Comparative Study of Silver and Zinc Oxide Nanoparticles Synthesized from Ethanolic Extract of Leaves of *Boerhavia diffusa* for their *in vitro* Antidiabetic and Antibacterial Activities

K.Govarthini

(17PBC005)

Thesis submitted to

Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore - 641 043

In Partial Fulfilment of the Requirement for the Degree of

Master of Science in Biochemistry

April, 2019

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Signature of the Head of the Department

Signature of the Supervisor

Acknowledgement

ACKNOWLEDGEMENT

"GRATITUDE IS THE MEMORY OF HEART"

The author owes her special tribute to **God Almighty** for the opportunity given to take up to complete her work successfully. In addition to the will of supreme divinity, the willingness of many subject experts and erudite scholars to extend their assistance and help for the completion of a work plays, indeed a vitally important role.

The author express her deep sense of gratitude to all Higher authorities of Avinashilingam Institute for Home Science and Higher Education for Women for their immense support.

The author expressing her sincere thanks to **Dr**. (**Thiru) P.R.Krishna Kumar**, Chancellor, Avinashilingam Institute for Home Science and Higher Education for women, Coimbatore, for providing the opportunity and infrastructure to undertake this investigation.

The author immensely thank **Dr. Premavathy Vijayan**, Vice Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for providing the entire facilities essential to carry out and complete the study.

The author record her sincere thanks to **Dr. S. Kowsalya**, Registrar, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for help rendered to carry out the work.

The author express her deep sense of gratitude to **Dr. K. Manimozhi**, Controller of Examinations, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for her sincere advice and suggestions given during my project work.

The author express her reverential thanks to **Dr. G.P. Jeyanthi**, Director, Research and Consultancy, AvinashilingamInstitute for Home Science and Higher Education for Women, Coimbatore, for the support and encouragement rendered towards the completion of my thesis work.

The author records her sincere thanks to **Dr. P.R. Padma**, Dean and Head, Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for providing the opportunity and timely help rendered to carry out the work successfully.

The author express her special gratitude to **Dr. S. Annapurani,** former Professor and Head, Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore and her sustained support throughout the study.

The author owe her indebtedness, profound and deepest thanks to her guide **Dr. S.Gayathri Devi,** Professor, Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for her incessant guidance, immense tolerance, good support, thoughtful advice, steady encouragement throughout the research and motivation right from the selection of topic to the completion of the work efficiently and effectively.

The author submit her sincere thanks to all **The Staff Members** of the department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for lending a helping hand and invaluable guidance during the course of this thesis work.

The author place her gratitude to foot of her parents and her brother for their immense support and guidance during the course of her study.

The author expresses her sincere heart bound thanks to her Friends, Department of Biochemistry, Biotechnology and Bioinformatics, for their affectionate advice, unconditional love and incredible supports for the completion of her project work.

The author acknowledge the contribution of all other unseen hands during the course of the study for help rendered in the successful completion of the study.

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Introduction

1.0 INTRODUCTION

Nanoscience and nanotechnology designate a rapidly expanding research area. The main goal of the nanoscience cover design, preparation, manipulation and application of materials and structures having at least one dimension within the interval from 1 to 100 nanometers (nm). (Luby *et al.,* 2015). Nanomedicine is that the preservation and improvement of human health using molecular tools and molecular knowledge of the human body. Nanomedicine can have extraordinary and comprehensive implications for the medical profession, for the definition of disease, for the diagnosis and treatment of medical conditions including aging and ultimately for the improvement and extension of natural human biological structure and function (Abeer, 2012). Nanomedicine is the integration of nanotechnology in medicine for the better human health care. It utilizes components as tiny as $1/80{,}000^{th}$ of the diameter of a human hair. At the scale of 1 nanometer (or 10 times the diameter of a hydrogen atom), materials and devices can interact with cells and biological molecules in a unique way (Woldu and Lenjisa, 2014).

Nanotechnologies are modern areas of research focusing on affecting matter at the atomic and molecular levels. It is absolutely that trendy medicine will benefit greatly from it therefore nanomedicine has become one in all the most branches of nanotechnological research. Currently it focuses on developing new methods of preventing, diagnosing and treating various diseases. Nanoscience may also be a resource of the needed development in the fight against atherosclerosis, since nanostructures may be used in both preventing and increasing the stability of atherosclerotic lesions. Other potential applications of nanotechnology in medicine include nanoadjuvants with immunomodulatory properties used to deliver vaccine antigens (Zdrojewicz *et al.,* 2015). The applications of nanomaterials to biology or medication are fluorescent biological labels, drug and gene delivery, biodetection of pathogens, detection of proteins, probing of DNA structure, tissue engineering, tumour destruction via heating (hyperthermia), separation and purification of biological molecules and cells, MRI contrast enhancement and phagokinetic studies (Salata, 2004).

Nanotechnology in diabetes research has facilitated the development of novel glucose measurement and insulin delivery modalities which hold the potential to dramatically improve quality of life for diabetics (Di Santo *et al.,* 2015). Nanotechnology holds a great deal of promise in diabetes management for efficient drug delivery, generation of smart drugs which only

activate when needed. Anti-diabetes therapy is significantly transformed by using nanotechnology in many ways such as in delivery of therapeutic molecules, fabrication of nanosensors to monitor dynamic changes in blood glucose level and development of smart imaging molecule to visualize and quantify beta cells (Mukhopadhyay and Mondal, 2018).

The nanotechnology and biomedical sciences unlocked the possibility for a wide variety of biological research field and medical uses at the molecular and cellular level. The biosynthesis of nanoparticles has been proposed as an inexpensive and environmentally friendly substitute to chemical and physical methods. Plant-mediated synthesis of nanoparticles is a green chemistry method which connects nanotechnology with plants. Plants are nature's "chemical factories". They are low price and require low maintenance (Parveen *et al.,* 2016). In the plant mediated green chemistry method, the reduction rate of metal salts is very quick and the procedure itself requires no specific conditions unlike the physical and chemical methods. Beyond, this biogenic approach of nanoparticles synthesis seems to be reproducible and the particles, produced through this environmentally friendly approach are found extremely stable (Chintamani *et al.,* 2018).

