{-1} ($\text{C}=\text{O}$), 1244 cm^{-1} ($\text{C}=\text{O}$), 1224 cm^{-1} ($\text{C}=\text{O}$), 1204 cm^{-1} ($\text{C}=\text{O}$), 1184 cm^{-1} ($\text{C}=\text{O}$), 1164 cm^{-1} ($\text{C}=\text{O}$), 1144 cm^{-1} ($\text{C}=\text{O}$), 1124 cm^{-1} ($\text{C}=\text{O}$), 1104 cm^{-1} ($\text{C}=\text{O}$), 1084 cm^{-1} ($\text{C}=\text{O}$), 1064 cm^{-1} ($\text{C}=\text{O}$), 1044 cm^{-1} ($\text{C}=\text{O}$), 1024 cm^{-1} ($\text{C}=\text{O}$), 1004 cm^{-1} ($\text{C}=\text{O}$), 984 cm^{-1} ($\text{C}=\text{O}$), 964 cm^{-1} ($\text{C}=\text{O}$), 944 cm^{-1} ($\text{C}=\text{O}$), 924 cm^{-1} ($\text{C}=\text{O}$), 904 cm^{-1} ($\text{C}=\text{O}$), 884 cm^{-1} ($\text{C}=\text{O}$), 864 cm^{-1} ($\text{C}=\text{O}$), 844 cm^{-1} ($\text{C}=\text{O}$), 824 cm^{-1} ($\text{C}=\text{O}$), 804 cm^{-1} ($\text{C}=\text{O}$), 784 cm^{-1} ($\text{C}=\text{O}$), 764 cm^{-1} ($\text{C}=\text{O}$), 744 cm^{-1} ($\text{C}=\text{O}$), 724 cm^{-1} ($\text{C}=\text{O}$), 704 cm^{-1} ($\text{C}=\text{O}$), 684 cm^{-1} ($\text{C}=\text{O}$), 664 cm^{-1} ($\text{C}=\text{O}$), 644 cm^{-1} ($\text{C}=\text{O}$), 624 cm^{-1} ($\text{C}=\text{O}$), 604 cm^{-1} ($\text{C}=\text{O}$), 584 cm^{-1} ($\text{C}=\text{O}$), 564 cm^{-1} ($\text{C}=\text{O}$), 544 cm^{-1} ($\text{C}=\text{O}$), 524 cm^{-1} ($\text{C}=\text{O}$), 504 cm^{-1} ($\text{C}=\text{O}$), 484 cm^{-1} ($\text{C}=\text{O}$), 464 cm^{-1} ($\text{C}=\text{O}$), 444 cm^{-1} ($\text{C}=\text{O}$), 424 cm^{-1} ($\text{C}=\text{O}$), 404 cm^{-1} ($\text{C}=\text{O}$), 384 cm^{-1} ($\text{C}=\text{O}$), 364 cm^{-1} ($\text{C}=\text{O}$), 344 cm^{-1} ($\text{C}=\text{O}$), 324 cm^{-1} ($\text{C}=\text{O}$), 304 cm^{-1} ($\text{C}=\text{O}$), 284 cm^{-1} ($\text{C}=\text{O}$), 264 cm^{-1} ($\text{C}=\text{O}$), 244 cm^{-1} ($\text{C}=\text{O}$), 224 cm^{-1} ($\text{C}=\text{O}$), 204 cm^{-1} ($\text{C}=\text{O}$), 184 cm^{-1} ($\text{C}=\text{O}$), 164 cm^{-1} ($\text{C}=\text{O}$), 144 cm^{-1} ($\text{C}=\text{O}$), 124 cm^{-1} ($\text{C}=\text{O}$), 104 cm^{-1} ($\text{C}=\text{O}$), 84 cm^{-1} ($\text{C}=\text{O}$), 64 cm^{-1} ($\text{C}=\text{O}$), 44 cm^{-1} ($\text{C}=\text{O}$), 24 cm^{-1} ($\text{C}=\text{O}$), 4 cm^{-1} ($\text{C}=\text{O}$).

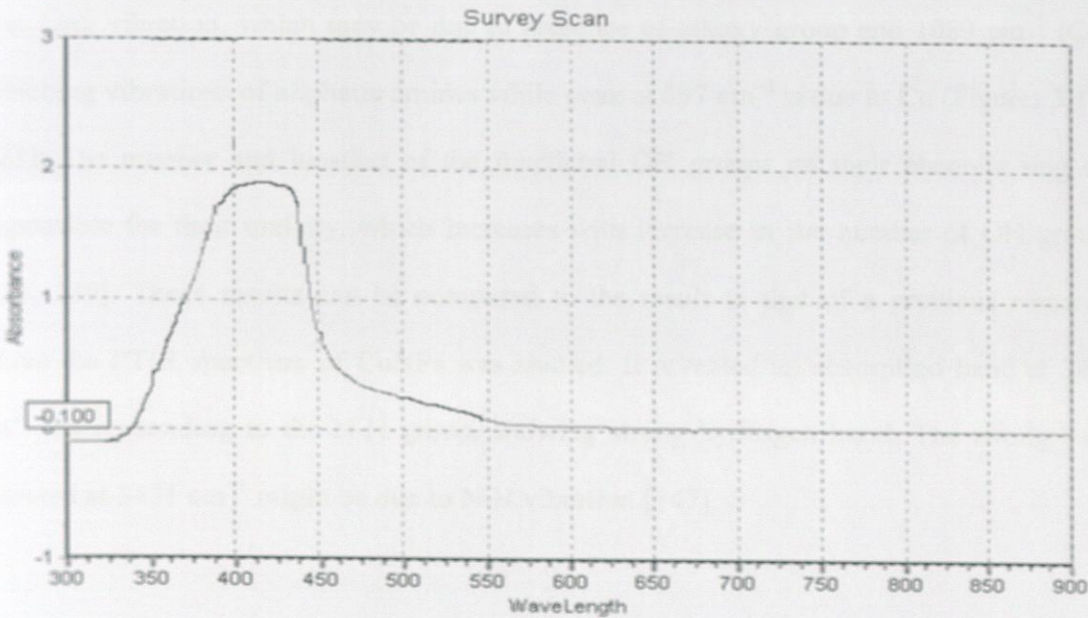


Figure 3.18: UV spectra of CuNPs

3.5.2. FTIR Analysis

FTIR spectra and their peaks for CuNPs are shown in Figures 3.19–3.21. The IR-spectrum indicates strong absorption bands at 3338 and 3298.28 cm^{-1} , corresponding to stretching vibrations of diatomic units, which may be due to hydroxyl or carboxyl groups. Different peak corresponds to different phenomenon; 1593 cm^{-1} (skeletal vibrations of C=C), 1386 cm^{-1} (tertiary C—OH), 1116.78 cm^{-1} (C—H in-plane bending vibration), 1066 cm^{-1} (C—O stretching vibration, which may be due to presence of alkoxy group and 1083 cm^{-1} (C—N stretching vibrations of aliphatic amines while peak at 597 cm^{-1} is due to Cu (Figures 3.19–3.21). The number and location of the functional OH groups on their phenolic ring are responsible for their toxicity, which increases with increase in the number of OH groups [135, 139]. These results can be compared to the result to that of a previous research, where the FTIR spectrum of CuNPs was studied. It revealed an absorption band at 3431 cm^{-1} , corresponding to the N-H group, showing strong hydrogen bond. The strong band detected at 3431 cm^{-1} might be due to N-H vibration [147].

Figure 3.19: FT-IR spectra of CuNPs.



Figure 3.20: FT-IR spectra of CuNPs.

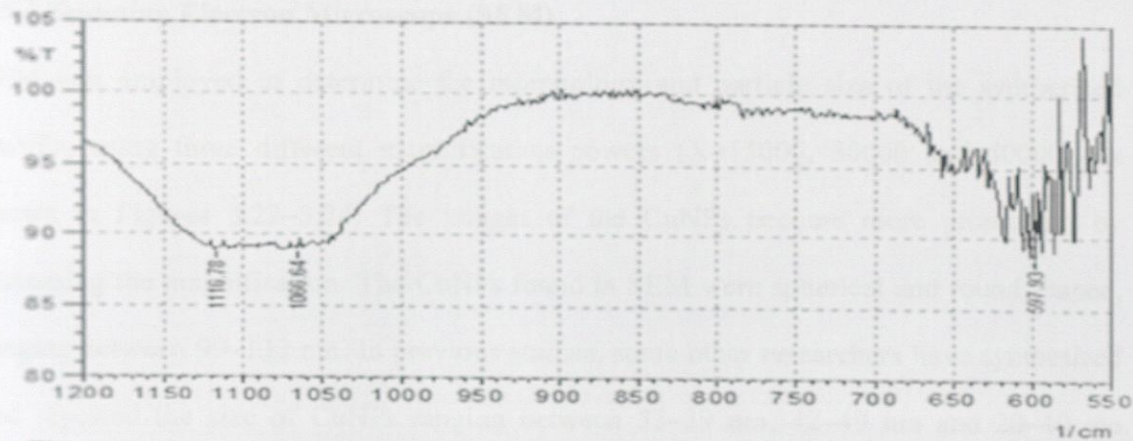


Figure: 3.19: FT-IR spectra of CuNPs

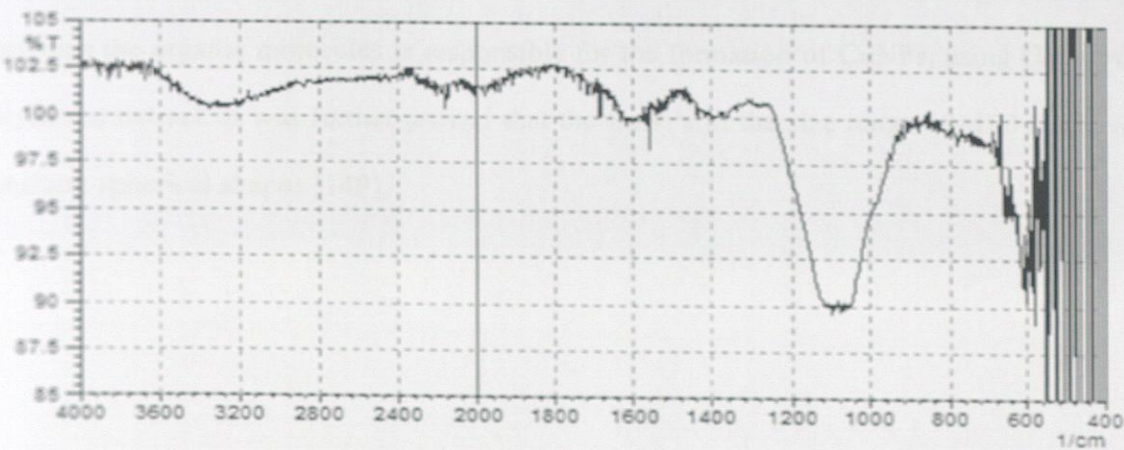


Figure: 3.20: FT-IR spectra of CuNPs

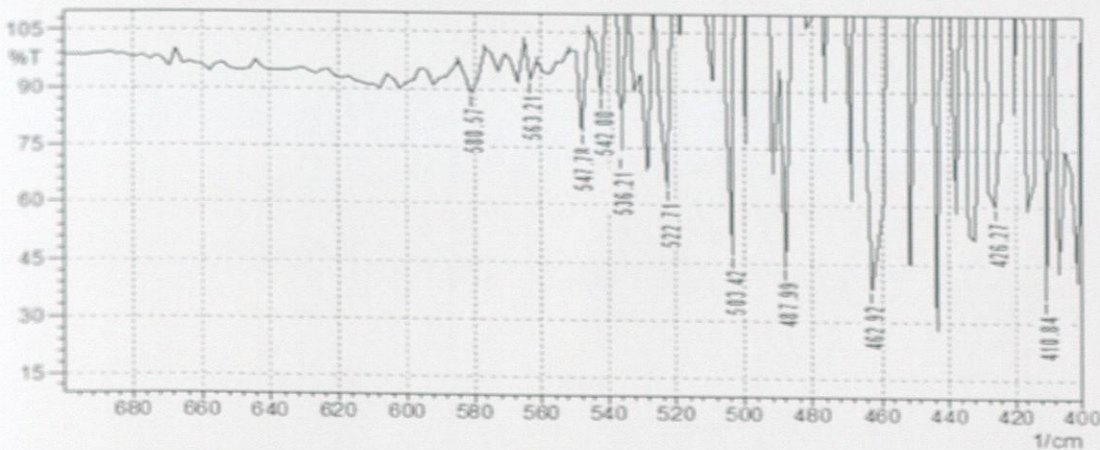


Figure 3.21: FT-IR spectra of CuNPs

3.5.3 Scanning Electron Microscope (SEM)

SEM was employed to determine the morphology and particle size of the synthesized CuNPs, using three different magnification powers ($X=15000$, 30000 and 50000), as shown in Figures 3.22–3.24. The images of the CuNPs become more prominent, by increasing the magnification. The CuNPs found in SEM were spherical and round shaped, ranging between $99\text{--}132\text{ nm}$. In previous studies, some other researchers have synthesized and reported the size of CuNPs ranging between $33\text{--}39\text{ nm}$, $42\text{--}49\text{ nm}$ and $20\text{--}40\text{ nm}$ [148]. The SEM study revealed that the electrostatic attractions and hydrogen bonding between the organic molecules is responsible for the formation of CuNPs, using *Capparis zeylanica* extract. It was further proved that the CuNPs in the size range $60\text{--}100\text{ nm}$ have uniform spherical shapes [149].

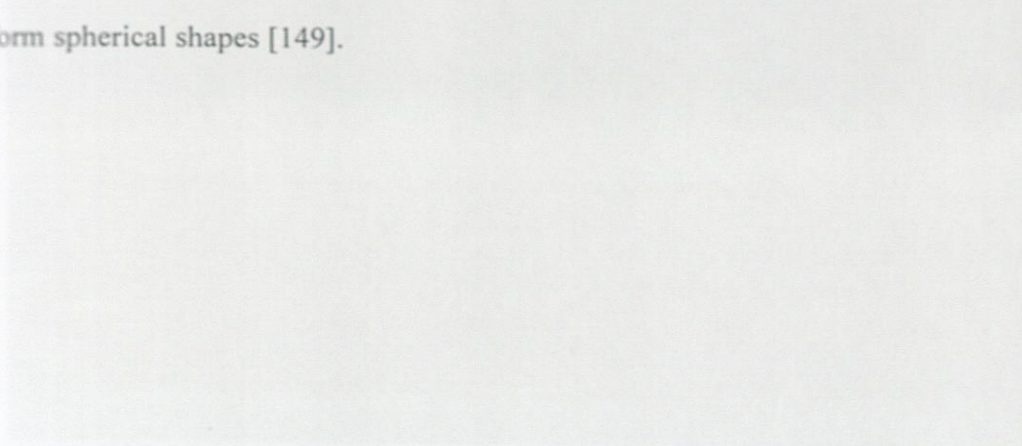


Figure 3.22: SEM image of CuNPs at 15000x.

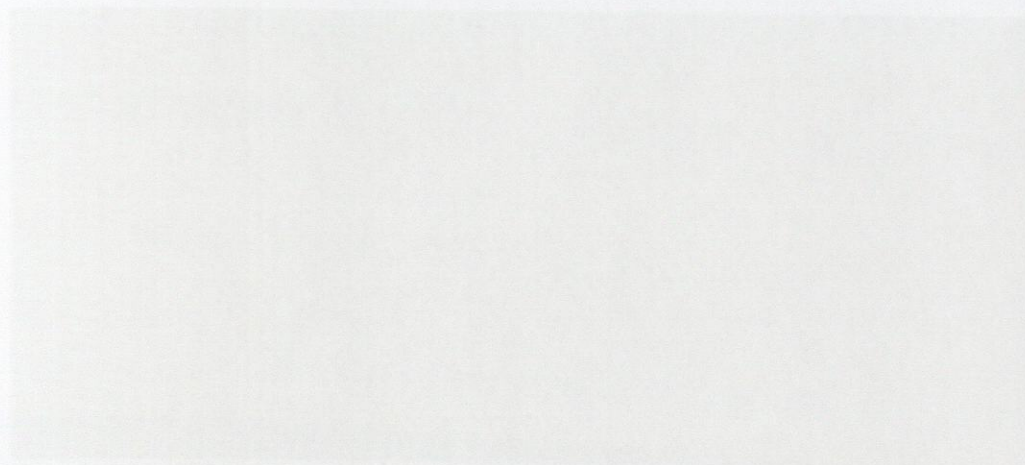


Figure 3.23: SEM image of CuNPs at 30000x.