Silver nanoparticles account for greater than 23 per cent of all nanoproducts and have been extensively used for diagnostic and therapeutic applications such as wound healing, arthritic disease. Silver nanoparticles have been extensively known for their antibacterial, antifungal and antiviral effects (Midha *et al.,* 2016). Green synthesis of silver nanoparticles (AgNPs) have proven to be good resources of agents effective in the treatment of diabetes mellitus (Prabhu *et al.,* 2018). The green synthesis of AgNPs could be a promising alternative to physicochemical methods, which limit the use of hazardous and toxic substances and are energy consuming. Nowadays, literature on the synthesis of AgNPs using plant extracts is receiving attention, because they act as both capping and stabilizing agents. Furthermore, it involves the reduction of silver ions (Ag^{+}) to silver atoms (Ag^{0}), resulting in colloidal nanoparticles. Bioactivity screening is a predominant part of the progress of new drugs for diabetes and several *in vivo* biological models are now available for this purpose (Campoy *et al.,* 2018).

Biocompatibility and stability of zinc oxide nanoparticles (ZnO NPs) synthesized using green approach is an attractive research area of study in nanotechnology, attributed to its extensively applications in biomedical, industrial, cell imaging, and biosensor fields. Zinc oxide nanoparticles (ZnO NPs), which are known to be nontoxic, chemically stable, biocompatible and

might be used as drug carriers, cell imaging agents, anticancer agents, antimicrobials, biosensors, antidiabetics and cosmetics because of their new physicochemical properties (Umar *et al.,* 2019). Zinc is a trace element and profusely found mineral in all human tissues and tissue fluids. Zinc is well known to keep the structural integrity of insulin and has a vigorous role within the secretion of insulin from pancreatic cells. It additionally participates in insulin synthesis, storage, and secretion (Jiang and Cai, 2018).

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Kharroubi and Darwish, 2015). Globally, diabetic foot ulcers are one of the crucial public health problems leading to socioeconomic burden to the suffering individuals. Around percent of all diabetic patients develop a foot ulcer which is highly unsafe to infections, at some time in their life.Foot ulcer infections usually expand rapidly on account of polymicrobial growth, predominantly consisting of aerobic, gram-positive and gram-negative organisms. In recent years, the number of the incidents and complications-related to diabetic foot infections (DFIs) has drastically increased because of increased incidence of multidrug-resistant organisms (Sekhar *et al.,* 2014).

One of the most vital applications of the metal nanoparticles, especially silver nanoparticles, in the field of medicine is using these nanoparticles as antimicrobial agents. The lethal activity of nanoparticles against broad spectrum of Gram-positive bacteria, Gram-negative bacteria and fungi has been approved (Soliman *et al.,* 2018). Antibacterial activity of zinc oxide nanoparticles (ZnO-NPs) has received consequential interest worldwide particularly by the implementation of nanotechnology to synthesize particles within the nanometer range. Many microorganisms exist within the vary from hundreds of nanometers to tens of micrometers. Zinc oxide nanoparticles exhibit attractive antibacterial properties due to increased specific surface area as the reduced particle size resulting in increased particle surface reactivity. Zinc oxide nanoparticles could be bio-safe material that possesses photo-oxidizing and photocatalysis impacts on chemical and biological species (Sirelkhatim *et al.,* 2015).

Boerharvia diffusa is a native plant of India belonging Nyctaginaceae family. *Boerharvia diffusa* in the Indian system of medicine, is a perennial herb found throughout the waste land of India. It has many ethnobotanical uses (the leaves are used as vegetable, the root juice is used to cure asthma, urinary disorders, leukorrhoea, rheumatism and encephalitis) and is medicinally

used in the traditional, ayurvedic system in India and Unani medicine in Arab countries (Vasundhara and Devi 2014). It is beneficial in treating obesity. Other advantage includes treatment of anemia, nervous weakness, paralysis, constipation and cough. Studies have revealed that punarnava is potent diuretic, anti-inflammatory, mild laxative and is a heart tonic. Punarnava could be used in treating obesity, improving appetite, jaundice and general fever (Bhowmik *et al.*, 2012).

With this background of information, the present study "Comparative Study of Silver and Zinc Oxide Nanoparticles Synthesized from Ethanolic Extract of Leaves of *Boerhavia diffusa* for their *in vitro* Antidiabetic and Antibacterial activities" was undertaken with the following objectives

- To synthesis silver and zinc oxide nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*
- To characterize the synthesized silver and zinc oxide nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*
- To determine the *in vitro* antidiabetic activity of silver and zinc oxide nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*
- To determine the antibacterial potential of the silver and zinc oxide nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*

REVIEW OF LITERATURE

2.0 REVIEW OF LITERATURE

Nanotechnology is defined as the understanding and control of matter at dimensions between 1 and 100 nm where unique phenomena enable novel applications. Although human exposure to nanoparticles has occurred throughout human history, it dramatically increased during the industrial revolution (Hulla, 2015).

The application of emerging nanotechnology to the practice of medicine represents a frontier of nanomedicine. Nanomedicine has been outlined as a science that emphasizes the employment of nanoscale tools in conjunction with background of the frame for diagnosing and treatment. By in operation at molecular, intracellular and intercellular levels, nanomedicine offers promising improvements in diagnostic utilities, preventive medicine, targeted pharmacotherapy, tissue regeneration. Although for the most part theoretical now a days, such enhancement have vital implications for clinical practice of emergency medicine (Pourmand *et al.*, 2012).

There is no longevity of life without plants. Plants are the essential base of medicine. Some important drugs that are still in use today are derived from traditional medicinal plants (Aslam and Ahmad, 2016). The use of medicinal plants not only for the treatment of diseases but also play an important role for maintaining good health (Oladeji, 2016**).** The herbal products are the mark of safety in compare to the synthetic drugs. Treatment with herbs is taken into account terribly safe as there is no or bottom facet effects. Herbal plants are thought-about as fashionable resources of ingredients which may be utilized in drug development either pharmacopoeia, non-pharmacopoeia or synthetic drugs. Apart from that, these plants play an essential role in the development of human cultures around the whole world. Moreover, some plants are considered as principle source of nutrition and as a result of that they are recommended for their therapeutic values (Khan, 2016).