3.2.4 Energy Dispersive X-ray Analysis

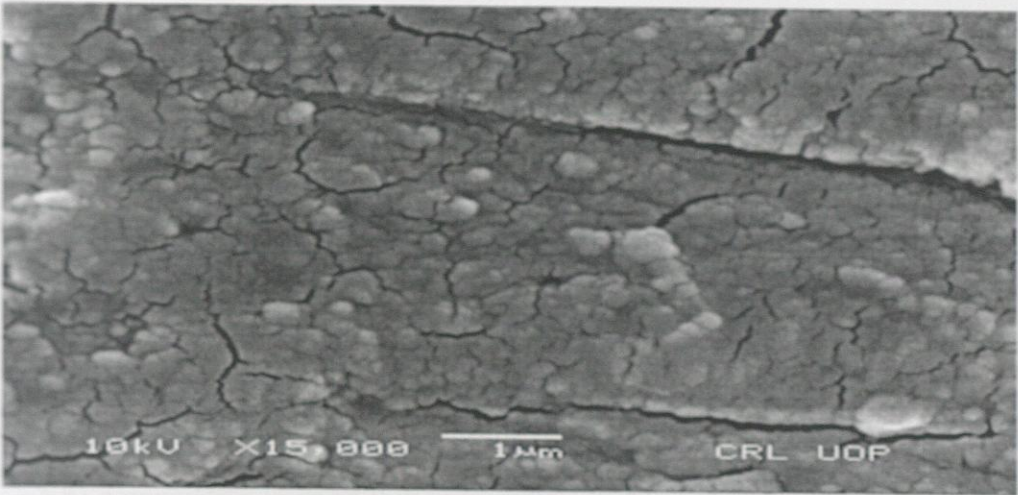


Figure 3.22: SEM image of CuNPs at 15000x

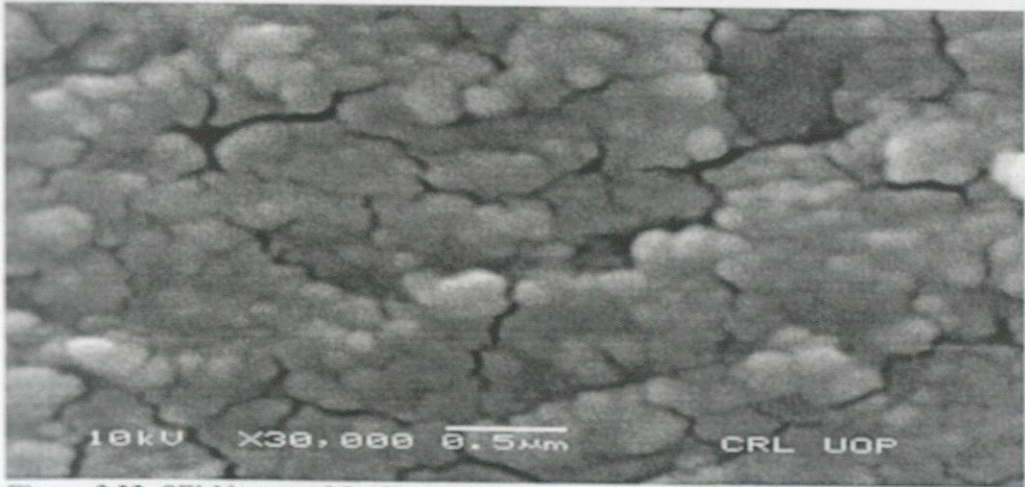


Figure 3.23: SEM image of CuNPs at 30000x

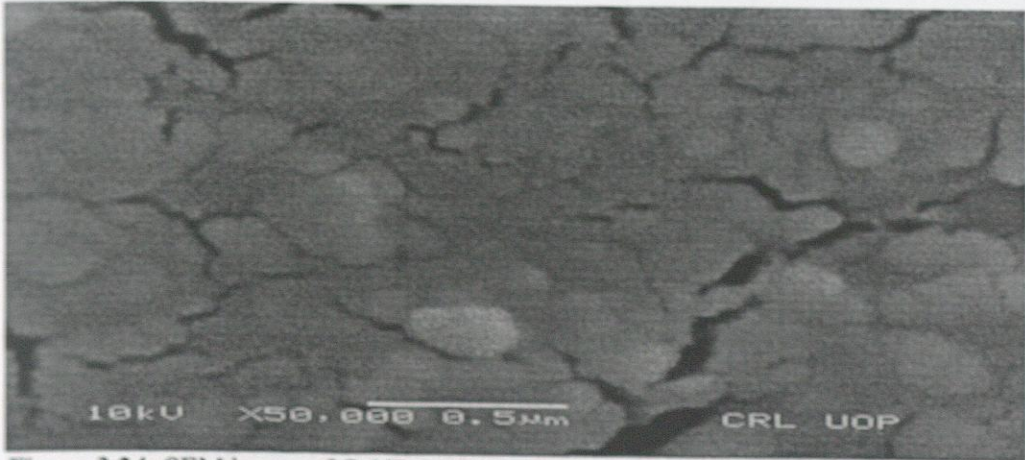


Figure 3.24: SEM image of CuNPs at 50,000x

3.5.4 Energy Dispersive X-ray Analysis

EDX was used to determine the presence of elemental Cu, which shows the reduction of Cu^{2+} . EDX profile showed a signal for elemental Cu at 8.0 keV, as shown in Figure 3.25. The EDX profile of CuNPs revealed absorbance peaks for Mg at 1, S at 2.2, Si at 1.2, K 3.2, Cl at 2.3 and Ca at 3.9 keV.

Other elemental composition of CuNPs, such as C, K, S and O were also detected. The X-ray counts are shown on the perpendicular axis, while energy (keV) on the parallel axis. The sharp and bright signals of CuNPs confirms the formation of NPs.

The CuNPs composition showed that by percent weight it contains C (41.10), Cu (47.06), Mg (0.79), S (3.66) and O (4.28), while the atomic percent values were found to be 51.40, 44.18, 0.49, 1.72 and 1.01, respectively. EXD recorded a high signal for Cu, followed by C. The Cu was found to be 47.06% by mass and 44.18 % by atomic mass. In another study, the graph of EDX detected the presence of Si, Ca and C in the EDX image of CuNPs. In EDX analysis due to SPR, the CuNPs showed higher absorption peaks [149].

Table 3.3. CuNPs Mass and Atomic weight percentage

Element	Mass%	Atom%
C K	41.10	51.40
Cu K	47.06	44.18
Mg K	0.79	0.49
S K	3.66	1.72
O K	4.28	1.01

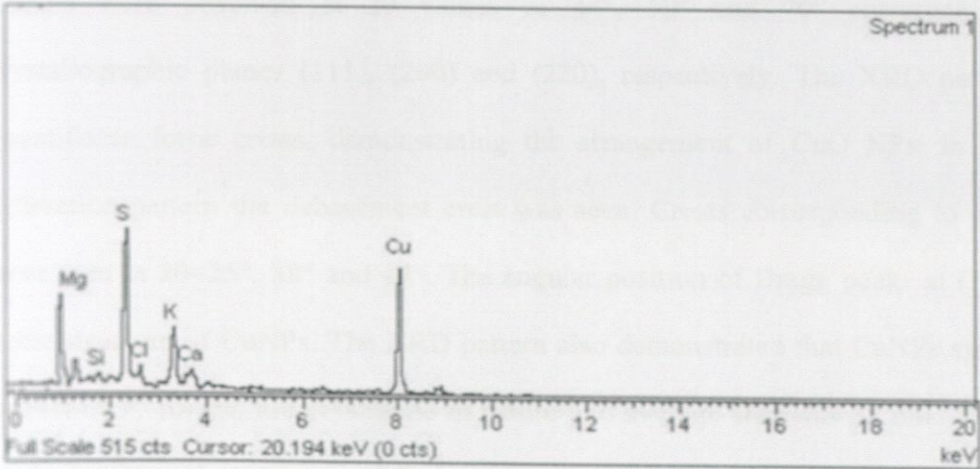


Figure 3.25: EDX spectra of CuNPs

3.5.5 X-ray Diffraction

The XRD pattern of the CuNPs, using aqueous leaf extracts of *F. carica*, is shown in Figure 3.26. Miller Indices (h, k l) to each peak were assigned by using the indexing process of the diffraction pattern in the first step. By studying the intensity of peaks, the degree of crystallinity of the CuNPs can be determined. The size of the CuNPs estimated from the Debye-Scherrer formula is 32 nm.

The XRD pattern revealed successful green synthesis of CuNPs, using *F. carica* as stabilizing and reducing agent, as shown in Figure 3.26. The sharpest diffraction peaks for CuNPs were observed at 2θ values of 44° , 50° and 70° corresponding to the crystallographic planes (111), (200) and (220), respectively. The XRD pattern showed quantifiable force crests, demonstrating the arrangement of CuO NPs. In the X-beam diffraction pattern the debasement crest was seen. Crests corresponding to copper oxide were seen at $2\theta=25^\circ$, 38° and 47° . The angular position of Bragg peaks at (1), reveal the cubic structure of CuNPs. The XRD pattern also demonstrated that CuNPs synthesized by extract of *F. carica*, are crystalline in nature and average size was 32 nm. Therefore, this study confirms that extracts of *F. carica* can reduce Cu ions into crystalline CuNPs. The XRD plots of CuNPs synthesized by using *Ocimum sanctum* leaf extract have also shown that they are highly crystalline in nature. Through Debye-Scherrer equation, the NP size was determined and was found to be 77 nm, indicating increased surface area and raised surface area/volume ratio of CuNPs in crystalline shape [150]. Two diffraction peaks were observed at 39.1° and 68.3° through XRD analysis, proving the cubic structure of CuNPs. In this study, the particle size determined by Debye-Scherrer equation was 5 nm [149].

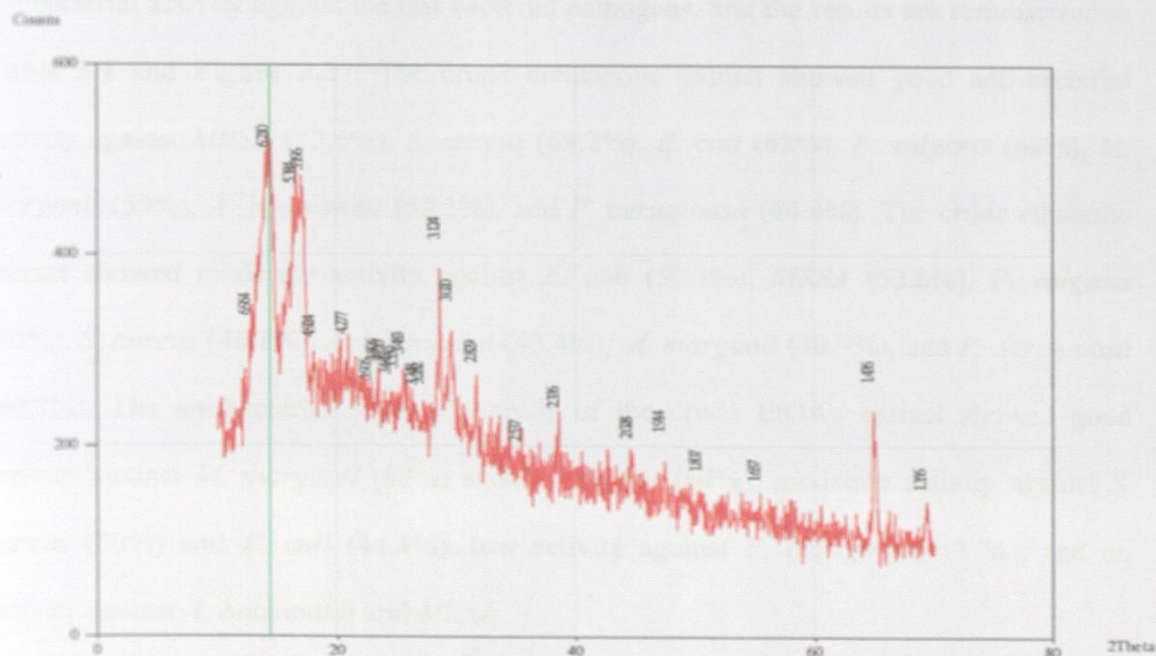


Figure: 3.26: XRD of CuNPs

3.6 Biological activities of the crude extracts, ZnNPs and CuNPs

3.6.1. Antibacterial activity of *E. japonica*

The crude methanolic, ethanolic, and EtOAc extracts of *E. japonica* were screened for their antibacterial activity against the test bacterial pathogens, and the results are summarized in Table 3.4 and Figure 3.27. The crude methanolic extract showed good anti-bacterial activity against MRSA (73.6%), *S. aureus* (69.2%), *E. coli* (62%), *P. vulgaris* (60%), *M. morganii* (59%), *A. baumannii* (52.1%), and *P. aeruginosa* (44.4%). The crude ethanolic extract showed moderate activity against *E. coli* (59.2%), MRSA (52.6%), *P. vulgaris* (52%), *S. aureus* (46.1%), *A. baumannii* (43.4%), *M. morganii* (40.9%), and *P. aeruginosa* (40.7%). The antibacterial activity analysis of the crude EtOAc extract showed good activity against *M. morganii* (68%) and *P. vulgaris* (64%), moderate activity against *S. aureus* (50%) and *E. coli* (44.4%), low activity against *P. aeruginosa* (37%), and no activity against *A. baumannii* and MRSA.

The results of the current study are comparable with those of another study wherein the antibacterial activity of leaves and branches of *E. japonica*, was determined against ESBL producing *K. pneumoniae* and *E. coli*. The results showed that the crude ethanolic extract and the other fractions (petroleum ether, dichloromethane, EtOAc, and aqueous fraction) of the selected plant possess good antibacterial activity [151]. The antimicrobial activity of ZnO NPs has also been studied by other researchers against *B. subtilis* and *P. fluorescens* and was found active against these bacteria [152]. In another study, ZnO NPs were also potentially active against *S. enteritidis* and *L. monocytogenes*. It was further reported that this antibacterial property is because of the rupturing of cell wall of bacterial cell by these NPs [153]. The production of H₂O₂ from the surface of ZnONPs is referred as a plausible explanation for bacterial growth inhibition. It is assumed that the number of ZnO particles

present per unit volume of powder slurry increases with a decrease in particle size resulting in increased surface area and hence increased production of H_2O_2 [154].

Table 3.6 Anticatalytic activity of *E. coli* strains

Name of Bacteria	pH	Methanol		Ethanol		Ethyl acetate	
		100% volume	50% volume	100% volume	50% volume	100% volume	50% volume
1. <i>E. coli</i>	21	17	23	41	52	10	24
2. <i>E. coli</i>	27	14	51.4	31	52.4	20	32
3. <i>E. coli</i>	28	15	49.1	21	49.1	13	31
4. <i>E. coli</i>	21	17	54	2	53.4	15	45
5. <i>E. coli</i>	23	12	33.7	10	41.4	1	2
6. <i>E. coli</i>	25	18	50	22	53	14	44
7. <i>E. coli</i>	19	14	75.6	15	34.6	0	0

Table 3.4: Antibacterial activity of *E. japonica*

Name of Bacteria	Amoxicillin 10gm/dis	Methanol		Ethanol		EtOAc	
		Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)
<i>E. coli</i>	27	17	62	16	59.2	12	44.4
<i>P. aeruginosa</i>	27	12	44.4	11	40.7	10	37
<i>S. aureus</i>	26	18	69.2	12	46.1	13	
<i>M. morganii</i>	22	13	59	9	40.9	15	68
<i>A. baumannii</i>	23	12	52.1	10	43.4	0	0
<i>P. vulgaris</i>	25	15	60	13	52	16	64
MRSA	19	14	73.6	10	52.6	0	0

3.2 Antibacterial activity of *E. japonica*

The antibacterial properties of *E. japonica* fruit *Exopolysaccharide* were tested against six bacterial pathogens. The results of this activity are presented in Table 3-5 and Figure 3.27. The *Exopolysaccharide* exhibited significant activity against MRSA (94.7%) and *P. aeruginosa* (93%), showed good activity against *S. aureus* (75%), and *M. morganii* (67%).

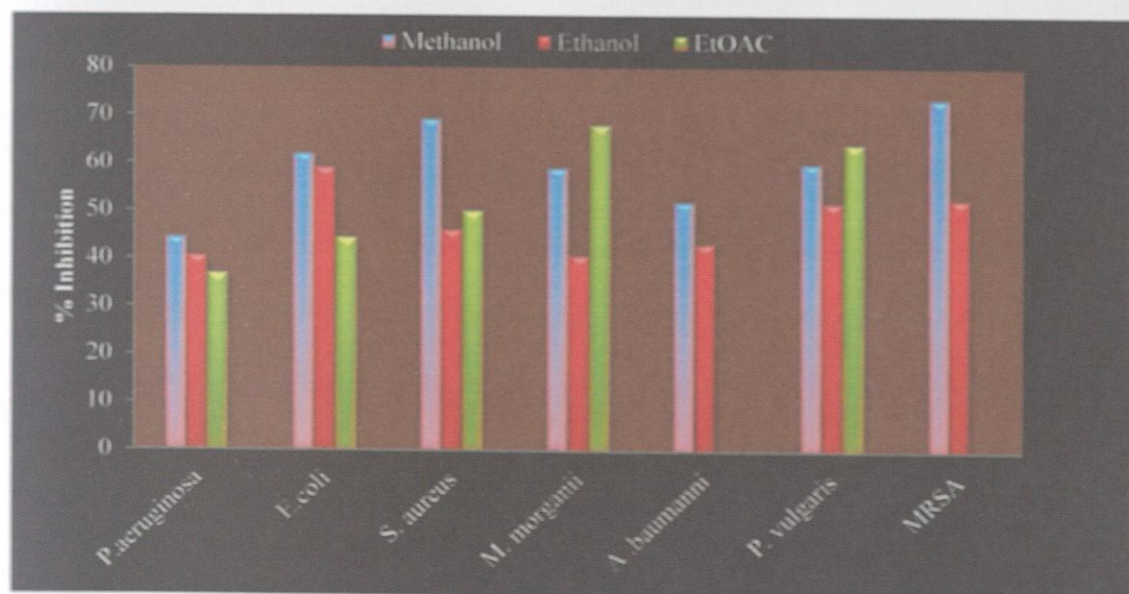


Figure 3.27: Antibacterial activity of crude methanolic, ethanolic and EtOAc fractions of *E. japonica*

3.6.2 Antibacterial activity of ZnNPs

The antibacterial properties of ZnNPs from *E. japonica* were studied against the selected bacterial pathogens. The results of this activity are presented in **Table 3.5** and **Figure 3.28**. The ZnNPs exhibited significant activity against MRSA (94.7%) and *P. vulgaris* (92%). It showed good activity against *S. aureus* (73%) and *M. morganii* (63.6%), moderate activity against *E. coli* (48.1%) and *A. baumannii*, (43.4%), while it presented a low activity against *P. aeruginosa* (37%).