The review of literature pertaining to the research entitled “Comparative Study of Silver and of Zinc Oxide Nanoparticles Synthesized from Ethanolic Extract of Leaves of *Boerhavia diffusa* for their *in vitro* Antidiabetic and Antibacterial Activities” is pertinently presented below the subsequent heading

2.1 NANOTECHNOLOGY

Nanoscale is commonly deal with to be at a size below 0.1 µm or 100 nm. Nanoscale science or nanoscience studies the phenomena, properties and responses of materials at atomic, molecular and macromolecular scales and normally at sizes between 1-100 nm. In this scale and particularly below 5 nm, the properties of matter differ significantly (that is quantum scale effects play a vital role) from that at a larger particulate scale. Nanotechnology is then the layout, the manipulation, the building, the production and application, by regulating the shape and size, the properties responses and functionality of structures, devices and systems of the order or less than 100 nm (Logothetidis, 2012).

Biomedical nanotechnology introduces revolutionary opportunities in fight against many diseases. An area with near term potential is identifying molecules associated with diseases such as cancer, diabetes mellitus and neurodegenerative diseases. Adding to these, nanotechnology is useful in identifying microorganisms and viruses associated with infections, such as pathogenic bacteria, fungi and human immunodeficiency virus (HIV) viruses. For example, within the field of cancer medical aid, promising innovative nanoparticles can reply to outwardly applied physical stimuli in ways that make them suitable therapeutics or therapeutic delivery systems (Kamal *et al.*, 2015).

Nanomedicine comprise usage of nanotechnology for the benefit of human health and well-being. The usage of nanotechnology in various region of therapeutics has revolutionized the field of medicine where nanoparticles of dimensions ranging between 1-100 nm are designed and

used for diagnostics, therapeutics and as biomedical tools for research. It is now probable to give therapy at a molecular level with the help of these tools, thus treating the disease and facilitating in study of the pathogenesis of disease (Surendiran *et al.,* 2009).

2.1.1 Nanoparticles

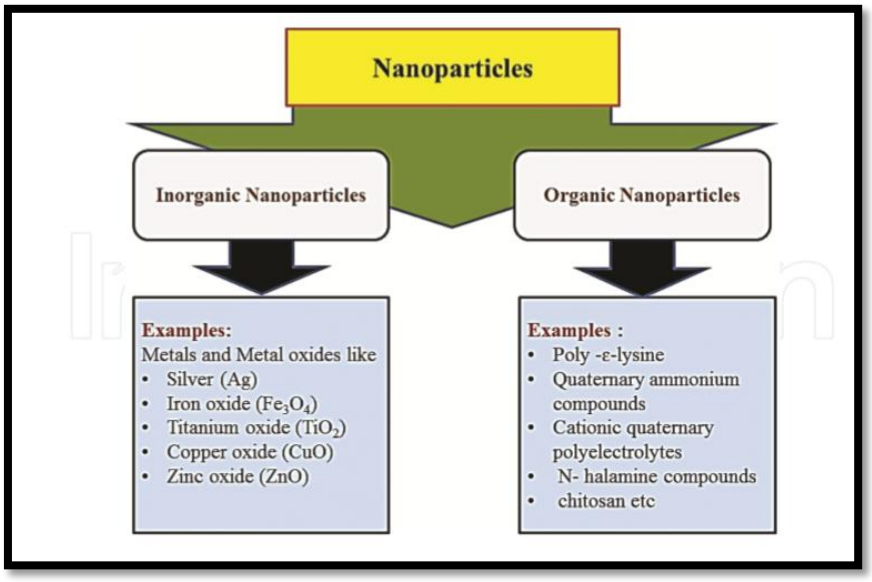
Nanoparticles are outlined as particulate dispersions or solid particles with a size varying in range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or connectedto a nanoparticle matrix. Depending upon the tactic of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are unit systems during which the drug is confined to a cavity encircled by a unique polymer membrane, whereas nanospheres are unit matrix systems during which the drug is physically and uniformly dispersed. In recent years, perishable polymeric nanoparticles, notably those coated with hydrophilic compounds like poly ethylene glycol (PEG) called long-circulating particles, are used as potential drug delivery devices because of their ability to circulate for a prolonged period time target a selected organ, as carriers of DNA in gene therapy and their ability to deliver proteins, peptides and genes. The major goals coming up with nanoparticles as a delivery system are to regulate particle size, surface properties and release of pharmacologically active agents so as to attain the site-specific action of the drug at the therapeutically optimum rate and dose regime (Mohanraj *et al.,* 2006).

2.1.2 Types of nanoparticles

There are two different kinds of nanoparticles, inorganic nanoparticles and organic nanoparticles. The inorganic nanoparticles are metal and metal oxides, which are very effective antibacterial agents. Metal oxide nanoparticles like silver, iron oxide, titanium oxide, copper oxide and zinc oxide are certain examples of inorganic nanoparticles. Organic nanoparticles like poly-Ɛ-lysine, quaternary ammonia compounds, cationic quaternary polyelectrolytes, N-halamine compounds and chitosan. Organic nanoparticles are typically less stable at high temperatures. Due this reason, inorganic nanoparticles are more preferred as antimicrobial polymers (Oscar *et al.,* 2016). Figure1 represents different type of nanoparticles.