AgNPs extracted from the aqueous extract of *Dioscorea bulbifera* showed good activity against *Bacillus cereus*, *B. subtilis*, *S. aureus*, and *P. aeruginosa*, while no activity was observed against *S. typhimurium* and *P. vulgaris* making it comparable with our study. The AgNPs exhibited antibacterial activities by interacting with the bacterial plasma membrane or cell wall, bacterial proteins or bacterial DNA [148, 155]. In addition to this, ethyl acetate extracts were only effective against *K. pneumoniae* and *S. epidermidis*; however, at concentration of 75%, it was also active against *E. faecalis*, *S. pyogenes*, and *P. aeruginosa* strains [156].

Table 3.5: Antibacterial activities of ZnNPs of *E. japonica*

Name of Bacteria	Amoxicillin 10gm/disc	ZnNPs	
		Zone of Inhibition (mm)	Inhibition (%)
<i>E.coli</i>	27	13	48.1
<i>P. aeruginosa</i>	27	10	37
<i>S. aureus</i>	26	19	73.0
<i>M. morganii</i>	22	14	63.6
<i>A. baumannii</i>	23	10	43.4
<i>P. vulgaris</i>	25	23	92.0
MRSA	19	18	94.7

3.4.3. Antibacterial activity of *E. japonica*

The antibacterial activity of ZnNPs synthesized using NaOH and EDTA as catalysts of *E. japonica* were determined against different bacterial strains, and the results are summarized in Table 3.4 and Figure 3.28. The ZnNPs showed good activity against *P. aeruginosa* (36.7%), *E. coli* (47.0%), *S. aureus* (71.0%), *M. morgani* (61.0%), *A. baumannii* (40.0%), *P. vulgaris* (88.0%) and MRSA (90.0%). The ZnNPs

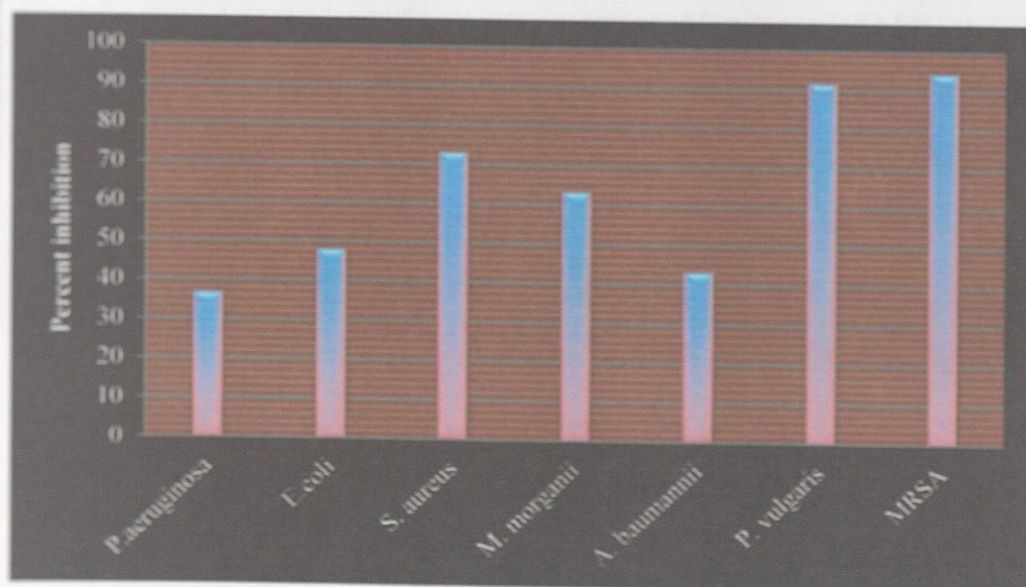


Figure 3.28: Antibacterial activity of ZnNPs of *E. japonica* against selected bacterial strains

of *E. japonica* in the aqueous phase [177]. In another study, *E. japonica* showed high quantity of ZnNPs and showed good anti-bacterial activity against several microorganisms [178].

3.6.3. Antibacterial activity of *F. carica*

The antibacterial activities of crude methanolic, ethanolic, and EtOAc extract of *F. carica* were determined against different bacterial strains, and the results are summarized in Table 3.6 and Figure 3.29. The crude methanolic extract showed good activity against *P. aeruginosa* (70.3%), *S. aureus* (65%), *P. vulgaris* (60%), and moderate activity against *M. morganii* (54.5%), *MRSA* (52.6%), *E. coli* (51.8%), and *A. baumannii* (43.4%). The crude ethanolic extract showed significant activity against *M. morganii* (81.8%) and good against *MRSA* (68.4.6%). It showed moderate activity against *A. baumannii* (56.5.4%), *P. vulgaris* (48%), *S. aureus* (46.1%), and *E. coli* (40.7%), while low activity was observed against *P. aeruginosa* (25.9%). The antibacterial activity assay of the crude EtOAc extract showed good activity against *MRSA* (78.9%), *S. aureus* (65.3%), *A. baumannii* (65.2%), and *M. morganii* (63.6%), and moderate activity against *P. vulgaris* (52%), *E. coli* (48.1%), and *P. aeruginosa* (48.1%).

The ethanolic latex extract of *F. carica* was investigated for its antibacterial activity against *S. typhi*, *S. aureus*, *S. pyogenes* and *K. pneumoniae*. The ethanolic extract exhibited good activity against *S. aureus* and *S. typhi* and the reason for this activity may be the presence of flavonoids in the selected plants [157]. In another study *F. carica* latex showed high quantity of coumarins and showed good anti-bactericidal activity against several pathogenic bacteria [158].

Table 3.6: Antibacterial activity of *F. carica*

Name of Bacteria	Amoxicillin 10gm/disc	Methanol		Ethanol		EtOAc	
		Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)
<i>E.coli</i>	27	14	51.8	11	40.7	13	48.1
<i>P. aeruginosa</i>	27	19	70.3	7	25.9	13	48.1
<i>S. aureus</i>	26	17	65.3	12	46.1	17	65.3
<i>M. morganii</i>	22	12	54.5	18	81.8	14	63.6
<i>A. baumannii</i>	23	10	43.4	13	56.5	15	65.2
<i>P. vulgaris</i>	25	15	60.0	12	48	13	52.0
MRSA	19	10	52.6	13	68.4	15	78.9

3.4.4 Antibacterial activity of *F. carica*

The antibacterial activity of *F. carica* was determined against different bacterial pathogens. The results are presented in Table 3.7 and Figure 3.29.

The Ca NP exhibited significant activity against MRSA (65%) and good against *S. aureus* (75%). It exhibited moderate activity against *A. baumannii* (54%), *E. coli* (47%), *P. vulgaris* (48%), *P. aeruginosa* (47%) and *P. mirabilis* (48%).

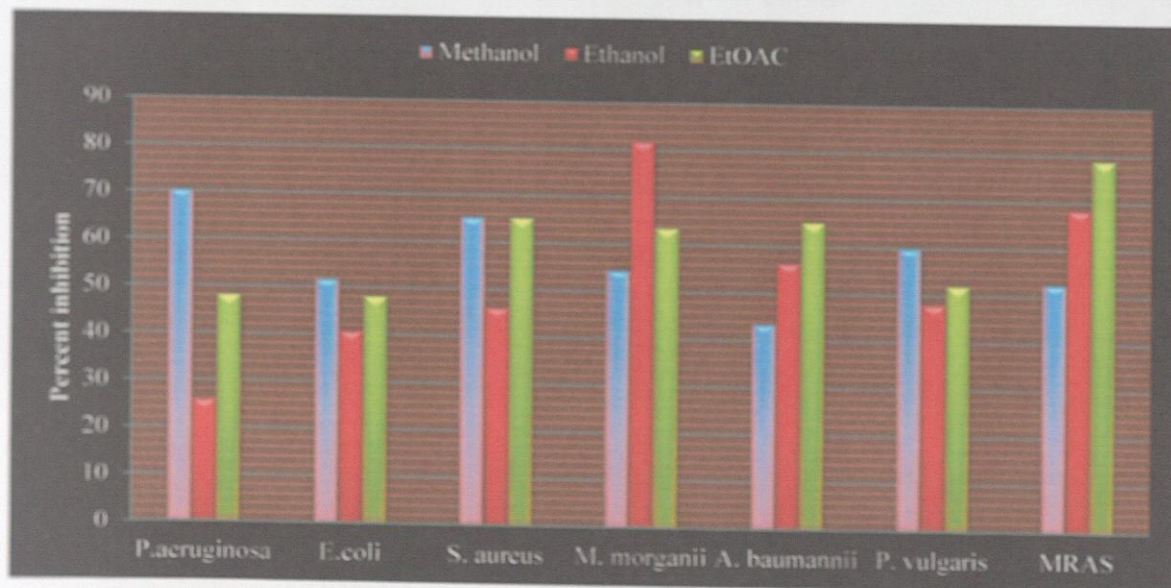


Figure 3.29: Antibacterial activity of crude methanolic, ethanolic and EtOAc fractions of *F. carica*

3.6.4 Antibacterial activity of CuNPs

The antibacterial activity of CuNPs from *F. carica* was determined against different bacterial pathogens. The results are presented in **Table 3.7** and **Figure 3.30**.

The Cu NPs exhibited significant activity against MRSA (89%) and good against *S. aureus* (73%). It presented moderate activity against *A. baumannii* (56.5%), *E. coli* (55.5%), *M. morganii* (54.5%), *P. aeruginosa* (44.4%), and *P. vulgaris* (44%).

In another study, the antibacterial activity of CuNPs was determined against MRSA, *P. aeruginosa*, and *Proteus*, and it was shown to exhibit good activity against these bacterial pathogens. The MIC of CuNPs, however, was very high [159]. In another study CuNPs exhibited good activity against Gram-positive than Gram-negative bacteria. The results of the study revealed the presence of carboxyl (-COO-) and amines (-NH₂) groups on the bacterial surface with NPs possessing a greater capability to combine with these functional groups [160–162].

Table 3.7: Antibacterial activities of CuNPs of *F. carica*

Name of Bacteria	Amoxicillin 10gm/disc	CuNPs	
		Zone of Inhibition (mm)	Inhibition (%)
<i>E.coli</i>	27	15	55.5
<i>P. aeruginosa</i>	27	12	44.4
<i>S. aureus</i>	26	19	73.0
<i>M. morganii</i>	22	12	54.5
<i>A. baumannii</i>	23	13	56.5
<i>P. vulgaris</i>	25	11	44
MRSA	19	17	89

3.3.3 Antibacterial activity of *F. carica*

The crude methanolic extracts and the leaf extracts of *F. carica* were screened against *E. pseudotuberculosis*, *E. coli*, *S. aureus*, *M. morganii*, *P. aeruginosa*, and *P. vulgaris* for their antibacterial activity. The results are presented in Table 3.4 and Figure 3.30. The Cr. Met. Ex. showed low activity against *E. pseudotuberculosis* (44%) and no activity against *P. aeruginosa*, *S. aureus*, *M. morganii*, *P. aeruginosa* and *P. vulgaris*.

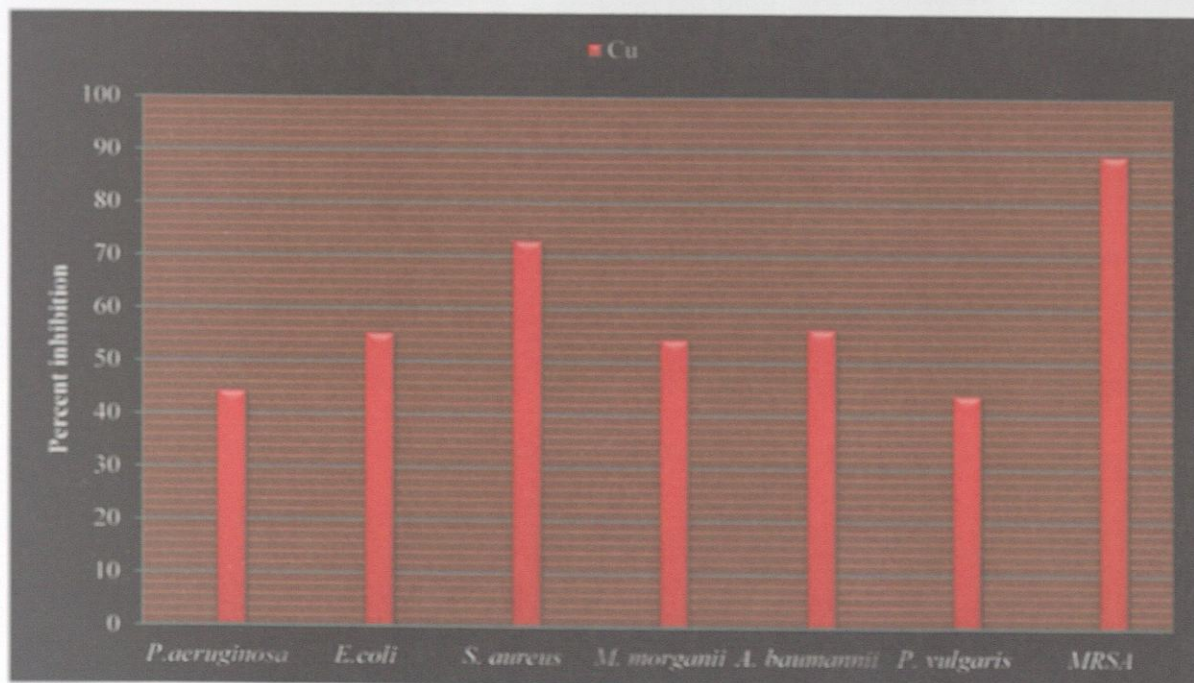


Figure 3.30: Antibacterial activity of CuNPs of *F. carica* against selected bacterial strains

3.7.1 Antifungal activity *E. japonica*

The crude methanolic, ethanolic, and EtOAc extracts of *E. japonica* were screened against *H. pseudocrispula*, *A. niger*, *A. parasiticus*, *P. notatum*, *V. longisporum*, and *P. chrysogenum* for their antifungal activity, the results being presented in **Table 3.8** and **Figure 3.31**. The Cr. Met. Ext showed low activity against *H. pseudocrispula* (10%) and no activity against *P. notatum*, *A. niger*, *A. parasiticus*, *P. chrysogenum* and *V. longisporum*. Low activity was exhibited against *A. niger* (23%) and *P. notatum* (13%) by crude ethanolic extract showed, while it showed no activity against *V. longisporum*, *H. pseudocrispula*, *P. chrysogenum* and *A. parasiticus*. The EtOAc extract exhibited low activity against *A. niger* (12%) and no activity against the rest of the test fungal pathogens. Different extracts of *Cynara scolymus* L were screened for their antifungal activity against *Alternaria* sp. The results indicated that the crude extracts exhibited good activity (75%) against *Alternaria* sp., which is in contrast to our study in which we found low activity in most of the cases [163].

Table 3.8: Antifungal activity of *E. japonica* against selected fungal pathogens

Name of Fungi	Percent Linear Growth Inhibition				
	-ive Control	+ive Control	Methanol	Ethanol	EtOAc
<i>A.niger</i>	0	100	0	23	12
<i>H. pseudocrispula</i>	0	100	10	0	0
<i>P.notatum</i>	0	100	0	13	0
<i>A.parasiticus</i>	0	100	0	0	0
<i>V.longisporum</i>	0	100	0	0	0
<i>P.chrysogenum</i>	0	100	0	0	0

3.3.2 Antifungal activity of *E. japonica*

The antifungal activities of *E. japonica* were determined against selected fungal pathogens, and the results are summarized in Table 3.9 and Figure 3.30. The *E. japonica* showed good activity (22%) against *A. niger*, moderate activity (12%) against *P. notatum*, and no activity (0%) against *A. parasiticus*, *V. longisporum*, and *P. chrysogenum*. The *E. japonica* also showed activity (10%) against *H. pseudocrispula*.

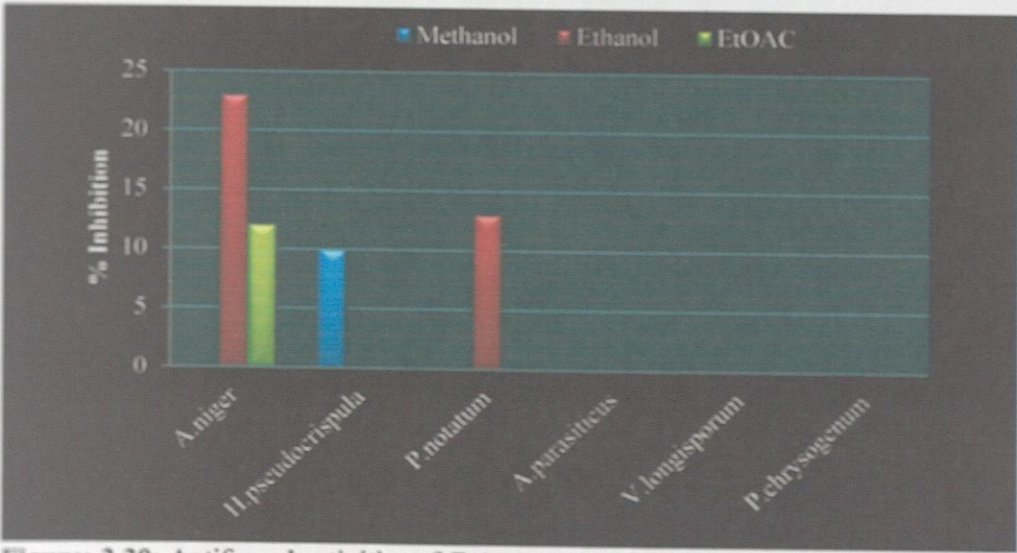


Figure: 3.30: Antifungal activities of *E. japonica* against selected fungal pathogens.

3.7.2 Antifungal activity of ZnNPs

The antifungal activities of ZnNPs from *E. japonica* were determined against different fungal pathogens, and the results are summarized in Table 3.9 and Figure 3.32. The ZnNPs showed good activity (60%) against *H. pseudocrispula*, moderate against *P. chrysogenum* (55%), *A. niger* (40%), and *P. notatum* (40%), low against *A. parasiticus* (30%), and was inactive against *V. longisporum*.