FIGURE 1

DIFFERENT TYPES OF NANOPARTICLES



(Oscar *et al.*, 2016)

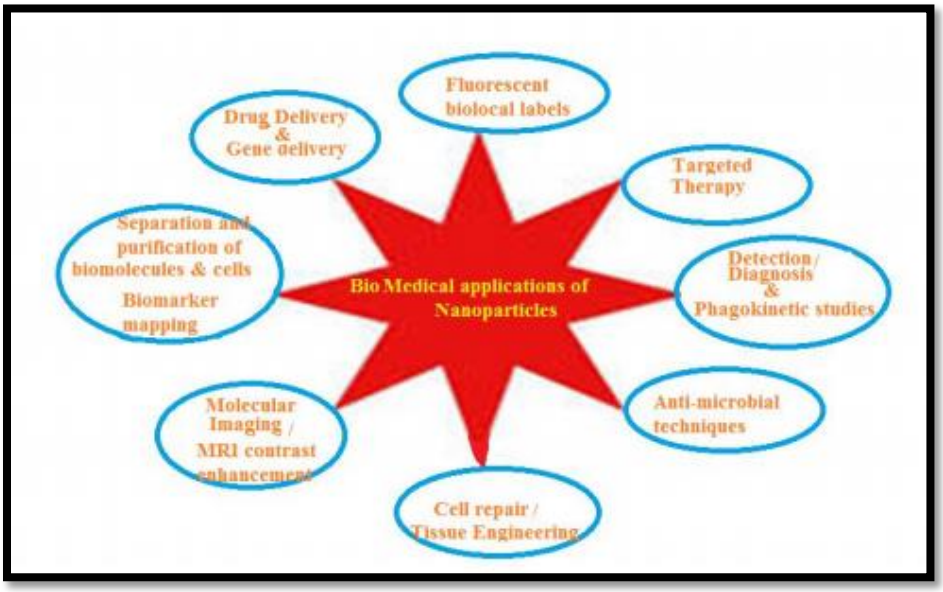
2.1.3 Application of Nanotechnology in medicine

Nanotechnology plays a vital role in the recent technological advances in the field of disease diagnosis, drug design and drug delivery. The nanotechnological applications to disease treatment, diagnosis, monitoring and to the regulate of biological systems that have been considered to as 'nanomedicine' (Moghimi *et al*., 2005). Research and development in different areas of nanomedicine is expected to revolutionize the disease diagnosis and treatment approaches in the near future. Nanosized distinction agents are anticipated to lead way to advancements in understanding biological processes at the molecular level. Nanomedical approaches to drug delivery focuses on developing nanoscale particles or molecules to enhance the bioavailability of a drug. Special attention has been given to bioassay applications such as biosensors, biomedical devices and biofuel cells using nanomaterials. Nanotechnology on a chip may be a new paradigm for total qualitative analysis systems. Nanorobotics and nanomanipulation technologies will ultimately allow moving and manipulating nanoscale materials and congregate them into nanosytems such as nanoscale robotics. The manipulation techniques can well be utilized in medicine for the investigation of structures and functioning

mechanisms of living things and their interactions at the molecular level (Saji *et al.*, 2010). Figure 2 represents biomedical applications of nanoparticles.

FIGURE 2

BIOMEDICAL APPLICATIONS OF NANOPARTICLES



(Lanka 2017)

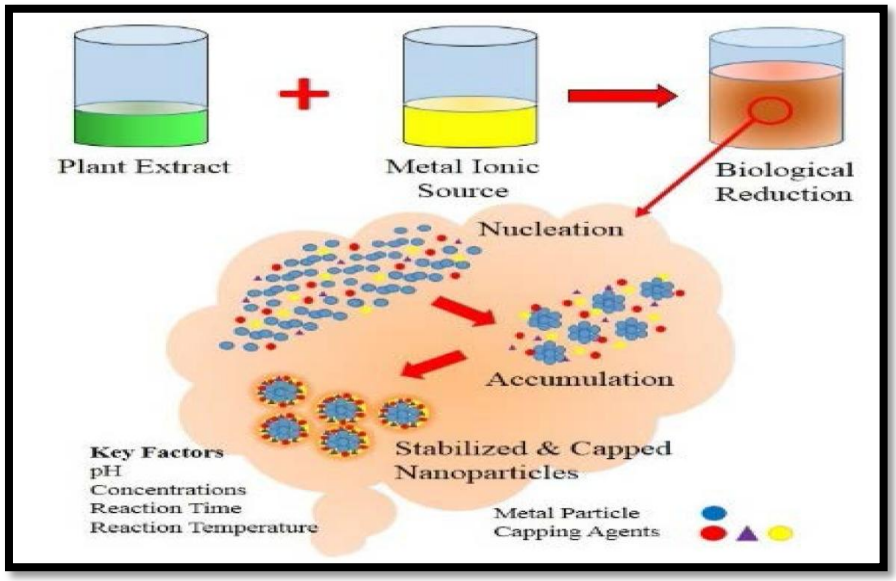
2.2 SYNTHESIS OF NANOPARTICLES

Nature has contrive numerous processes for the synthesis of nano and micro length scaled inorganic materials that have contributed within the growth of comparatively new and largely unfamiliar space of research based on the biosynthesis of the nanomaterials. Synthesis using bio-organisms is suitable with the green chemistry principles. “Green synthesis” of nanoparticles makes quality of environmental friendly, harmless and safe reagents. Nanoparticles synthesized with the help of biological techniques or green technology have various natures, with higher stability and proper dimensions since they are synthesized using a one-step procedure. Nanoparticles can also be synthesized using different kinds of methods including chemical, physical, biological and hybrid techniques (Parveen *et al.*, 2016).

Importantly, the synthesis of nanoparticles from reducing metal salts via plants is a comparatively straightforward room temperature process. The process begins by combining a sample of plant extract with a metal salt solution. Biochemical reduction of the salts starts

instantly and the formation of nanoparticles referred by a change in the colour of the reaction mixture. In the time of synthesis, there is primary activation period when process metal ions are converted from their mono or divalent oxidation states to zero-valent states and nucleation of the reduced metal atoms takes place. This is immediately followed by a period of growth when smaller neighboring particles merge to form larger nanoparticles that are thermodynamically more stable while further biological reduction of metal ions takes place. As growth progresses nanoparticles combine to form a variety of morphologies like cubes, spheres, triangles, hexagons, pentagons, rods and wire. In the final stage of synthesis, the plant extracts ability to stabilize the nanoparticle eventually determines it's most energetically favorable and stable morphology. Properties of the plant extract like, its concentration, metal salt concentration, reaction time, reaction solution, pH and temperature considerably influence the quality, size and morphology of the synthesized nanoparticles (Shah, 2015). Figure 3 illustrates biological synthesis of nanoparticles using plant extracts.