In a reported study, the antifungal activity of ZnONPs was investigated against *Penicillium expansum* and *Botrytis cinerea* and exhibited good activity. ZnONPs have been recommended as fungicide in agriculture and food industry on the basis of these results [164]. In another research, titanium oxide nanoparticles and ZnNPs were studied for their antifungal activities against *Trichophyton*, *Fonsecaea*, *A. niger*, *Ramichloridium schulzeri*, *Aspergillus flavus* and *Rhizopus oryzae*. The above NPs were found active against these fungal strains. The comparative study proved that ZnNPs and titanium oxide nanoparticles were far more effective than amphotericin-B and miconazole [165].

Table 3.9: Antifungal activity of ZnNPs of *E. japonica*

Name of Fungi	Percent Linear Growth Inhibition		
	-ive Control	+ive Control	ZnNPs
<i>A.niger</i>	0	100	40
<i>H.pseudocrispula</i>	0	100	60
<i>P.notatum</i>	0	100	40
<i>A.parasitiCus</i>	0	100	30
<i>V.longisporum</i>	0	100	0
<i>P.chrysogenum</i>	0	100	55

3.3.3 Antifungal activity of ZnNPs

The *Aspergillus niger*, *Aspergillus fumigatus*, and *Aspergillus terreus* are three common species of filamentous fungi. *A. niger* is a common fungus found in the environment. *A. fumigatus* is a common fungus found in the environment. *A. terreus* is a common fungus found in the environment. The antifungal activity of ZnNPs against these three species is presented in Table 3.10 and Figure 3.32. The ZnNPs showed high antifungal activity against *A. niger* and *A. fumigatus*, while *A. terreus* showed low antifungal activity. The antifungal activity of ZnNPs against *A. niger* and *A. fumigatus* was 50% and 55%, respectively. The antifungal activity of ZnNPs against *A. terreus* was 28%.

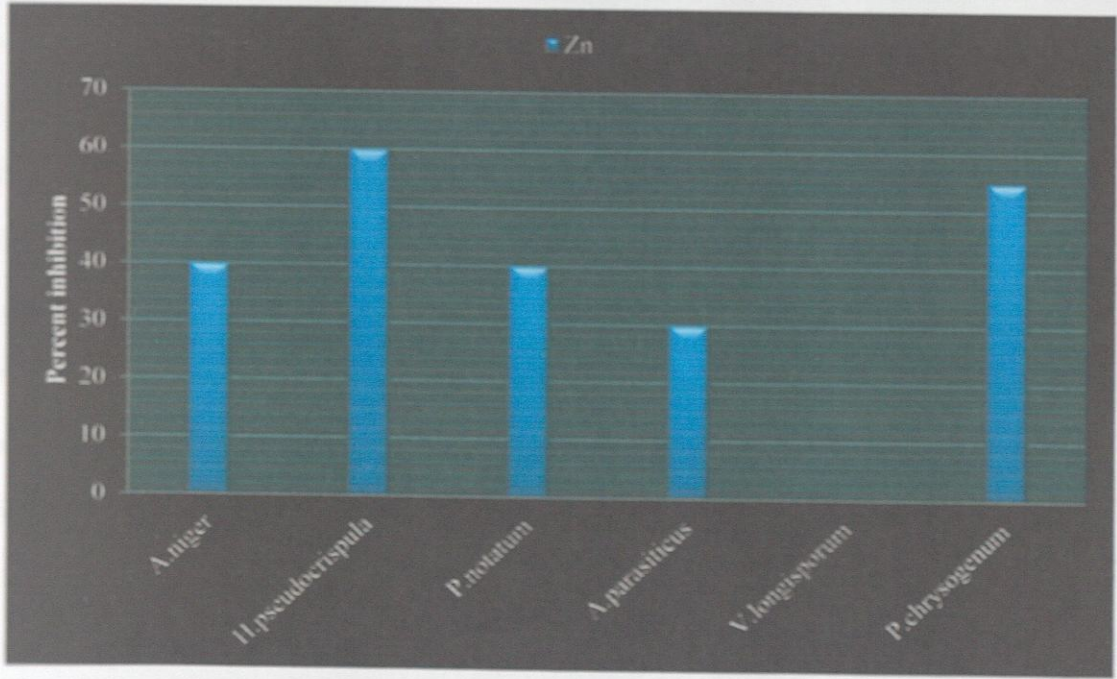


Figure 3.32: Antifungal activity of ZnNPs against selected fungal strains.

3.7.3 Antifungal activity of *F. carica*

The crude methanolic, ethanolic, and EtOAc extracts of *F. carica* were screened against *A. parasiticus*, *V. longisporum*, *A. niger*, *H. pseudocrispula*, *P. notatum* and *P. chrysogenum* for their antifungal activity and the results are presented in Table 3.10 and Figure 3.33.

The Cr. Met. Ext showed low activity against *A. parasiticus* (14%) and no activity against *H. pseudocrispula*, *P. notatum*, *A. niger*, *V. longisporum* and *P. chrysogenum*. The crude ethanolic extract showed low activity against *V. longisporum* (20%) and no activity against *P. chrysogenum*, *A. niger*, *H. pseudocrispula*, *P. notatum*, and *A. parasiticus*. The EtOAc extract exhibited low activity against *P. chrysogenum* (32%) and *A. niger* (11%), and no activity against *H. pseudocrispula*, *P. notatum*, *V. longisporum* and *A. parasiticus*.

A study reported the antifungal activity of methanolic extract of *F. carica* against *P. notatum*, *F. oxysporum*, *A. flavus*, *A. alternata* and *T. harzianum* and was found effective against these species making them in contradiction with our study [166]. The *n*-hexane, CHCl_3 , EtOAc, and methanolic extracts of *F. carica* latex were investigated for their antifungal properties against seven different strains of fungi. The minimal inhibitory concentration (MIC) of the methanol fraction was found to be 500 $\mu\text{g/mL}$ which showed total inhibition against *C. albicans* (100%). The methanolic extract (75%) strongly inhibited *Microsporum canis* at a concentration of 750 $\mu\text{g/mL}$ [167].

Table 3.10: Antifungal activity of *F. carica* against selected fungal pathogens

Name of Fungi	Percent Linear Growth Inhibition				
	-ive Control	+ive Control	Methanol	Ethanol	EtOAc
<i>A. Niger</i>	0	100	0	0	11
<i>H. pseudocrispula</i>	0	100	0	0	0
<i>P. notatum</i>	0	100	0	0	0
<i>A. Parasiticus</i>	0	100	14	0	0
<i>V. longisporum</i>	0	100	0	20	0
<i>P. chrysogenum</i>	0	100	0	0	32

3.3.4 Antifungal activity of *F. carica*

The antifungal activity of *F. carica* against *A. niger*, *H. pseudoerispula*, *P. notatum*, *A. parasiticus*, *V. longisporum*, and *P. chrysogenum* is summarized in Table 3.31 and Figure 3.33. *F. carica* showed moderate activity against *A. niger* (50%), *H. pseudoerispula* (25%), *A. parasiticus* (25%), *V. longisporum* (40%), and low against *P. longisporum* (10%) and *P. chrysogenum* (10%).

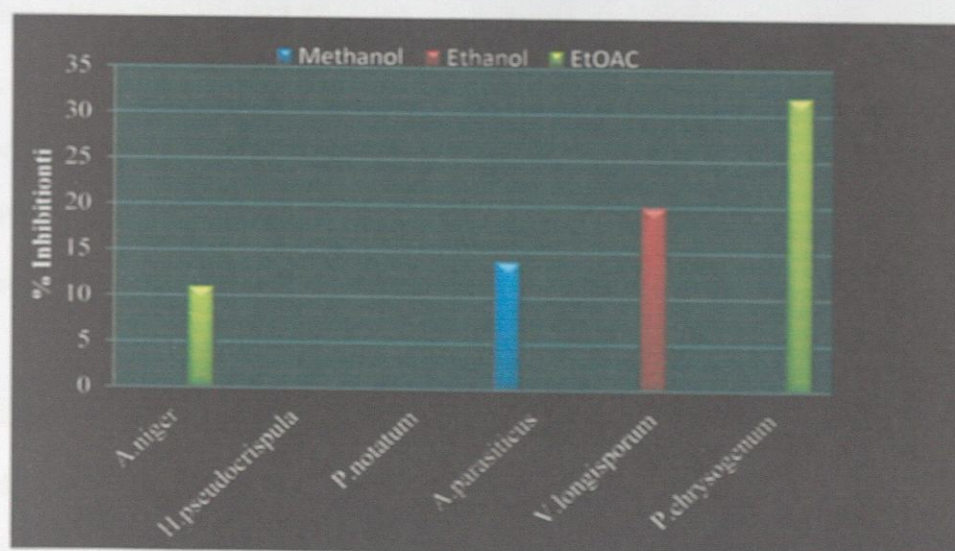


Figure: 3.33 Antifungal activity of *F. carica* against selected fungal pathogens

3.7.4 Antifungal activity of CuNPs

The antifungal activity of CuNPs from *F. carica* was determined against different fungal pathogens as presented in Table 3.11 and Figure 3.34. The CuNPs showed moderate activity against *A. niger* (50%), *H. pseudocrispula* (50%), *A. parasiticus* (50%), *P. notatum* (40%), and low against *V. longisporum* (10%) and *P. chrysogenum* (10%).

CuNPs have been investigated for use in biotechnological applications and scientists have attempted to use CuNPs in combination with polymer materials to release the metal in a sustained way to render fungal and other pathogens inactive [169]. The antifungal activity of CuNPs has been determined against various species of *S. cerevisiae* [168]. In another study, the researchers determined the fungicidal action of Cu-based zeolites extracted from marble against *Cladosporium cladosporioides*, *Phaeococcomyces chersonesus* and *Ulocladium chartarum* and showed good activity [169]. The antifungal properties of hyper-stretched polyamine CuNPs was considered against *C. albicans* and was observed that colloidal hyper branched polyamine NPs repressed the development of *C. albicans* even at a low concentration of 1.4 g/100 L [170]. This high antimicrobial activity of CuNPs is proposing them in different formulations and textile fabrics. One of the research groups assessed the antimicrobial properties of CuNPs by soil internment test. The results plainly demonstrated that the treatment with CuNPs improved the resistance of cotton fabrics to microbial attack. In a similar study, another group examined the antifungal action of CuNPs against the fungi, *A. flavus*, *A. niger*, and *C. albicans*. The CuNPs were most active against *C. albicans*, while least activity was seen for *A. flavus* [171].

Table 3.11: Antifungal activity of CuNPs against selected fungal pathogens

Name of Fungi	Percent Linear Growth Inhibition		
	-ive Control	+ive Control	CuNPs
<i>A. niger</i>	0	100	50
<i>H. pseudocrispula</i>	0	100	50
<i>P. notatum</i>	0	100	40
<i>A. parasiticus</i>	0	100	50
<i>V. longisporum</i>	0	100	10
<i>P. chrysogenum</i>	0	100	10

3.3.1 Fungicidal activity of *F. carica*

The phytochemical analysis of *F. carica* showed the presence of various fractions of *F. carica* against *A. niger* and *A. parasiticus* (Table 3.3 and Figure 3.34). The methanolic extract (60, 50 and 40%) showed 100% inhibition against *A. niger* and *A. parasiticus* (60, 50 and 40% methanolic extract showed 100%, 100% and 100% inhibition, respectively). The ethanolic extract showed 100% inhibition against *A. niger* and



Figure 3.34: Antifungal activity of CuNPs of *F. carica* against selected fungal pathogens

3.8.1 Phytotoxic activity of *E. japonica*

The phytotoxicity analysis of methanolic, ethanolic, and ethyl acetate extracts and various other fractions of *E. japonica* against *L. minor* are summarized in **Table 3.12** and **Figure 3.35**. The methanolic extract (60, 50, and 40%), ethanolic extract (70, 50, and 30%), and ethyl acetate fractions (60, 50, and 30%) inhibited growth at concentrations 1000, 100, and 10 $\mu\text{g/mL}$, respectively. The methanolic extract showed good activity at 1000, and moderate at 100 and 10 $\mu\text{g/mL}$. The ethanolic extract showed good activity at 1000, moderate at 100 $\mu\text{g/mL}$, and low activity at 10 $\mu\text{g/mL}$. The EtOAc fraction showed low activity at 10 $\mu\text{g/mL}$, moderate at 100, and good at 1000 $\mu\text{g/mL}$. The results of the current study are in contrast to another report where different fractions of *Ricinus communis*, *Chrozophora tinctoria*, *Peganum harmala*, *Tribulus terrestris*, and *Fagonia cretica* were found effective against *L. minor* [172].

Table 3.13: Phytotoxic activity of *E. japonica* against *L. minor*

Name of Plant	Conc. of sample ($\mu\text{g/ml}$)	No. of fronds survived			Conc. Of Std. drug * ($\mu\text{g/ml}$)
		Methanol	Ethanol	EtOAc	
<i>Lemna Minor</i>	1000	4	3	4	0.015
	100	5	5	5	
	10	6	7	7	

Figure 3.13: Phytotoxic activity of *E. japonica* against *L. minor*

3.1.2 Phytotoxic Activity of *E. japonica*

The ethanolic (10, 40, and 100%), methanolic (25, 50, and 200%) and EtOAc (10, 20, and 100%) fractions showed growth inhibition at 1000, 100, and 10 $\mu\text{g/ml}$, respectively. The methanolic, ethanolic, and EtOAc fractions showed significant activity at high concentrations, while at low concentrations low activity was observed. The results are shown in Table 3.13 and Figure 3.35. Low activity is not observed for the dilutions of natural compounds and synthetic compounds. The activity of one of the isolates showed

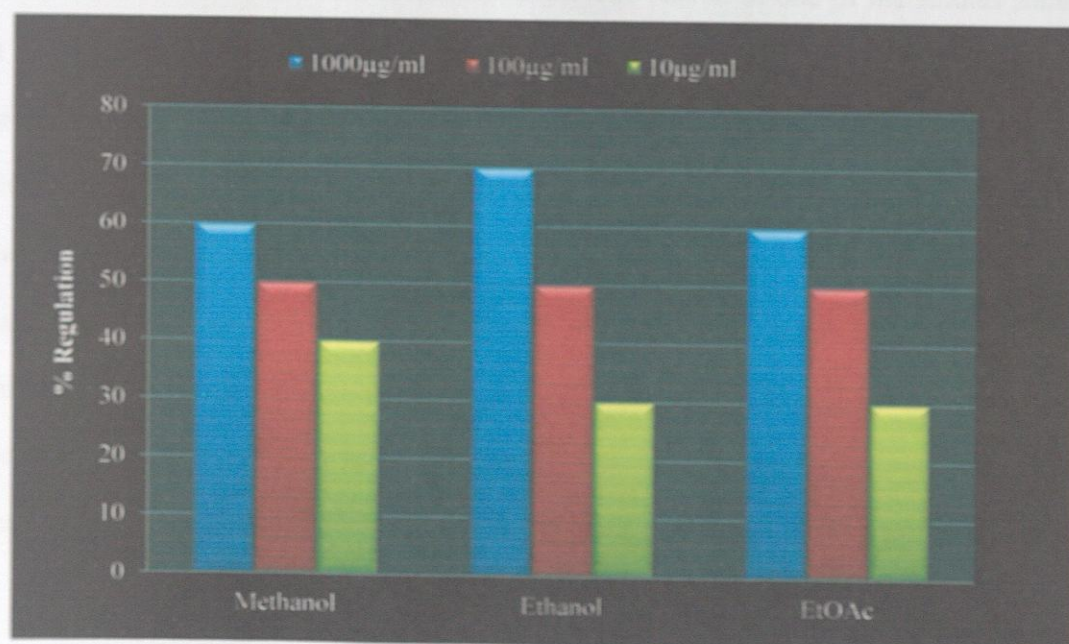


Figure 3.35: Phytotoxic activity of *E. japonica* against *L. minor*

3.8.2 Phytotoxic Activity of *F. carica*

The ethanolic (60, 40, and 30%), methanolic (50, 40, and 20%) and EtOAc (50, 30, and 10%) fractions showed growth inhibition at 1000, 100, and 10 $\mu\text{g/mL}$, respectively. The methanolic, ethanolic, and EtOAc fractions showed moderate activity at high concentrations, while at low concentration low activity was observed. The results are shown in Table 3.13 and Figure 3.36. Lemna assay is one of the methods for the detection of natural phytotoxic and antitumor compounds. The results of one of the studies showed that *F. carica* contains phytotoxic compounds inhibiting germination and of *L. minor*, thus demonstrating a good allelo-chemical potential [173]. Hameed et al. determined the toxicity profiles of methanolic extracts of *Datura innoxia*, *Withania coagulans*, *Solanum surattense*, *Solanum nigrum*, and *Withania somnifera* and found that *D. innoxia* and *W. coagulans* showed low phytotoxic activity at maximum doses. In contrast, moderate, and significant phytotoxic activities were detected for the extracts of both plants at concentrations of 1000, 100, and 10 $\mu\text{g/mL}$. The ethanolic extracts of *S. nigrum*, *W. somnifera*, and *S. surattense* exhibited moderate toxic activity at 1000 $\mu\text{g/mL}$ [174].

Table 3.13: Phytotoxic activity of *F. carica* against *L. minor*

Name of Plant	Conc. of sample ($\mu\text{g/ml}$)	No. of fronds survived			Conc. Of Std. drug * ($\mu\text{g/ml}$)
Lemna Minor		Methanol	Ethanol	EtOAc	
	1000	5	4	5	0.015
	100	6	6	7	
	10	8	7	9	

Figure 3.13: Phytotoxic activity of *F. carica* against *L. minor*

3.3.3 Phytotoxic Activity of *F. carica* against *L. minor*

The phytotoxic activities of *F. carica* against the model weed species *L. minor* were tested. The *F. carica* showed positive growth regulation of 49, 38 and 19% against *L. minor* at concentrations of 1000, 100 and 10 $\mu\text{g/ml}$, respectively. *F. carica* showed positive growth regulation of 48, 27 and 7% at concentrations of 1000, 100 and 10 $\mu\text{g/ml}$, respectively (Table 3.33 and Figure 3.36).

The *F. carica* showed positive activity of 49, 38 and 19% against *L. minor* at concentrations of 1000, 100 and 10 $\mu\text{g/ml}$, respectively. The *F. carica* showed positive activity of 48, 27 and 7% against *L. minor* at concentrations of 1000, 100 and 10 $\mu\text{g/ml}$, respectively.

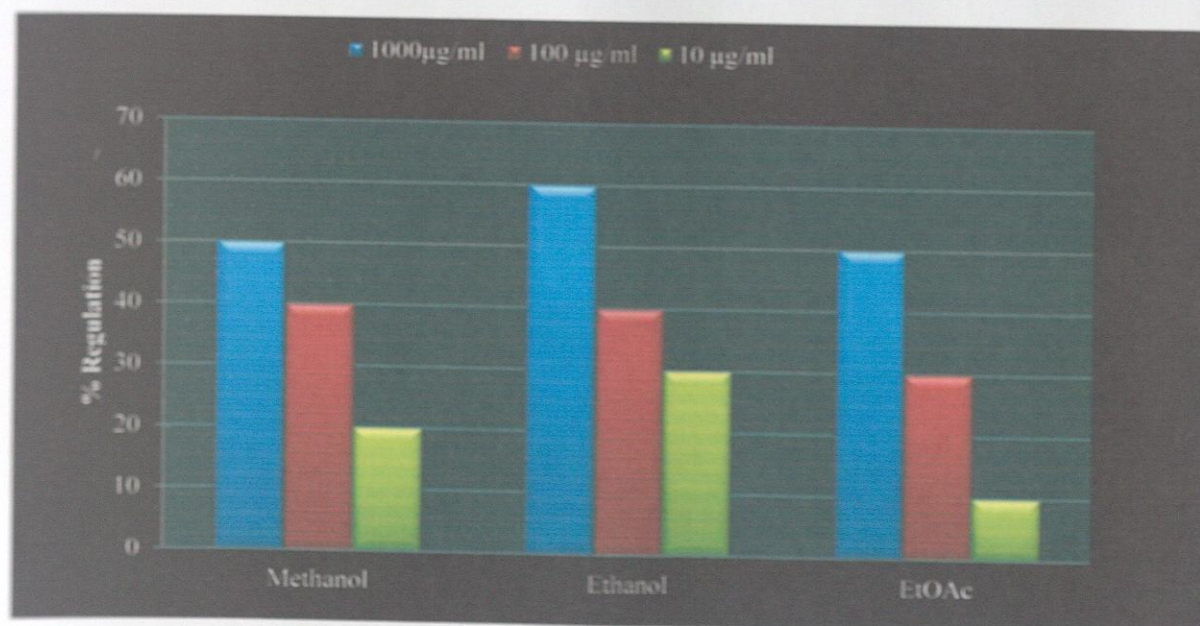


Figure 3.36: Phytotoxic activity of *F. carica* against *L. minor*

3.8.3 Phytotoxic Activity of ZnNPs and CuNPs

The phytotoxic activities of ZnNPs and CuNPs were evaluated against *L. minor*. The ZnNPs showed percent growth regulations of 70, 50, and 40 at concentrations of 1000, 100, and 10 $\mu\text{g/mL}$, respectively. CuNPs showed percent growth regulations of 70, 60, and 50 at concentrations of 1000, 100 and 10 $\mu\text{g/mL}$, respectively (Table 3.14 and Figure 3.37).

The ZnNPs showed good activity at concentrations of 1000 and 100 $\mu\text{g/mL}$, while moderate activity was observed at a concentration of 10 $\mu\text{g/mL}$. Cu NPs showed good activity at high concentrations (1000 and 100 $\mu\text{g/mL}$) and moderate activity at 10 $\mu\text{g/mL}$. These results suggest that ZnNPs and CuNPs possess phytotoxic activities at varying degrees. In another study, it was shown that CuONPs enhanced the expression of the exogenous gene encoding the Bt toxin protein in leaves and roots, especially at low CuONPs concentrations, providing an important benefit for Bt cotton of insect resistance [175].

3.4.1 Inhibitory activity of ZnNPs

Several studies have shown the phytotoxic activity of ZnNPs against *L. minor*. The phytotoxic activity of ZnNPs was evaluated by measuring the growth of *L. minor* in the presence of ZnNPs. The results showed that the growth of *L. minor* was significantly inhibited by ZnNPs at concentrations of 1000, 100, and 10 µg/ml. The phytotoxic activity of ZnNPs was also evaluated by measuring the chlorophyll content of *L. minor*. The results showed that the chlorophyll content of *L. minor* was significantly reduced by ZnNPs at concentrations of 1000, 100, and 10 µg/ml.

Table 3.14: Phytotoxic activity of ZnNPs and CuNPs against *L. minor*

Name of Plant	Conc. of sample (µg/ml)	No. of fronds survived		Conc. of Std. drug * (µg/ml)
		ZnNPs	CuNPs	
Lemna Minor	1000	3	3	0.015
	100	5	4	
	10	6	5	

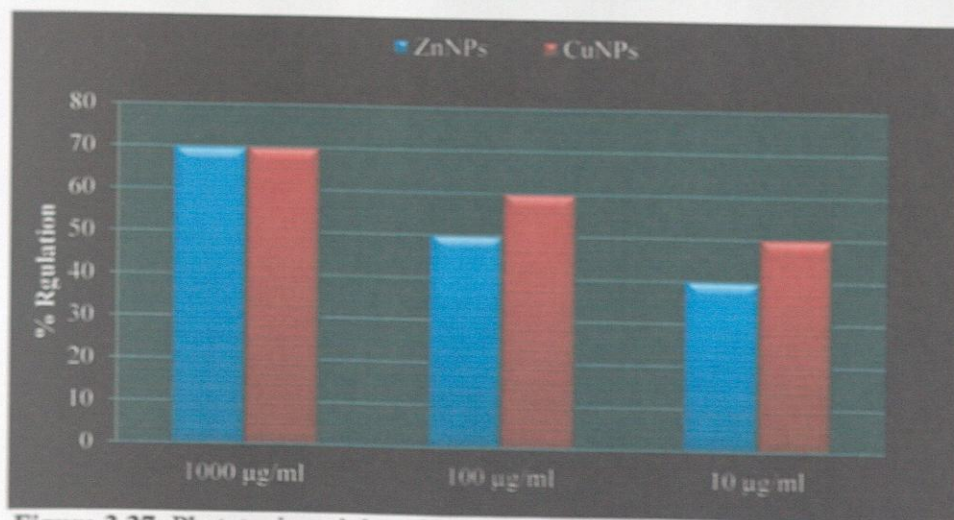


Figure 3.37: Phytotoxic activity of ZnNPs and CuNPs against *L. minor*

3.9.1 Insecticidal activity of *E. japonica*

Interest is being shown to develop environment friendly insecticides from natural resources due to the toxic effects of synthetic insecticides to the environment. The insecticidal activities of EtOAc, methanolic and ethanolic extracts were determined against *T. castaneum*, *T. confusum* and *C. maculatus* and the results are being summarized in **Table 3.15** and **Figure 3.38**. The methanolic extract of *E. japonica* showed moderate activity against *T. confusum* (40%), while it was inactive against *C. maculatus* and *T. castaneum*. The crude ethanolic extract of *E. japonica* showed moderate activity against *T. confusum* (50%), low activity against *T. castaneum* (20%) while no activity was observed for *C. maculatus*. The EtOAc extract showed moderate activity against *T. confusum* (50%) and *C. maculatus* (40%), and low activity against *T. castaneum* (10%). In another study, it was reported that different extracts of *E. japonica* were effective against *Meloidogyne incognita* and *Cephalobus littoralis* [176].

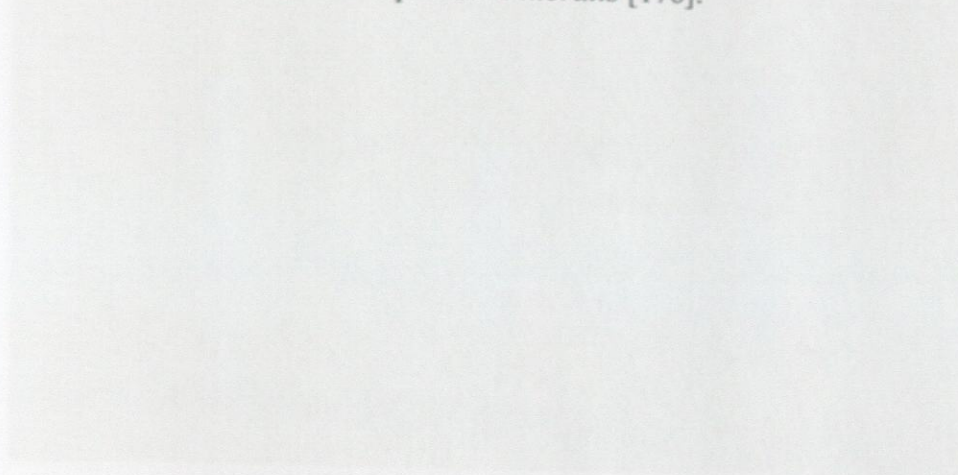
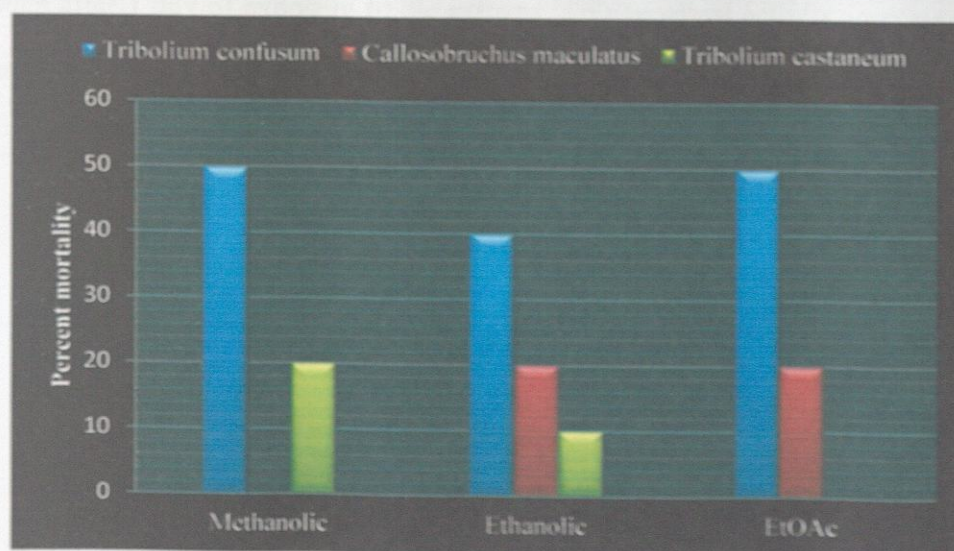


Figure 3.38 Insecticidal activity of *E. japonica* against *T. castaneum*, *T. confusum* and *C. maculatus*.

Table 3.15: Insecticidal activity of *E. japonica* against selected insects

Name of Insect	Percent Mortality of test insects				
	Control (Positive)	Control (Negative)	Methanol	Ethanol	EtOAc
<i>Tribolium confusum</i>	100	0	40	50	50
<i>Callosobruchus maculatus</i>	100	0	0	0	40
<i>Tribolium castaneum</i>	100	0	0	20	10

Figure 3.38: Insecticidal activity of different extracts of *E. japonica*

3.9.2 Insecticidal activity of *F. carica*

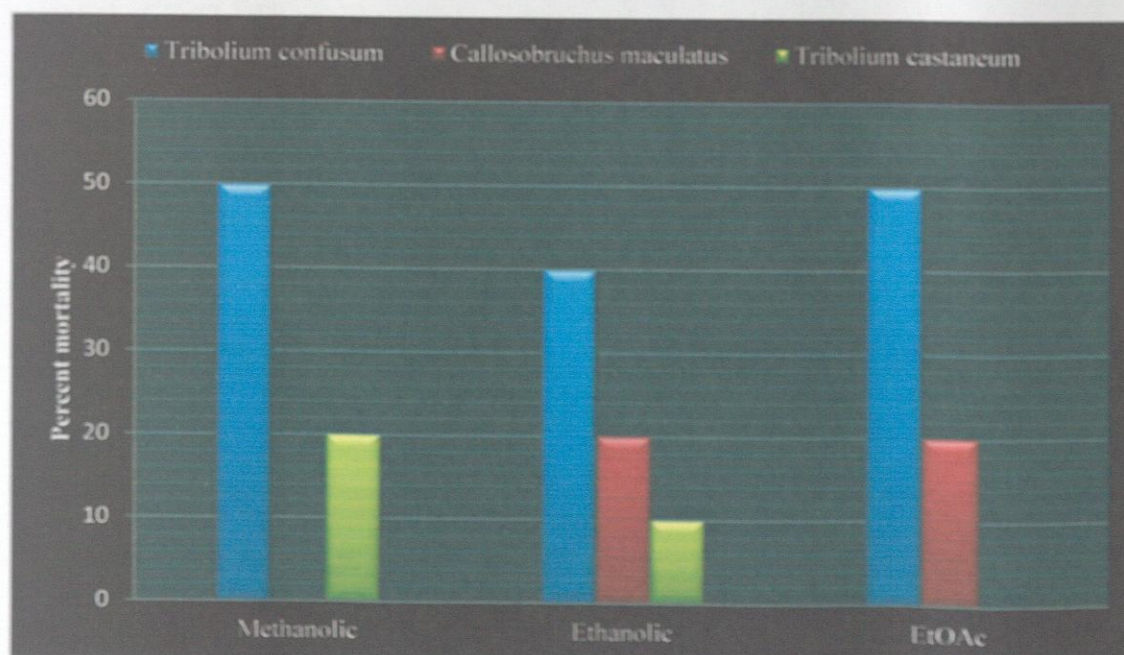
The insecticidal activity of EtOAc, methanolic and ethanolic extracts were determined against *C. maculatus*, *T. confusum* and *T. castaneum* and being summarized in **Table 3.16** and **Figure 3.39**. The methanolic extract of *F. carica* showed moderate activity against *T. confusum* (50%), low activity against *T. castaneum* (20%) but it was inactive against *C. maculatus*. The crude ethanolic extract of *F. carica* showed moderate activity against *T. confusum* (40%), and low activity against *C. maculatus* (20%) and *T. castaneum* (10%). The EtOAc extract showed moderate activity against *T. confusum* (50%), low activity against *C. maculatus* (20%), and no activity against *T. castaneum*. Different extracts (*n*-hexane and methylene chloride) of *Alpinia officinarum* were tested against *Tribolium castaneum* (adults). Both the extracts showed significant activity against *Tribolium castaneum* after 1 day of exposure to the extracts. This repellent activity increased with an increase in the time of exposure and quantity [177].

3.3.3 Insecticidal activity of *F. carica* extracts

The insecticidal activity of *F. carica* extracts was tested against the test insects.

Table 3.16: Insecticidal activity of *F. carica*

Name of Insect	% Mortality of test insects				
	Control (Positive)	Control (Negative)	Methanol	Ethanol	EtOAc
<i>Tribolium confusum</i>	100	0	50	40	50
<i>Callosobruchus maculatus</i>	100	0	0	20	20
<i>Tribolium castaneum</i>	100	0	20	10	0

Figure 3.39: Insecticidal activity of different extracts of *F. carica*

3.9.3 Insecticidal activity of ZnNPs and CuNPs

The insecticidal activities of ZnNPs and CuNPs were determined against the tested insect to determine their insecticidal potential. ZnNPs showed 60%, 50%, and 30% inhibition against *C. maculates*, *T. confusum*, and *T. castaneum*, respectively. Thus it can be concluded that ZnNPs possess good activity against *C. maculates*, moderate against *T. confusum*, and low activity against *T. castaneum*.

CuNPs showed good activity against *T. castaneum* and moderate activities against *T. confusum* and *C. maculates* (Table 3.17 and Figure 3.40).

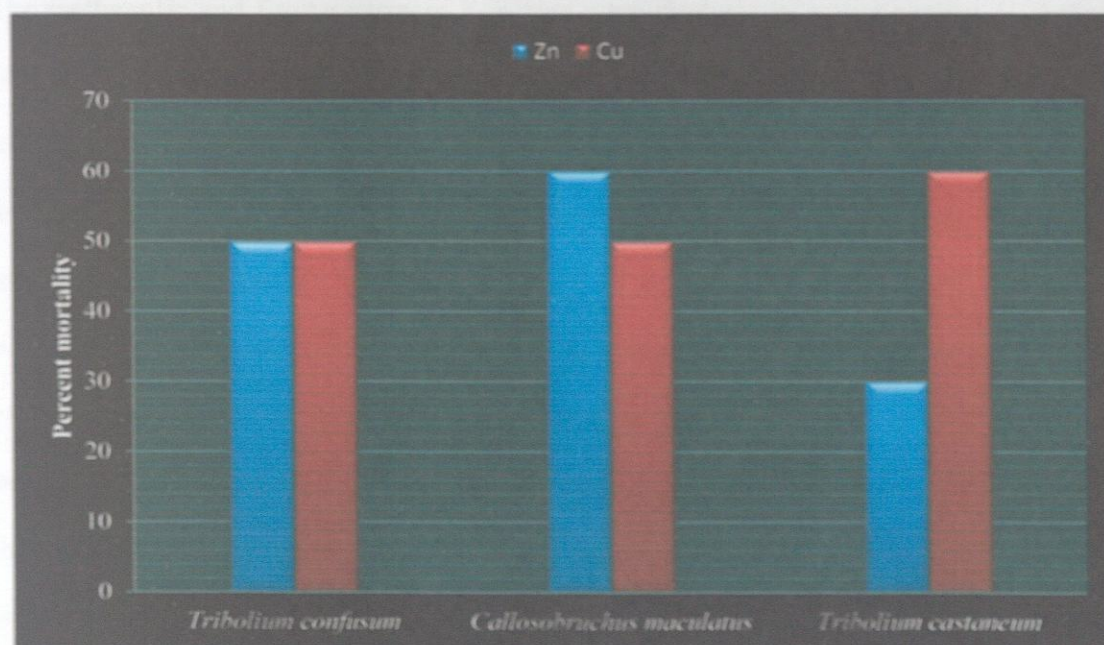
One of the studies reported that ZnONPs exhibited good activity against *Galleria mellonella*, the wax moth [178]. Another study was conducted to determine the insecticidal potential of ZnO NPs against certain pests and the most significant activity was observed against *humanus capitis*, hatchlings of *A. subpictus* and *C. quinquefasciatus* [179].