FIGURE 3
BIOLOGICAL SYNTHESIS OF NANOPARTICLES USING PLANT EXTRACTS



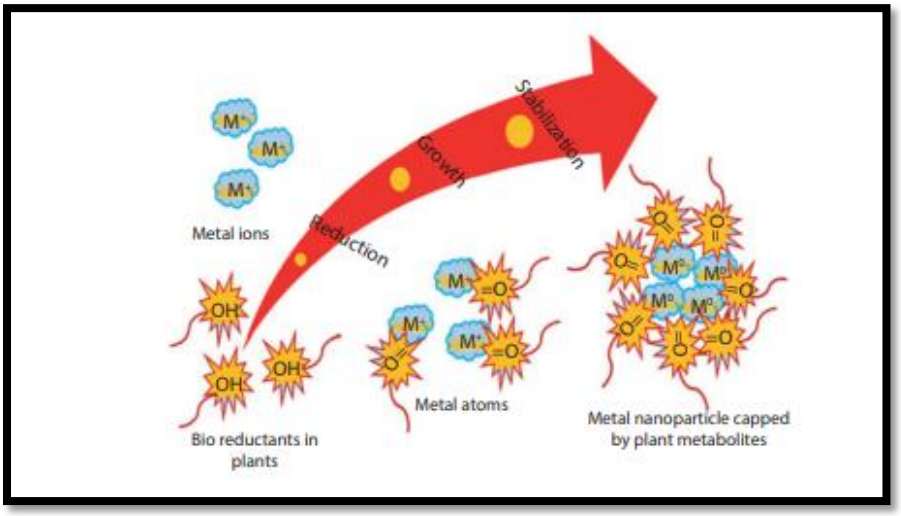
(Shah, 2015)

Metal nanoparticles obtained from plant extracts are prepared from living plant extracts. Plant parts, such as root, leaf, latex, seed and stem are broadly used for metal-based nanoparticle

synthesis. In addition, plant extracts comprise bioactive polyphenols, alkaloids, proteins, sugars, phenolic acids and terpenoids which are made up to have a vital role in initially reducing the metallic ions and then stabilizing them. The difference in conformation and concentration of these energetic biomolecules among many plants and their resulting collaboration with aqueous metal ions are thought to be one of the key factors associated with the diverse of nanoparticle sizes and shapes fabricated (Chokkareddy *et al.,* 2018). Figure 4 represents green synthesis mechanism.

FIGURE 4

MECHANISM OF GREEN SYNTHESIS



(Chokkareddy *et al.*, 2018)

2.2.1 Synthesis of silver nanoparticles

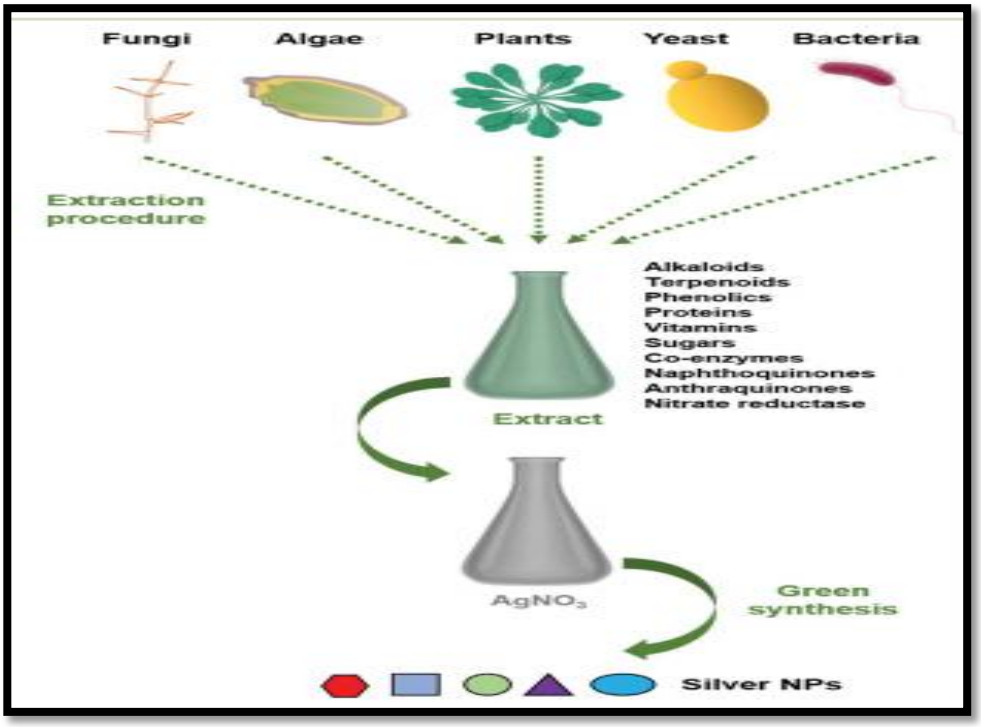
The use of plants as the production assembly of silver nanoparticles has drawn attention, because of its rapid, eco-friendly, non-toxic, economical protocol and affording a single step technique for the biosynthetic processes. The reduction and stabilization of silver ions by amalgamation of biomolecules like proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins tannins, phenolics, saponins, terpenoids and vitamins which are already established in the plant extracts having medicinal values and are environmental benign, yet chemically complex structures (Ahmed *et al.*, 2016).

Most commonly used biomolecules arise from plant extracts. Apart from the plant extract, biomolecules from microbial (bacteria, fungi, yeasts and actinomycetes), algal and

cyanobacterial inception have also been successfully used for the synthesis of nanoparticles. These biological molecules extracted from different origin are externally added to metal salts to make nanoparticles. To synthesize silver nanoparticles, biological extract in situ reduces silver salts (Ag^{+}) to metallic silver, Ag^{0}. In the method of nanoparticle synthesis in a green way, biological molecules not only reduce the metal salts but also cover the formed nanoparticles or acts as *in situ* reducing and capping agent. This capping is advantageous over as it acts as multifunctional way; (i) prevents the agglomeration of the nanoparticles, (ii) reduces the toxicity, and (iii) improves antimicrobial activity (Roy *et al.*, 2019). Figure 5 illustrate schematic representation of the procedure for green synthesis of silver nanoparticles using various biological entities.