In another study, the CuONPs activity was determined against Bt-transgenic and conventional cotton and was found that CuONPs retard the developments of insects effecting the metabolism of abscisic acid (ABA) and indole-3-acetic acid (IAA). The results revealed that CuNPs were deposited on the epidermis layer and leaves and gain entry into the cells by endocytosis. CuONPs improved the exogenous gene expression of Bt toxin protein making them beneficial for cotton offering insect resistance [175]

Figure 3.40 Insecticidal activity of ZnNPs and CuNPs

Table 3.17: Insecticidal activity of ZnNPs and CuNPs of *E. japonica* and *F. carica*

Name of Insect	% Mortality of test insects			
	Control (Positive)	Control (Negative)	ZnNPs	CuNPs
<i>Tribolium confusum</i>	100	0	50	50
<i>Callosobruchus maculatus</i>	100	0	60	50
<i>Tribolium castaneum</i>	100	0	30	60

**Figure: 3.40:** Insecticidal activity of ZnNPs and CuNPs

3.10.1 Antitermite activity of *E. japonica* and *F. carica* and its ZnNPs and CuNPs

The anti-termite activity of crude methanolic, ethanolic, and EtOAc extracts were carried out against the *Heterotermes indicola* at the termites lab at the Nuclear Institute of Food and Agriculture (NIFA), Peshawar, Khyber Pakhtunkhwa, Pakistan. All the experiments were performed in triplicate. The results are presented in Table 6.18.

The methanolic and EtOAc extracts of *E. japonica* showed low activities against *H. indicola* of 30% and 10%, respectively, while the ethanolic extract was moderately active against *H. indicola* (40%).

The methanolic and EtOAc extracts *F. carica* were moderately active against *H. indicola* (40%), while the ethanolic extract was inactive.

In a separate report, *C. decidua* has been demonstrated to be effective against penetration and feeding of termites. [180] Another study reported the antitermite activities of the extracts from *Polygonum hydropiper*, *Diospyros sylvatica*, *Allium sativum*, *Pogostemon parviflorus* (Benth), *Aleurites fordii* and *Euphorbia kansui* [181]. The termiticidal activity exhibited by Cajput oil (*Melunuca cajputi*) against *Coptotermes formosanus* is due to the presence of monoterpene, diterpenes, sesquiterpene, and hydrocarbons. It was concluded that the plant species under study have diverse biological compounds one or a few of which can be effective against termites [182].

The anti-termite activity of ZnNPs was also observed against the insect, *H. indicola*. ZnNPs showed moderate activity against *H. indicola* (40%). While CuNPs showed good activity against termites (*H. indicola* 60%). In one study, ZnONPs (ZnO) were screened against *Galleria mellonella* and revealed that ZnONPs possess good insecticidal activity against it [178]. In another study, the CuONPs were checked for their activity against Bt-transgenic and conventional cotton. CuONPs improved the gene expression of the Bt-toxin

protein. This protein is found in leaves and roots, and imparts resistance against insects [175].

Table 3.10: Antimicrobial Activity of *Trichostema aegyptium* Extracts

Class of Insect	Concentration					Zone of Inhibition (mm)
	Control (Positive)	Control (Negative)	100 µg/ml	200 µg/ml	400 µg/ml	
Housefly (<i>Musca domestica</i>)	100	0	20	40	40	2.5 mm
	100	0	20	40	40	2.5 mm
	100	0	20	40	40	2.5 mm
	100	0	20	40	40	2.5 mm

3.11.1 Bioinsecticidal Activity of *E. japonica* and *F. carica* and Their ZnNPs and CuNPs

The crude methanolic, ethanolic, EtOAc extracts of *E. japonica*, *F. carica*, ZnNPs and CuNPs were subjected for antitermite bioassay against *RMD* termites groups. The results are presented in Table 3.17 and 3.18. The results revealed that extracts extracted the *termites* and ZnNPs and CuNPs activity indicating a degree of phytotoxicity.

Table 3.18: Antitermite activity of *E. japonica* and *F. carica*

Name of Insect	% Mortality					Name of the plants / Nanoparticle
	Control (Positive)	Control (Negative)	Methanol	Ethanol	EtOAc	
<i>Heterotermes indicola</i>	100	0	30	40	0	<i>E. japonica</i>
	100	0	40	0	40	<i>F. carica</i>
	100	0	40			ZnNPs
	100	0	60			CuNPs

3.11.1 Haemagglutination Activity of *E. japonica* and *F. carica* and their ZnNPs and CuNPs

The crude methanolic, ethanolic, EtOAc extracts of *E. japonica*, *F. carica*, Zn NPs, and Cu NPs were screened for hemagglutination against ABO blood groups. The results are presented in Table 3.19 and 3.20. The results revealed that plants extracts and the NPs do not exhibit hemagglutination activity indicating the lack of phytolectins.

Table 3.19: Haemagglutination activity of *E. japonica* and ZnNPs

Blood Groups	AB -ive, AB +ive, O +ive, O -ive, A-ive , A +ive, B-ive, B +ive			
Dilutions	1:2	1:4	1:8	1:16
Methanol	-	-	-	-
Ethanol	-	-	-	-
EtOAc	-	-	-	-
ZnNPs	-	-	-	-

Table 3.20: Haemagglutination activity of *F. carica* and CuNPs

Blood Groups	AB -ive, AB +ive, O +ive, O -ive, A-ive , A +ive, B-ive, B +ive			
Dilutions	1:2	1:4	1:8	1:16
Methanol	-	-	-	-
Ethanol	-	-	-	-
EtOAc	-	-	-	-
CuNPs	-	-	-	-



Figure 3.41: Haemagglutination activity of *E. japonica* and *F. carica* crude extracts and their ZnNPs and CuNPs



Figure: 3.42 Haemagglutination activity of *E. japonica* and *F. carica* crude extracts and their ZnNPs and CuNPs

Conclusion

The current study was aimed at screening the methanolic, ethanolic and ethyl acetate (EtOAc) fractions of *Eriobotrya japonica* and *Ficus carica* leaves and Zinc nanoparticles (ZnNPs) of *E. japonica* and Copper nanoparticles (CuNPs) of *F. carica* for various pharmacological activities; antibacterial, antifungal, phytotoxic, insecticidal, anti-termite, haemagglutination and their comparison.

The ZnNPs and CuNPs were characterized using reported procedures utilizing aqueous leaves extract of *E. japonica* and *F. carica* respectively and different conditions like temperature, pH and incubation time were optimized. The synthesized nanoparticles were characterized using UV-Visible spectrophotometry, Fourier Transform Infrared spectroscopy (FTIR), Scanning Electron Microscope (SEM), Energy Dispersive X-ray (EDX), X-ray diffraction (XRD) and Thermogravimetric/Differential Thermal Analysis (TGA/DTA). The result of antibacterial activity of *E. japonica* revealed good activities against *E. coli*, *S. aureus*, *M. morganii*, *P. vulgaris* (60, 52 and 64%) and MRSA. The crude extract also revealed some good activities against *S. aureus*, *P. aeruginosa*, *M. morganii*, *A. baumannii*, *P. vulgaris* and MRSA. The order of antibacterial activity of ZnNPs and CuNPs against *E. coli* was (48.1 and 40.7 %), *S. aureus* (73 and 72%), *P. aeruginosa* (37 and 44.4%), *M. morganii* (64 and 54.5%), *A. baumannii* (43 and 56%), *P. vulgaris* (92 and 44%) and MRSA (94 and 89%). The methanolic, ethanolic and EtOAc fractions of *F. carica* and *E. japonica* showed low (<24%) and no antifungal activity against the test fungal pathogens; *A. niger*, *H. pseudocrispula*, *P. notatum*, *A. parasiticus*, *V. longisporum* and *P. chrysogenum*. The antifungal activity of ZnNPs and CuNPs was found against all the test fungal strains from low to moderate. The methanolic, ethanolic and EtOAc extract of *E. japonica* and *F. carica* showed moderate activity at higher concentrations while low activity at other test concentrations. Phytotoxic activity of Zn-NPs and Cu-NPs against *L. minor* at 1000, 100

and 10 µg/ml was 70, 50 and 40% and 70, 60 and 50%, respectively. The insecticidal activity of methanol, ethanol and EtOAc fractions was determined against *T. confusum*, *C. maculatus* and *T. castaneum* and was moderate to low for both *E. japonica* and *F. carica*. The results indicated that the selected plants lack phyto-lectins, as no haemagglutination was observed for any of the test samples and nanoparticles.

CONCLUSION

The current study was aimed at screening the methanolic, ethanolic and ethyl acetate (EtOAc) fractions of *Eriobotrya japonica* and *Ficus carica* leaves and Zinc nanoparticles (ZnNPs) of *E. japonica* and Copper nanoparticles (CuNPs) of *F. carica* for various pharmacological activities; antibacterial, antifungal, phytotoxic, insecticidal, anti-termite, haemagglutination and their comparison.

The ZnNPs and CuNPs were characterized using reported procedures utilizing aqueous leaves extract of *E. japonica* and *F. carica* respectively and different conditions like temperature, pH and incubation time were optimized. The synthesized nanoparticles were characterized using UV-Visible spectrophotometry, Fourier Transform Infrared spectroscopy (FTIR), Scanning Electron Microscope (SEM), Energy Dispersive X-ray (EDX), X-ray diffraction (XRD) and Thermogravimetric/Differential Thermal Analysis (TGA/DTA). The result of antibacterial activity of *E. japonica* revealed good activities against *E. coli*, *S. aureus*, *M. morganii*, *P. vulgaris* (60, 52 and 64%) and MRSA. The crude extract also revealed some good activities against *S. aureus*, *P. aeruginosa*, *M. morganii*, *A. baumannii*, *P. vulgaris* and MRSA. The order of antibacterial activity of ZnNPs and CuNPs against *E. coli* was (48.1 and 40.7 %), *S. aureus* (73 and 72%), *P. aeruginosa* (37 and 44.4%), *M. morganii* (64 and 54.5%), *A. baumannii* (43 and 56%), *P. vulgaris* (92 and 44%) and MRSA (94 and 89%). The methanolic, ethanolic and EtOAc fractions of *F. carica* and *E. japonica* showed low (<24%) and no antifungal activity against the test fungal pathogens; *A. niger*, *H. pseudocrispula*, *P. notatum*, *A. parasiticus*, *V. longisporum* and *P. chrysogenum*. The antifungal activity of ZnNPs and CuNPs was found against all the test fungal strains from low to moderate. The methanolic, ethanolic and EtOAc extract of *E. japonica* and *F. carica* showed moderate activity at higher concentrations while low activity at other test concentrations. Phytotoxic activity of Zn-NPs and Cu-NPs against *L.*

minor at 1000, 100 and 10 $\mu\text{g/ml}$ was 70, 50 and 40% and 70, 60 and 50%, respectively. The insecticidal activity of methanol, ethanol and EtOAc fractions was determined against *T. confusum*, *C. maculatus* and *T. castaneum* and was moderate to low for both *E. japonica* and *F. carica*. The results indicated that the selected plants lack phyto-lectins, as no haemagglutination was observed for any of the test samples and nanoparticles.

REFERENCES

1. Benn, T., Cavanagh, B., Hristovski, K., Posner, J. D., & Westerhoff, P. (2010). The release of nanosilver from consumer products used in the home. *Journal of environmental quality*, 39(6), 1875-1882.
2. Aitken, R. J., Creely, K. S., & Tran, C. L. (2004). *Nanoparticles: An occupational hygiene review. Prepared by the Institute of Occupational Medicine for Health and Safety Executive*. Edinburgh.
3. Arnall, A. H. (2003). *Future Technologies, Today's Choices: Nanotechnology, Artificial Intelligence and Robotics; A technical, political and institutional map of emerging technologies*. Greenpeace Environmental Trust.
4. Dürrenberger, F., Höck, J., & Höhener, K. (2004). Overview of completed and ongoing activities in the field: safety and risks of nanotechnology. *TEMAS AG*.
5. Colvin, V. L. (2003). The potential environmental impact of engineered nanomaterials. *Nature biotechnology*, 21(10), 1166-1170.
6. Takacs, L. (2003). M. Carey Lea, the father of mechanochemistry. *Bulletin of Historical Chemistry*, 28(1), 26-34.
7. Frens, G., Overbeek, J. T. Carey Leas colloidal silver. *Kolloid Z. Z. Polym.* (1969), 233 (1-2), 922-&.
8. Henglein, A., & Giersig, M. (1999). Formation of colloidal silver nanoparticles: capping action of citrate. *The Journal of Physical Chemistry B*, 103(44), 9533-9539.
9. Boese, K. (1921). Über Collargol, seine Anwendung und seine Erfolge in der Chirurgie und Gynäkologie. *Langenbeck's Archives of Surgery*, 163(1), 62-84.
10. Bogdanchikova, N. E., Kurbatov, A. V., Tret'yakov, V. V., & Rodionov, P. P. (1992). Activity of colloidal silver preparations towards smallpox virus. *Pharmaceutical Chemistry Journal*, 26(9), 778-779.

11. Nowack, B., Krug, H. F., & Height, M. (2011). Reply to Comments on "120 Years of Nanosilver History: Implications for Policy Makers". *Environmental science & technology*, 45(17), 7593-7595.
12. Moudry, Z., V. (1953) Process of producing oligodynamic metal biocides. United States Patent 2,927,052.
13. Milton, M. (1968). *U.S. Patent No. 3,374,608*. Washington, DC: U.S. Patent and Trademark Office.
14. Ko, F. W., Lai, C. K., Woo, J., Ho, S. C., Ho, C. W., Goggins, W., & Hui, D. S. (2006). 12-year change in prevalence of respiratory symptoms in elderly Chinese living in Hong Kong. *Respiratory medicine*, 100(9), 1598-1607.
15. Zhang, C., Khoshmanesh, K., Mitchell, A., & Kalantar-Zadeh, K. (2010). Dielectrophoresis for manipulation of micro/nano particles in microfluidic systems. *Analytical and bioanalytical chemistry*, 396(1), 401-420.
16. Reddy, V. R. (2006). Gold nanoparticles: synthesis and applications. *Synlett*, 2006 (11), 1791-1792.
17. Bhattacharjee, C. R., Purkayastha, D. D., Bhattacharjee, S., & Nath, A. (2011). Homogeneous chemical precipitation route to ZnO nanosphericals. *Assam University Journal of Science and Technology*, 7(2), 122-127.
18. Wang, L., & Muhammed, M. (1999). Synthesis of zinc oxide nanoparticles with controlled morphology. *Journal of Materials Chemistry*, 9 (11), 2871-2878.
19. Yadugiri, V. T., & Malhotra, R. (2010). 'Plenty of room'-fifty years after the Feynman lecture. *Current Science* (00113891), 99 (7).
20. Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical microbiology reviews*, 12(4), 564-582.

21. Ducamp-Sanguesa, C., Herrera-Urbina, R., & Figlarz, M. (1992). Synthesis and characterization of fine and monodisperse silver particles of uniform shape. *Journal of Solid State Chemistry*, 100(2), 272-280.
22. Kim, D., Jeong, S., & Moon, J. (2006). Synthesis of silver nanoparticles using the polyol process and the influence of precursor injection. *Nanotechnology*, 17(16), 4019-4024.
23. Fu, Z., Yang, B., Li, L., Dong, W., Jia, C., & Wu, W. (2003). An intense ultraviolet photoluminescence in sol-gel ZnO-SiO₂ nanocomposites. *Journal of Physics: Condensed Matter*, 15(17), 2867.
24. Khodja, A. A., Sehili, T., Pilichowski, J. F., & Boule, P. (2001). Photocatalytic degradation of 2-phenylphenol on TiO₂ and ZnO in aqueous suspensions. *Journal of Photochemistry and Photobiology A: Chemistry*, 141(2), 231-239.
25. Reddy, V. R., Currao, A., & Calzaferri, G. (2007). Gold and silver metal nanoparticle-modified AgCl photocatalyst for water oxidation to O₂. In *Journal of Physics: Conference Series* 61, (1) 960.
26. Dehaghi, S. M., Rahmanifar, B., Moradi, A. M., & Azar, P. A. (2014). Removal of permethrin pesticide from water by chitosan-zinc oxide nanoparticles composite as an adsorbent. *Journal of Saudi Chemical Society*, 18(4), 348-355.
27. Shankar, S. S., Rai, A., Ahmad, A., & Sastry, M. (2004). Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. *Journal of colloid and interface science*, 275(2), 496-502.
28. Wöll, C. (2007). The chemistry and physics of zinc oxide surfaces. *Progress in Surface Science*, 82(2), 55-120.
29. Leela, A., & Vivekanandan, M. (2008). Tapping the unexploited plant resources for the synthesis of silver nanoparticles. *African Journal of Biotechnology*, 7(17).