FIGURE 5

SCHEMATIC REPRESENTATION OF THE PROCEDURE FOR GREEN SYNTHESIS OF SILVER NANOPARTICLES USING VARIOUS BIOLOGICAL ENTITIES



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(Roy *et al.*, 2019)

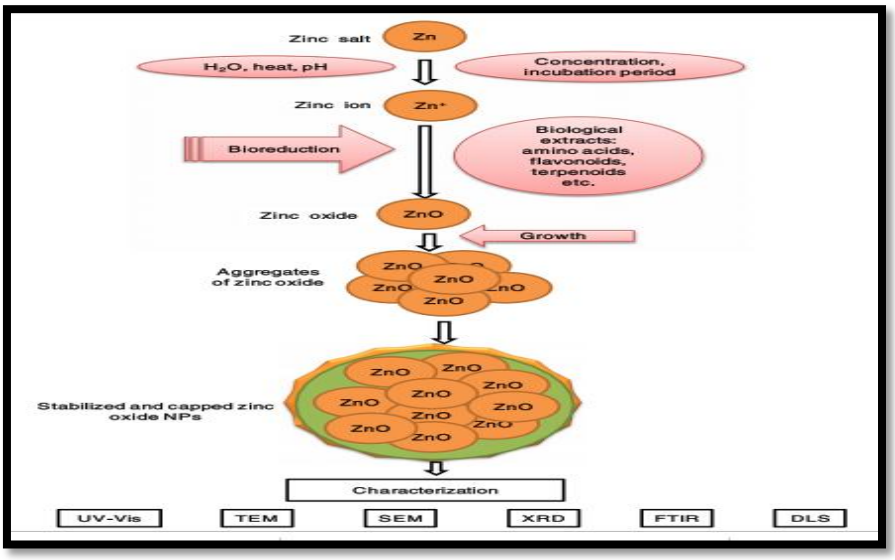
2.2.2. Synthesis of zinc oxide nanoparticles

Zinc oxide (ZnO) is a kind of inorganic metal oxides available and show a broad range of nanostructures. Photocatalytic and photooxidizing power against chemical and biological species are used to characterize these metal oxides. In expensive, UV blocking properties, greater catalytic activity, large surface area, white appearance and their remarkable applications in the area of medicine and agriculture are the advantages of ZnO particles. Recently, ZnO have been used broadly in environmental remediation and antibacterial activity (Parthasarathy *et al.,* 2016).

Biological synthesis of nanoparticles has benefit over conventional chemical and physical methods. In recently, ‘‘green’’ method for the synthesis of nanoparticles has become an area of utmost interest. The focus is applied in this direction because the use of conventional chemical methods is costly and requires the use of chemical compounds or organic solvents as reducing agents. Conventional chemical reduction method involves various toxic chemicals for the synthesis of nanoparticles which can later be a reason for diverse health issues due to their toxicity, while green synthesis method is an eco-friendly method to produce NPs. Green synthesis approach is free of contaminants thus benefit for biological applications where purity is a matter of concern. The use of toxic chemicals on the surface of nanoparticles and non-polar diluents in the chemical synthesis method constrain their applications in clinical and biological fields. Nanoparticles synthesized by plants are more stable and biocompatible due to the process of coating with biogenic surfactants or capping agents and they also have reduced environmental consequences, show variations in shape and size and have a faster rate of synthesis in comparison with the nanoparticles produced by other organisms. The faster rate of synthesis is owed to the biological capacity to act as a catalyst for reactions in aqueous mediate standard temperature and pressure conditions (Singh *et al.,* 2018). Figure 6 represents possible mechanism involved in biological synthesis of zinc oxide nanoparticles.

FIGURE 6

MECHANISM INVOLVED IN BIOLOGICAL SYNTHESIS OF ZINC OXIDE NANOPARTICLES



(Singh *et al.*, 2018)

2.3 CHARACTERIZATION OF SILVER AND ZINC OXIDE NANOPARTICLES

Characterization of nanoparticles is important to understand and regulate nanoparticle synthesis and application. Characterization is perform using variety of different techniques like transmission and scanning electron microscopy, atomic force microscopy, dynamic light scattering, X-ray photoelectron spectroscopy, powder X-ray diffractometry, Fourier transform infrared spectrometry and UV spectroscopy. These techniques are used for determination of different parameters like particle size, shape, crystallinity, fractal dimension, pore size and surface area. Moreover, orientation, intercalation and dispersion of nanoparticles and nanotubes in nanocomposite materials could be determined by these techniques (EI-Nour *et al.*, 2010).

2.3.1 UV-Visible absorbance spectroscopy

UV-visible spectroscopy is a very beneficial and trustworthy technique for the initial characterization of synthesized nanoparticles, which is also used to monitor the synthesis and stability nanoparticles. Nanoparticles have distinctive optical properties which make them

strongly interact with particular wavelengths of light. In addition, UV-visible spectroscopy is quick, easy, simple, sensitive and selective for different class of nanoparticles, needs only a short time for measurement and finally a calibration is not required for particle characterization of colloidal suspensions. In nanoparticles, the conduction band and valence band lie very close to each other in which electrons move freely. These free electrons increase surface plasmon resonance absorption band, occurring due to the converge oscillation of electrons of nano particles in resonance with the light wave (Zhang *et al.*, 2016).

It measures the intensity of light reflected from a sample and compares it to the intensity of light reflected from a reference material. Nanoparticles have optical properties that are unit sensitive to size, shape, concentration, agglomeration state and refractive index close to the nanoparticles surface that makes UV-Visible spectroscopy significant tool to spot, characterize and investigate these materials and evaluate the stability of NP colloidal solutions (Mourdikoudis *et al*., 2018).

2.3.2 Fourier transform infrared (FT-IR)

Fourier transform infrared (FT-IR) spectroscopy is generally employed to use the expression of characteristic spectral bands to reveal nanomaterial–biomolecule conjugation. Proteins bound to nanoparticle surfaces and to illustrate the conformational states of the bound proteins. Moreover, FT-IR has also been expanded to study nano-scaled materials, such as confirmation of functional molecules covalently grafted onto carbon nanotubes (Lin *et al.*, 2014).

The FT-IR spectrometers attain the IR spectrum by Fourier transformation of the signal from an interferometer with a moving mirror to produce an optical transform of the infrared signal. Numerical Fourier examintion gives the relation of intensity and frequency, that is, the IR spectrum. The FT-IR technique can be used to analyze gases, liquids, and solids with minute preparation (Barrios *et al.*, 2012).

2.3.3 Scanning Electron Microscopy (SEM)

The conventional Everhart–Thornley (E-T) and the In-Lens detector of modern SEMs are capable to image sample surface morphology at high dimensional resolution to a sub-nm scale. Imaging that means of transmitted electrons in an SEM exploits the high signal-to-noise ratio resulting from the dominant “forward” electron scattering within a nanoscale sample. In addition, the transmitted electrons suffer reduced scattering events inside thin sample, leading to an

intense electron signal in transmission mode. Surface effects, like charging and contamination are not as critical in comparison with the secondary electron (SE) imaging mode. Hence, the SEM in transmission mode (T-SEM) provides a science tool for dimensional (lateral) measurements of nanoparticles that is particle size distribution, however is additionally well suited for "in-depth" observation of structured nanoparticles (Hodoroaba *et al*., 2016).

2.3.4. Energy Dispersive X-ray (EDX)

The Energy Dispersive X-ray (EDX) analysis is involved in different biomedical fields of study due to its high sensitivity in identifying the different elements in tissues. In fact, EDX technique is made particularly useful in the study of drugs delivery in which the EDX is major tool in order to detect nanoparticles. Conventionally, EDX is supreme technique allowing an elemental analysis of the surface of the samples (Scimeca *et al*., 2018).

2.3.5 X-ray diffraction (XRD)

XRD is one of the most extensively used techniques for the characterization of nanoparticles. (Mourdikoudis *et al*., 2018). It could be a powerful methodology for the study of nanomaterials (materials with structural options of minimum of one dimension within vary of 1-100 nm). The wavelength of X-rays is on the atomic scale, X-ray diffraction (XRD) is an initial tool for probing structure of nano-materials. XRD offers alone unparalleled accuracy with in the measurement of atomic spacing and is that the technique of selection for deciding strain states in thin films. The intensities measured with XRD will offer quantitative, accurate information on the atomic arrangements at interfaces. With lab-based equipment, surface sensitivities down to a thickness of ~50A^0 are achievable, but synchrotron radiation allows the characterization of much thinner films and for many materials, monoatomic layers can be analyzed (Sharma *et al.*, 2012)

2.4 ROLE OF SILVER AND ZINC OXIDE NANOPARTICLES IN MEDICINE

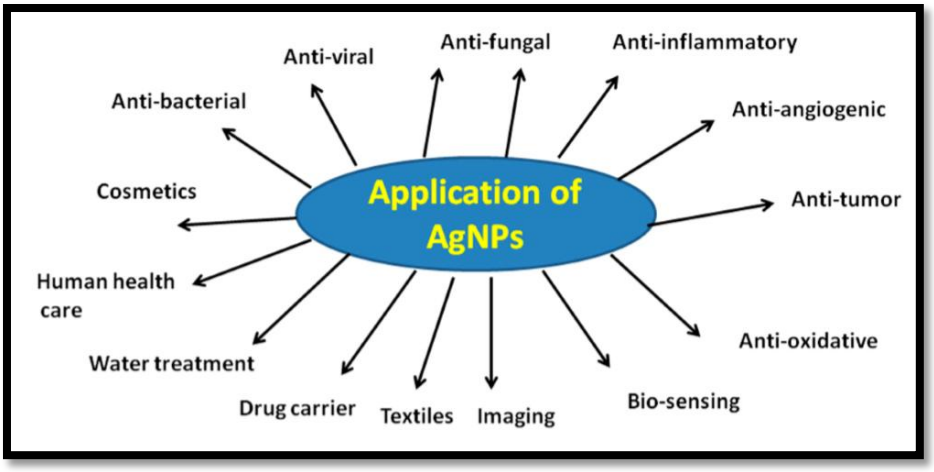
Recent studies of silver nanoparticles lead to utilization in some important applications like diagnostic imaging, therapy, bio-sensing and cancer diagnosis. Silver nanoparticles are considered to be used as drug delivery vehicles and cancer therapeutic agents. Interferon gamma

and tumor necrosis can also be inhibited by silver nanoparticles. Nano silver may be used for destroying unwanted cells due to its plasmonic nature (Nurani *et al.*, 2015).

Due to their unique properties, silver nanoparticles have been used extensively in household utensils, the health care industry, and in food storage, environmental and biomedical applications. Several reviews and book chapters are dedicated in varied areas of the applying of silver nanoparticles. The applications of silver nanoparticles in various biological and biomedical applications, like antibacterial, antifungal, antiviral, anti-inflammatory, anti-cancer and anti-angiogenic (Zhang *et al.*, 2016). Figure 7 illustrate applications of silver nanoparticles.

FIGURE 7

APPLICATIONS OF SILVER NANOPARTICLES



(Zhang *et al.*, 2016)