30. John, A. A. (1992). Chromatographic analysis of pharmaceuticals. *Marcel Dekker, Inc. Chromatographic science series*, 74, 135-184.
31. Newman, R. A., Lansky, E. P., & Block, M. L. (2007). *Pomegranate: The Most Medicinal Fruit*. Basic Health Publications.
32. Baránek, M., Raddová, J., & Pidra, M. (2006). Comparative analysis of genetic diversity in *Prunus L.* as revealed by RAPD and SSR markers. *Scientia horticulturae*, 108(3), 253-259.
33. Gardea-Torresdey, J. L., Gomez, E., Peralta-Videa, J. R., Parsons, J. G., Troiani, H., & Jose-Yacamán, M. (2003). Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles. *Langmuir*, 19(4), 1357-1361.
34. Daniel, S. K., Vinothini, G., Subramanian, N., Nehru, K., & Sivakumar, M. (2013). Biosynthesis of Cu, ZVI, and Ag nanoparticles using *Dodonaea viscosa* extract for antibacterial activity against human pathogens. *Journal of nanoparticle research*, 15(1), 1-10.
35. Bakar, N. H. H. A., & Tan, W. L. (2016). Natural Composite Membranes for Water Remediation: Toward a Sustainable Tomorrow. In *Renewable Energy and Sustainable Technologies for Building and Environmental Applications*(pp. 25-49).
36. Gardea-Torresdey, J. L., Tiemann, K. J., Gamez, G., Dokken, K., Tehuacanero, S., & Jose-Yacamán, M. (1999). Gold nanoparticles obtained by bio-precipitation from gold (III) solutions. *Journal of Nanoparticle Research*, 1(3), 397-404.
37. Song, J. Y., Jang, H. K., & Kim, B. S. (2009). Biological synthesis of gold nanoparticles using *Magnolia kobus* and *Diopyros kaki* leaf extracts. *Process Biochemistry*, 44(10), 1133-1138.
38. Ramesh, P., Rajendran, A., & Meenakshisundaram, M. (2014). Green Synthesis of Zinc Oxide Nanoparticles Using Flower Extract *Cassia Auriculata*. *Journal of Nano Science and Nano Technology*, 2(1), 41-45.

39. Herrera, I., Gardea-Torresdey, J. L., Tiemann, K. J., Peralta-Videa, J. R., Armendariz, V., & Parsons, J. G. (2003). Binding of silver (I) ions by alfalfa biomass (*Medicago sativa*): batch pH, time, temperature, and ionic strength studies. *Journal of Hazardous Substance Research*, 4(1), 1_1.
40. Vigneshwaran, N., Kumar, S., Kathe, A. A., Varadarajan, P. V., & Prasad, V. (2006). Functional finishing of cotton fabrics using zinc oxide-soluble starch nanocomposites. *Nanotechnology*, 17(20), 5087.
41. Nemmar, A., Hoylaerts, M. F., Hoet, P. H., & Nemery, B. (2004). Possible mechanisms of the cardiovascular effects of inhaled particles: systemic translocation and prothrombotic effects. *Toxicology letters*, 149(1), 243-253.
42. Kleiner, K., & Hogan, J. (2003). How safe is nanotech?. *New Scientist*, 177(2388), 14-15.
43. Kreuter, J. (2001). Nanoparticulate systems for brain delivery of drugs. *Advanced drug delivery reviews*, 47(1), 65-81.
44. Bonazzi, M., von Bose, H., & Tokamanis, C. (2010). Communicating nanotechnology. Why, to whom, saying what and how. *European Commission, Brussels*.
45. Dhas, N. A., Raj, C. P., & Gedanken, A. (1998). Synthesis, characterization, and properties of metallic copper nanoparticles. *Chemistry of materials*, 10(5), 1446-1452.
46. Ren, G., Hu, D., Cheng, E. W., Vargas-Reus, M. A., Reip, P., & Allaker, R. P. (2009). Characterisation of copper oxide nanoparticles for antimicrobial applications. *International journal of antimicrobial agents*, 33(6), 587-590.
47. Eastman, J. A., Choi, S. U. S., Li, S., Yu, W., & Thompson, L. J. (2001). Anomalously increased effective thermal conductivities of ethylene glycol-based nanofluids containing copper nanoparticles. *Applied physics letters*, 78(6), 718-720.
48. Luo, X., Morrin, A., Killard, A. J., & Smyth, M. R. (2006). Application of nanoparticles in electrochemical sensors and biosensors. *Electroanalysis*, 18 (4), 319-326.

49. Lee, Y., Choi, J. R., Lee, K. J., Stott, N. E., & Kim, D. (2008). Large-scale synthesis of copper nanoparticles by chemically controlled reduction for applications of inkjet-printed electronics. *Nanotechnology*, 19(41), 415604.
50. Dwivedi, Y. K., Wastell, D., Laumer, S., Henriksen, H. Z., Myers, M. D., Bunker, D., & Srivastava, S. C. (2015). Research on information systems failures and successes: Status update and future directions. *Information Systems Frontiers*, 17(1), 143-157.
51. Borm, P. J., & Kreyling, W. (2004). Toxicological hazards of inhaled nanoparticles—potential implications for drug delivery. *Journal of nanoscience and nanotechnology*, 4(5), 521-531.
52. Kamat, P. V., & Meisel, D. (2003). Nanoscience opportunities in environmental remediation. *Comptes Rendus Chimie*, 6(8), 999-1007.
53. Cuenca, A. G., Jiang, H., Hochwald, S. N., Delano, M., Cance, W. G., & Grobmyer, S. R. (2006). Emerging implications of nanotechnology on cancer diagnostics and therapeutics. *Cancer*, 107(3), 459-466.
54. Maynard, A. D., & Kuempel, E. D. (2005). Airborne nanostructured particles and occupational health. *Journal of nanoparticle research*, 7(6), 587-614.
55. Luo, X.; Morrin, A.; Killard, A. J.; Smyth, M. R., (2006). Application of nanoparticles in electrochemical sensors and biosensors. *Electroanalysis*, 18 (4), 319-326.
56. Tiwari, D. K., & Behari, J. (2009). Biocidal nature of combined treatment of Ag-nanoparticle and ultrasonic irradiation in Escherichia coli DH5. *Advances in Biological Research*, 3(3-4), 89-95.
57. Yamamoto, O., Komatsu, M., Sawai, J., & Nakagawa, Z. E. (2004). Effect of lattice constant of zinc oxide on antibacterial characteristics. *Journal of materials science: materials in medicine*, 15(8), 847-851.

58. Sven Landrein, Renata Borosova and Joanna Osborne. (2011). The Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey, U.K
59. Uniyal, S. K., Singh, K. N., Jamwal, P., & Lal, B. (2006). Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalaya. *Journal of Ethnobiology and Ethnomedicine*, 2(1), 1.
60. Davidse, G., M. Sousa Sánchez, S. Knapp & F. Chiang Cabrera. (2014). Saururaceae a Zygophyllaceae. 2(3): ined. In G. Davidse, M. Sousa Sánchez, S. Knapp & F. Chiang Cabrera (eds.) *Flora Mesoamericana*. Universidad Nacional Autónoma de México, México D.F
61. Badenes, M., Martínez-Calvo, J., & Llácer, G. (2000). *Euphytica*, 114(3), 187-194.
62. Vilanova, S., Badenes, M. L., Martínez-Calvo, J., & Llácer, G. (2001). Analysis of loquat germplasm (*Eriobotrya japonica* Lindl) by RAPD molecular markers. *Euphytica*, 121(1), 25-29.
63. Hussain, A. Z. H. A. R., Abbasi, N. A., Hafiz, I. A., & Akhtar, A. (2009). Morpho-physical characteristics of eight loquat genotypes cultivated in Chakwal district, Pakistan. *Pakistan Journal of Botany*, 41, 2841-2849.
64. Janick, J. (2006). Genetic alteration associated with fruit domestication. In *II International Symposium on Loquat 750* (pp. 27-36).
65. Soriano, J. M., Romero, C., Vilanova, S., Llácer, G., & Badenes, M. L. (2005). Genetic diversity of loquat germplasm (*Eriobotrya japonica* (Thunb) Lindl) assessed by SSR markers. *Genome*, 48(1), 108-114.
66. Lin, S., Huang, X., Cuevas, J., & Janick, J. (2007). Loquat: an ancient fruit crop with a promising future. *Chronica Hort*, 47(2), 12-15.
67. Zhuang, F. Y., Chen, J. F., Staub, J. E., & Qian, C. T. (2004). Assessment of genetic relationships among *Cucumis* spp. by SSR and RAPD marker analysis. *Plant Breeding*, 123(2), 167-172.

68. Sakuramata, Y., Oe, H., Kusano, S., & Aki, O. (2004). Effects of combination of Caiapo® with other plant-derived substance on anti-diabetic efficacy in KK-Ay mice. *BioFactors*, 22(1-4), 149-152.
69. Nishioka, Y., Yoshioka, S., Kusunose, M., Cui, T., Hamada, A., Ono, M., ... & Kyotani, S. (2002). Effects of extract derived from *Eriobotrya japonica* on liver function improvement in rats. *Biological and Pharmaceutical Bulletin*, 25(8), 1053-1057.
70. Takuma, D., Yokota, J., Hamada, A., Yoshioka, S., Kusunose, M., Miyamura, M., ... & Nishioka, Y. (2005). Effects of *Eriobotrya japonica* seed extract in a model rat of lipopolysaccharide-induced inflammation. *Journal of the Japanese Society for Food Science and Technology (Japan)*.
71. Zohary, D., Hopf, M., & Weiss, E. (2012). *Domestication of Plants in the Old World: The origin and spread of domesticated plants in Southwest Asia, Europe, and the Mediterranean Basin*. Oxford University Press on Demand.
72. Karadeniz, T. (2002). Loquat (*Eriobotrya japonica* lindl.) growing in Turkey. In *First International Symposium on Loquat* (pp. 11-13).
73. Husain, S. Z., Malik, R. N., Javaid, M., & Bibi, S. (2008). Ethnobotanical properties and uses of medicinal plants of Morgah biodiversity park, Rawalpindi. *Pak J Bot*, 40(5), 1897-1911.
74. Rønsted, N., Weiblen, G. D., Savolainen, V., & Cook, J. M. (2008). Phylogeny, biogeography, and ecology of *Ficus* section *Malvanthera* (Moraceae). *Molecular Phylogenetics and Evolution*, 48(1), 12-22.
75. Janzen, D. H. (1979). How to be a fig. *Annual review of ecology and systematics*, 10, 13-51.
76. Jeong, W. S., & Lachance, P. A. (2001). Phytosterols and fatty acids in fig (*Ficus carica*, var. Mission) fruit and tree components. *Journal of food science*, 66(2), 278-281.

77. Mars, M. (2001). Fig (*Ficus carica* L.) genetic resources and breeding. In *II International Symposium on Fig 605* (pp. 19-27).
78. Wagner, W. L., Herbst, D. R., & Sohmer, S. H. (1999). *Manual of the Flowering Plants of Hawai'i, Vols. 1 and 2* (No. Edn 2). University of Hawai'i and Bishop Museum Press.
79. Duke, J. A. (2002). *Handbook of medicinal herbs*. CRC press.
80. WEIPING, Y., Hongming, C., Tianxin, W., & Mengshen, C. (1997). Research on the chemical structure and anticancer activity of 9, (19) 24, 25.
81. Mohan, G. K., Pallavi, E., Kumar, R., Ramesh, M., & Venkatesh, S. (2007). Hepatoprotective activity of *Ficus carica* Linn leaf extract against carbon tetrachloride-induced hepatotoxicity in rats. *DARU Journal of Pharmaceutical Sciences*, 15(3), 162-166.
82. Joseph, B., & Raj, S. J. (2011). Pharmacognostic and phytochemical properties of *Ficus carica* Linn—An overview. *International Journal of PharmTech Research*, 3(1), 8-12.
83. Kavitha, K. S., Baker, S., Rakshith, D., Kavitha, H. U., Yashwantha Rao, H. C., Harini, B. P., & Satish, S. (2013). Plants as green source towards synthesis of nanoparticles. *Int Res J Biol Sci*, 2(6), 66-76.
84. Singh, C., Baboota, R. K., Naik, P. K., & Singh, H. (2012). Biocompatible synthesis of silver and gold nanoparticles using leaf extract of *Dalbergia sissoo*. *Adv Mater Lett*, 3(4), 279-285.
85. Asmathunisha, N., & Kathiresan, K. (2013). Rapid biosynthesis of antimicrobial silver and gold nanoparticles by in vitro callus and leaf extracts from *lycopersicon esculentum* mill. *Int J Pharm Bio Sci*, 4(1), 334-344.
86. Kalpana, D., Pichiah, P. T., Sankarganesh, A., Park, W. S., Lee, S. M., Wahab, R., ... & Lee, Y. S. (2013). Biogenesis of gold nanoparticles using plant powders and assessment of in vitro cytotoxicity in 3T3-L1 cell line. *Journal of Pharmaceutical Innovation*, 8(4), 265-275.

87. F. A. Anand Raj and Jayalakshmy. ERaj L. F. AJayalakshmy E. (2015). *Orient J Chem* 31(1),124-127
88. Saranyaadevi, K., Subha, V., Ernest Ravindran, R. S., & Renganathan, S. (2014). Synthesis and characterization of copper nanoparticle using Capparis zeylanica leaf extract. *Int J ChemTech Res*, 6(10), 4533-4541.
89. Kulkarni, V. D., & Kulkarni, P. S. (2013). Green synthesis of copper nanoparticles using Ocimum sanctum leaf extract. *Int J Chem Stud*, 1(3), 1-4.
90. Sivaraj, R., Rahman, P. K., Rajiv, P., & Venckatesh, R. (2014). Biogenic zinc oxide nanoparticles synthesis using Tabernaemontana Divaricate leaf extract and its anticancer activity against MCF-7 breast cancer cell Lines. *studies*, 16, 18.
91. Patel, V. K., Sundriyal, P., & Bhattacharya, S. (2016). Aloe-Vera vs Poly (Ethylene) glycol-based Synthesis and Relative Catalytic Activity Investigations of ZnO Nanorods in Thermal Decomposition of Potassium Perchlorate. *Particulate Science and Technology*, (just-accepted).
92. Hawthorne, J., Musante, C., Sinha, S. K., & White, J. C. (2012). Accumulation and phytotoxicity of engineered nanoparticles to Cucurbita pepo. *International journal of phytoremediation*, 14(4), 429-442.
93. Angrasan, J. K. V. M., & Subbaiya, R. (2014). Biosynthesis of copper nanoparticles by Vitis vinifera leaf aqueous extract and its antibacterial activity. *Int J Curr Microbiol Appl Sci*, 3(9), 768-774.
94. Gopinath, M., Subbaiya, R., Selvam, M. M., & Suresh, D. (2014). Synthesis of copper nanoparticles from Nerium oleander leaf aqueous extract and its antibacterial activity. *Int J Curr Microbiol App Sci*, 3(9), 814-818.
95. Devi, R. S., & Gayathri, R. (2014). Green Synthesis of zinc oxide nanoparticles by using Hibiscus rosa-sinensis. *Int J Curr Eng Technol*, 4, 2444-2446.

96. Elumalai, K., Velmurugan, S., Ravi, S., Kathiravan, V., & Ashokkumar, S. (2015). Green synthesis of zinc oxide nanoparticles using *Moringa oleifera* leaf extract and evaluation of its antimicrobial activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 143, 158-164.
97. Bala, N., Saha, S., Chakraborty, M., Maiti, M., Das, S., Basu, R., & Nandy, P. (2015). Green synthesis of zinc oxide nanoparticles using *Hibiscus subdariffa* leaf extract: effect of temperature on synthesis, anti-bacterial activity and anti-diabetic activity. *RSC Advances*, 5(7), 4993-5003.
98. Sagar Raut , Dr. P. V. Thorat , Rohini Thakre (2015) International Journal of Science and Research.4 (5) 1225-1228
- 99.
100. Ahmad, B., Shireen, F., Bashir, S., Khan, I., & Azam, S. (2016). Green synthesis, characterisation and biological evaluation of AgNPs using *Agave americana*, *Mentha spicata* and *Mangifera indica* aqueous leaves extract. *IET Nanobiotechnology*.
101. Singh, P. P., & Bhakat, C. (2012). Green synthesis of gold nanoparticles and silver nanoparticles from leaves and bark of *Ficus carica* for nanotechnological applications. *Int. J. Sci. Res. Publ*, 2, 1-4.
102. Awwad, A. M., Salem, N. M., & Abdeen, A. O. (2013). Biosynthesis of silver nanoparticles using Loquat leaf extract and its antibacterial activity. *Advanced Materials Letters*, 4(5), 338-342.
103. Sett, A., Gadewar, M., Sharma, P., Deka, M., & Bora, U. (2016). Green synthesis of gold nanoparticles using aqueous extract of *Dillenia indica*. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, 7(2), 025005.

104. Kolawole, O. M. (2006). The effect of *Bridelia ferruginea* and *Senna alata* on plasma glucose concentration in normoglycemic and glucose induced hyperglycemic rats. *Ethnobotanical leaflets*, (1), 22.
105. Savithramma, N., Ankanna, S., & Bhumi, G. (2012). Effect of nanoparticles on seed germination and seedling growth of *Boswellia ovalifoliolata* an endemic and endangered medicinal tree taxon. *Nano Vision*, 2(1), 2.
106. Yin, L., Colman, B. P., McGill, B. M., Wright, J. P., & Bernhardt, E. S. (2012). Effects of silver nanoparticle exposure on germination and early growth of eleven wetland plants. *PLoS One*, 7(10), e47674.
107. Babu, R., & Murugan, K. (1998). Interactive effect of neem seed kernel and neem gum extracts on the control of *Culex quinquefasciatus* Say. *Neem Newslett*, 15(2), 9-11.
108. Ngoy, J. M., Iyuke, S. E., Neuse, W. E., & Yah, C. S. (2011). Covalent functionalization for multi-walled carbon nanotube (f-MWCNT)-folic acid bound bioconjugate. *Journal of Applied Sciences*, 11(15), 2700-2711.
109. Pan, Y., Leifert, A., Ruau, D., Neuss, S., Bornemann, J., Schmid, G., ... & Jahnen-Dechent, W. (2009). Gold nanoparticles of diameter 1.4 nm trigger necrosis by oxidative stress and mitochondrial damage. *Small*, 5(18), 2067-2076.
110. Yang, R. Z., & Tang, C. S. (1988). Plants used for pest control in China: a literature review. *Economic botany*, 42(3), 376-406.
111. Pascual-Villalobos, M. J., & Robledo, A. (1998). Screening for anti-insect activity in Mediterranean plants. *Industrial crops and products*, 8(3), 183-194.
112. Reed, D. K., Jacobson, M., Warthen Jr, J. D., Uebel, E. C., Tromley, N. J., Jurd, L., & Freedman, B. (1981). Cucumber beetle antifeedants: laboratory screening of natural products [*Diabrotica undecimpunctata howardi*, *Acalymma vittata*, *Erwinia tracheiphila*]. *Technical Bulletin-US Dept. of Agriculture (USA)*. no. 1641.

113. Tsao, R., Romanchuk, F. E., Peterson, C. J., & Coats, J. R. (2002). Plant growth regulatory effect and insecticidal activity of the extracts of the Tree of Heaven (*Ailanthus altissima* L.). *BMC ecology*, 2(1), 1.
114. Ngoy, J. M., Iyuke, S. E., Neuse, W. E., & Yah, C. S. (2011). Covalent functionalization for multi-walled carbon nanotube (f-MWCNT)-folic acid bound bioconjugate. *Journal of Applied Sciences*, 11(15), 2700-2711.
115. Pan, Y., Leifert, A., Ruau, D., Neuss, S., Bornemann, J., Schmid, G., ... & Jahnhen-Dechent, W. (2009). Gold nanoparticles of diameter 1.4 nm trigger necrosis by oxidative stress and mitochondrial damage. *Small*, 5(18), 2067-2076.
116. Boué, S. M., & Raina, A. K. (2003) Effects of plant flavonoids on fecundity, survival, and feeding of the Formosan subterranean termite. *Journal of chemical ecology*, 29(11), 2575-2584.
117. Ohmura, W., Doi, S., Aoyama, M., & Ohara, S. (2000). Antifeedant activity of flavonoids and related compounds against the subterranean termite *Coptotermes formosanus* Shiraki. *Journal of wood science*, 46(2), 149-153.
118. Kohata, K., Yamauchi, Y., Ujihara, T., & HORIE, H. (2004). Growth inhibitory activity of tea-seed saponins and glyphosate to weed seedlings. *Japan Agricultural Research Quarterly: JARQ*, 38(4), 267-270.
119. Kapusta, I., Janda, B., Stochmal, A., & Oleszek, W. (2005). Determination of saponins in aerial parts of barrel medic (*Medicago truncatula*) by liquid chromatography-electrospray ionization/mass spectrometry. *Journal of agricultural and food chemistry*, 53(20), 7654-7660.
120. Meyer J.R. (2005). Isoptera. Department of Entomology. NC State University. Online at. <http://www.cals.ncsu.edu/course/ent425/compendium/termites.html>.

121. Verma, M., Sharma, S., & Prasad, R. (2009). Biological alternatives for termite control: a review. *International Biodeterioration & Biodegradation*, 63(8), 959-972.
122. Myles, T. G. (2005). Termite biology. *Urban Entomology Programme*, online at <http://www.utoronto.ca/forest/termite/termite.htm>.
123. Ravi, S., Breshears, D. D., Huxman, T. E., & D'Odorico, P. (2010). Land degradation in drylands: Interactions among hydrologic–aeolian erosion and vegetation dynamics. *Geomorphology*, 116(3), 236-245.
124. Kolawole, O. M. (2006). The effect of *Bridelia ferruginea* and *Senna alata* on plasma glucose concentration in normoglycemic and glucose induced hyperglycemic rats. *Ethnobotanical leaflets*, 2006(1), 22.
125. Rauf, A., Uddin, G., & Khan, H. (2014). Preliminary antioxidant profile of *Pistacia integerrima* Stewart. *Pakistan journal of pharmaceutical sciences*, 27(4).
126. Usman, H., Abdulrahman, F. I., & Usman, A. (2009). Qualitative phytochemical screening and in vitro antimicrobial effects of methanol stem bark extract of *Ficus thonningii* (Moraceae). *African Journal of Traditional, Complementary and Alternative Medicines*, 6(3).
127. Ahmad, B., Ali, N., Bashir, S., Choudhary, M. I., Azam, S., & Khan, I. (2009). Parasitocidal, antifungal and antibacterial activities of *Onosma griffithii* Vatke. *African Journal of Biotechnology*, 8(19).
128. Prabhu, N., Raj, D. T., Yamuna, G. K., Ayisha, S. S., Puspha, J., & Innocent, D. (2010). SYNTHESIS OF SILVER PHYTO NANOPARTICLES AND THEIR ANTIBACTERIAL EFFICACY. *Digest Journal of Nanomaterials & Biostructures (DJNB)*, 5(1).
129. Salihah, Z., Khatoon, R., Khan, A., & Alamzeb, S. A. (1993). A termite trap, NIFA Termap for capturing large number of field population of *Heterotermes indicola*. *Proc. Pakistan Congr. Zool*, 13, 395-400.

130. Ahmad, B., Azam, S., Bashir, S., Khan, I., Adhikari, A., & Choudhary, M. I. (2010). Anti-inflammatory and enzyme inhibitory activities of a crude extract and a pterocarpan isolated from the aerial parts of *Vitex agnus-castus*. *Biotechnology journal*, 5(11), 1207-1215.
131. Prabhu, S., & Poulose, E. K. (2012). Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. *International Nano Letters*, 2(1), 1-10.
132. Van Hyning, D. L., Klemperer, W. G., & Zukoski, C. F. (2001). Silver nanoparticle formation: predictions and verification of the aggregative growth model. *Langmuir*, 17(11), 3128-3135.
133. Ahmad, B., Ali, J., & Bashir, S. (2013). Optimization and effects of different reaction conditions for the bioinspired synthesis of silver nanoparticles using *Hippophae rhamnoides* linn. leaves aqueous extract. *World Applied Sciences Journal*, 22(6), 836-843.
134. Sathishkumar, M., Sneha, K., & Yun, Y. S. (2010). Immobilization of silver nanoparticles synthesized using *Curcuma longa* tuber powder and extract on cotton cloth for bactericidal activity. *Bioresource technology*, 101(20), 7958-7965.
135. Singh, C., Sharma, V., Naik, P. K., KHandelwal, V., & Singh, H. (2011). A green biogenic approach for synthesis of gold and silver nanoparticles using *Zingiber officinale*. *Digest Journal of Nanomaterials and Biostructures*, 6(2), 535-542.
136. Veerasamy, R., Xin, T. Z., Gunasagaran, S., Xiang, T. F. W., Yang, E. F. C., Jeyakumar, N., & Dhanaraj, S. A. (2011). Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. *Journal of Saudi Chemical Society*, 15(2), 113-120.
137. Ghosh, S., Patil, S., Ahire, M., Kitture, R., Jabgunde, A., Kale, S., ... & Chopade, B. A. (2011). Synthesis of gold nanoanisotropes using *Dioscorea bulbifera* tuber extract. *Journal of Nanomaterials*, 2011, 45.

138. Jia, Z., Yue, L., Shengs, Y., & Xu, Z. (2008). Rod-like zinc oxide constructed by nanoparticles: synthesis, characterization and optical properties. *Materials Chemistry and Physics*, 107(1), 137-141.
139. Geissman, T. A., (1963). Flavonoid compounds, tannins, lignins and related compounds. in pyrrole pigments, isoprenoid compounds and phenolic plant constituents; Florkin, M., Stotz, E.H., Eds.; Elsevier: New York, NY, USA. 172- 190.
140. Sadauskas, E., Jacobsen, N. R., Danscher, G., Stoltenberg, M., Vogel, U., Larsen, A., ... & Wallin, H. (2009). Biodistribution of gold nanoparticles in mouse lung following intratracheal instillation. *Chemistry Central Journal*, 3(1), 1.
141. Ashe, B. (2011). A Detail investigation to observe the effect of zinc oxide and Silver nanoparticles in biological system. *National Institute of Technology. M. Sc.(Roll NO-607bm004)*.
142. Theivasanthi, T., & Alagar, M. (2010). X-ray diffraction studies of copper nanopowder. *arXiv preprint arXiv:1003.6068*.
143. Przybyszewska, M., & Zaborski, M. (2009). The effect of zinc oxide nanoparticle morphology on activity in crosslinking of carboxylated nitrile elastomer. *Express Polym. Lett*, 3, 542-552.
144. Romero-Romero, T., Anaya, A. L., & Cruz-Ortega, R. (2002). Screening for effects of phytochemical variability on cytoplasmic protein synthesis pattern of crop plants. *Journal of chemical ecology*, 28(3), 617-629.
145. Waterman, P. G., & Mole, S. (1994). *Analysis of phenolic plant metabolites*. Blackwell Scientific.
146. Sharma, N. C., Sahi, S. V., Nath, S., Parsons, J. G., Gardea-Torresde, J. L., & Pal, T. (2007). Synthesis of plant-mediated gold nanoparticles and catalytic role of biomatrix-embedded nanomaterials. *Environmental science & technology*, 41(14), 5137-5142.

147. Angrasan, J. K. V. M., & Subbaiya, R. (2014). Biosynthesis of copper nanoparticles by *Vitis vinifera* leaf aqueous extract and its antibacterial activity. *Int J Curr Microbiol Appl Sci*, 3(9), 768-774.
148. Balasubramanian, S. K., Poh, K. W., Ong, C. N., Kreyling, W. G., Ong, W. Y., & Liya, E. Y. (2013). The effect of primary particle size on biodistribution of inhaled gold nano-agglomerates. *Biomaterials*, 34(22), 5439-5452.
149. Saranyaadevi, K., Subha, V., Ernest Ravindran, R. S., & Renganathan, S. (2014). Synthesis and characterization of copper nanoparticle using *Capparis zeylanica* leaf extract. *Int J ChemTech Res*, 6(10), 4533-4541.
150. Kulkarni, V. D., & Kulkarni, P. S. (2013). Green synthesis of copper nanoparticles using *Ocimum sanctum* leaf extract. *Int J Chem Stud*, 1(3), 1-4.
151. Morita, H., Miyata, K., Mamitsuka, K., Fukuda, T., Noguchi, K., Kiyofuji, J., & Yamamoto, S. (2007). Antibiotic effects of *Eriobotrya japonica* seed extract. *Journal of Antibacterial and Antifungal Agents (Japan)*.
152. Jiang, W., Mashayekhi, H., & Xing, B. (2009). Bacterial toxicity comparison between nano-and micro-scaled oxide particles. *Environmental pollution*, 157(5), 1619-1625.
153. Jin, T., Sun, D., Su, J. Y., Zhang, H., & Sue, H. J. (2009). Antimicrobial efficacy of zinc oxide quantum dots against *Listeria monocytogenes*, *Salmonella Enteritidis*, and *Escherichia coli* O157: H7. *Journal of food science*, 74(1), M46-M52.
154. Brayner, R., Ferrari-Iliou, R., Brivois, N., Djediat, S., Benedetti, M. F., & Fiévet, F. (2006). Toxicological impact studies based on *Escherichia coli* bacteria in ultrafine ZnO nanoparticles colloidal medium. *Nano Letters*, 6(4), 866-870.
155. Chaloupka, K., Malam, Y., & Seifalian, A. M. (2010). Nanosilver as a new generation of nanoparticle in biomedical applications. *Trends in biotechnology*, 28(11), 580-588.

156. Mahesh, B., & Satish, S. (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World journal of agricultural sciences*, 4(5), 839-843.
157. Rashid, K. I., Mahdi, N. M., Alwan, M. A., & Khalid, L. B. (2014). Antimicrobial activity of fig (*Ficus carica* Linn.) leaf extract as compared with latex extract against selected bacteria and fungi. *Journal of Babylon University/Pure and Applied Sciences*, 22(5), 1620.
158. Jeong, M. R., Kim, H. Y., & Cha, J. D. (2009). Antimicrobial activity of methanol extract from *Ficus carica* leaves against oral bacteria. *Journal of Bacteriology and Virology*, 39(2), 97-102.
159. Jiang, W., Mashayekhi, H., & Xing, B. (2009). Bacterial toxicity comparison between nano-and micro-scaled oxide particles. *Environmental pollution*, 157(5), 1619-1625.
160. Ren, G., Hu, D., Cheng, E. W., Vargas-Reus, M. A., Reip, P., & Allaker, R. P. (2009). Characterisation of copper oxide nanoparticles for antimicrobial applications. *International journal of antimicrobial agents*, 33(6), 587-590.
161. Cioffi, N., Torsi, L., Ditaranto, N., Tantillo, G., Ghibelli, L., Sabbatini, L., ... & Traversa, E. (2005). Copper nanoparticle/polymer composites with antifungal and bacteriostatic properties. *Chemistry of Materials*, 17(21), 5255-5262.
162. Ruparelia, J. P., Chatterjee, A. K., Duttagupta, S. P., & Mukherji, S. (2008). Strain specificity in antimicrobial activity of silver and copper nanoparticles. *Acta biomaterialia*, 4(3), 707-716.
163. Dellavalle, P. D., Cabrera, A., Alem, D., Larrañaga, P., Ferreira, F., & Dalla Rizza, M. (2011). Antifungal activity of medicinal plant extracts against phytopathogenic fungus *Alternaria* spp. *Chilean Journal of Agricultural Research*, 71(2), 231.

164. He, L., Liu, Y., Mustapha, A., & Lin, M. (2011). Antifungal activity of zinc oxide nanoparticles against *Botrytis cinerea* and *Penicillium expansum*. *Microbiological research*, 166(3), 207-215.
165. Sara A George, M Shailaja Raj, Diana Solomon, and Roselin P. (2014) A Comparative Study of the Anti-Fungal Activity of Zinc Oxide and Titanium Dioxide Nano and Bulk Particles" Research And Reviews: Journal Of Microbiology And Biotechnology.3(3), 23-30.
166. Nasar Ali, Pushpendra Kumar Vishwakarma, Ikhan, M Sohaib (2014). *In Vitro* Antibacterial, Antifungal and Phytotoxic Activities of *Ficus Carica* Methanolic Leaves Extracts. *Int.J.Curr.Biotechnol* 2; (2.1-12).
167. Aref, H. L., Salah, K. B., Chaumont, J. P., Fekih, A., Aouni, M., & Said, K. (2010). In vitro antimicrobial activity of four *Ficus carica* latex fractions against resistant human pathogens (antimicrobial activity of *Ficus carica* latex). *Pak J Pharm Sci*, 23(1), 53-58.
168. Kamat, P. V., & Meisel, D. (2003). Nanoscience opportunities in environmental remediation. *Comptes Rendus Chimie*, 6(8), 999-1007.
169. Ingle, A. P., Duran, N., & Rai, M. (2014). Bioactivity, mechanism of action, and cytotoxicity of copper-based nanoparticles: a review. *Applied microbiology and biotechnology*, 98(3), 1001-1009.
170. Roco, M. C., & Bainbridge, W. S. (2003). *Converging technologies for improving human performance: Nanotechnology, biotechnology, information technology and cognitive science*. Kluwer Academic Publishers.
171. Ramyadevi, J., Jeyasubramanian, K., Marikani, A., Rajakumar, G., & Rahuman, A. A. (2012). Synthesis and antimicrobial activity of copper nanoparticles. *Materials Letters*, 71, 114-116.
172. Einhellig, F. A., Leather, G. R., & Hobbs, L. L. (1985). Use of *Lemna minor* L. as a bioassay in allelopathy. *Journal of chemical Ecology*, 11(1), 65-72.

173. Ahmad, B., Khan, I., Bashir, S., Azam, S., & Hussain, F. (2011). Screening of Zizyphus jujuba for antibacterial, phytotoxic and haemagglutination activities. *African Journal of Biotechnology*, 10(13), 2514-2519.
174. Hameed, I., Hussain, F., Sher, Z., Bacha, N., & Khan, A. A. (2013). Cytotoxicity and phytotoxicity of some selected medicinal plants of family Solanaceae. *Pak. J. Bot*, 45(5), 1749-1754.
175. Al-Samarrai, A. (2012). Nanoparticles as alternative to pesticides in management plant diseases. *International Journal of Scientific and Research Publications*, 2(4), 1-4.
176. Sultana, N., Akhtar, M., Ferheen, S., Bina, R. S., & Ahmed, G. (2014). Effect of different concentrations of Eriobotrya japonica extract on control of infection by Meloidogyne incognita and Cephalobus litoralis. *Journal of Entomology and Nematology*, 6(2), 27-31.
177. Lü, J., Guo, X., & Zhang, L. (2012). Bioactivity of two extracts from Alpinia officinarum rhizome against Tribolium castaneum (Herbst) adults. *African Journal of Biotechnology*, 11(16), 3788-3792.
178. Dastagir, G., & Hussain, F. (2013). Phytotoxic and insecticidal activity of plants of family Zygophyllaceae and Euphorbiaceae. *Sarhad J. Agric*, 29(1), 83-91.
179. Rahman, M. F., Karim, M. R., Alam, M. J., Islam, M. F., Habib, M. R., Uddin, M. B., & Hossain, M. T. (2011). Insecticidal effect of oyster mushroom (Pleurotus ostreatus) against Tribolium castaneum (Herbst). *NPALJ*, 7(4), 187-190.
180. Boué, S. M., & Raina, A. K. (2003). Effects of plant flavonoids on fecundity, survival, and feeding of the Formosan subterranean termite. *Journal of chemical ecology*, 29(11), 2575-2584.

181. Hutchins, R. A. (1997). Evaluation of the natural antitermitic properties of *Aleurites fordii* (tung tree) extracts. *J. of the Mississippi Academy of Sci*, 42(3), 165-172.
182. Kaakeh, W. (2005). Survival and feeding responses of *Anacanthotermes ochraceus* (Hodotermitidae: Isoptera) to local and imported wood. *Journal of economic entomology*, 98(6), 2137-2142.