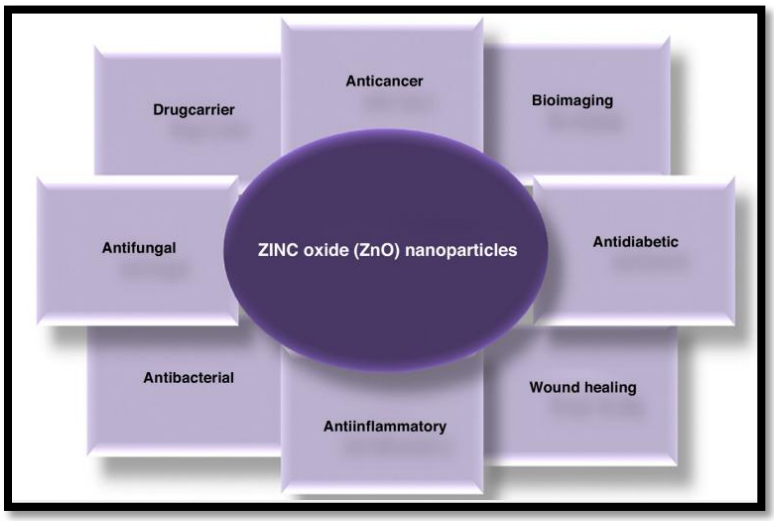
Zinc oxide nanoparticles has a vast range of biomedical applications like drug delivery, anti-cancer, antidiabetic, antibacterial, antifungal and agricultural properties. Although Zinc oxide is used for targeted drug delivery, it has the limitation of cytotoxicity which is yet to be resolved. Zinc oxide nanoparticles have a very strong antibacterial effect against gram negative and gram positive bacteria at a very low concentration. Further, they have shown strong antibacterial effect than the chemically synthesized Zinc oxide nanoparticle (Malaikozhundan, 2017).

Zinc oxide nanoparticles have also been successfully used in wound dressings owing to their strong antimicrobial properties and the epithelialization-stimulating effect of zinc. Nano-Zinc oxide was also able to better suppress local skin inflammation and induced the

systemic production of IgE antibodies. The antidiabetic activity of Zinc oxide nanoparticles has been explored based on the fact that zinc has an important role in insulin synthesis, storage and secretion (Mishra *et al.,* 2017). Figure 8 represents Biomedical applications of zinc oxide nanoparticles.

FIGURE 8

BIOMEDICAL APPLICATIONS OF ZINC OXIDE NANOPARTICLES



(Mishra *et al.*, 2017)

2.5 DIABETES MELLITUS

Diabetes mellitus (DM) – Type 2 is a crucial chronic metabolic disease afflicting a large proportion of the population worldwide. Diabetes is an endocrine disorder characterized by hyperglycemia and dyslipidemia. It is also recognized for associated complications such as diabetic nephropathy, neuropathy and retinopathy and hepatic damage. These complications have been attributed to the morbidity and mortality in DM. The WHO recognizes DM as a growing global epidemic which could be a main cause of disease and disability in the next quarter of the century. Worldwide around 230 million people have been affected by diabetes and the number are expected to reach around 366 million by 2030. The management of DM-2 thus is a challenge both for the patients and the medical fraternity. The conventional therapy includes a lifestyle management, nutritional intervention and pharmacological management. An alternative method to the management strategy of the disease has been the use of medicinal herbs.

Ethanopharmacological surveys indicate that more than 1200 plants are used in traditional medicine for their allied hypoglycemic activity (Virk, 2018).

2.5.1 Alpha amylase

Diabetes mellitus is one between the main diseases prevailing worldwide. New therapeutic methods are being investigated to regulate postprandial glucose levels owing to severe side effects of commercially available anti-diabetic medications. Alpha-amylase is accountable for postprandial glucose levels therefore, different plant extracts with alpha amylase inhibitory activity are being investigated that might decrease postprandial blood glucose levels, thus being an interesting and novel therapeutic target for diabetes treatment. A possible strategy to block dietary carbohydrate absorption is to use natural resources as carbohydrate digestive enzyme inhibitors as they have fewer side effects than synthetic drugs (Agarwal and Gupta, 2016).

2.5.2 Non enzymic glycosylation of hemoglobin

Glucose reacts nonenzymatically with proteins *in vivo*, chemically forming covalently attached glucose-addition products and crosslinks between proteins. The immoderate concentration of rearranged late-glucose-addition products, or advanced glycosylation end products, is confide to contribute to the severe complications of hyperglycemia. Thus, the discovery and investigation of compounds with an advanced glycosylation end products (AGEs) inhibitor activity, would certainly offer a potential therapeutic approach for the prevention of diabetes or other pathogenic complications (Gutierrez, 2012).

2.5.3 Protein glycation

Non enzymatic protein glycation in the body results vascular and renal complications of hyperglycemia. Diabetic patients tend to accumulate glycated proteins in their body tissues as a result of their blood glucose concentration is more than that in healthy people. The initial chemical modification step is that the reaction between the free amino group of proteins and carbonyl group of glucose, that results in formation of fructosamines via Schiff bases, followed by the Amadori rearrangement. The fructosamines are successively oxidized, dehydrated and condensed to make cross-linked proteins and eventually advanced glycation end products (AGEs). Various attempts are created to spot effective glycation inhibitors. Aminoguanidine has

the capability to prevent the diabetes-induced formation of AGEs, together the inhibition of protein cross-linking. Aspirin as well as vitamin B_6, taurine, quercetin and other natural inhibitors have also been reported (Matsuura *et al.*, 2014).

2.5.4 Glucose Uptake

The antidiabetic plant-based therapeutics could benefit the diabetic condition by promoting glucose uptake into tissues and enhancing insulin secretion from pancreatic β-cells. Glucose uptake by peripheral tissues is the most common mechanism by which, high glucose in the blood stream is reduced after a meal. Search for new anti-diabetic agents that can promote glucose uptake and enhance insulin secretion is one of the important aspects in diabetes research (Joladarash *et al.*, 2014).

2.5.5. Glucose diffusion

The blood sugar level in hypergycemic patients tends to rise enormously due to the cell membrane's inability to retain the glucose molecules. Certain viscous components present in plant extracts have shown to decrease glucose diffusion across the membrane. The plant extracts showed great potential in inhibiting the extent of glucose diffusion across the dialysis membrane; therefore, they will act as a possible barrier in lowering the blood glucose level by inhibiting the movement of glucose molecule across the plasma membrane into the blood vessel (Akhtar *et al.*, 2016).

2.6 ANTIDIABETIC ACTIVITY OF SILVER AND ZINC OXIDE NANOPARTICLES

Many researches prove the part of metals in glucose metabolism and the association of their deficiency with diabetes. Vanadium, chromium, magnesium and zinc have been reported to play a major role in blood sugar level maintenance and have been included in diabetes therapy. Zinc, an essential metal, is an activator for more than three hundred enzymes in the body, and plays a key role in different metabolic pathways including glucose metabolism. Zinc assist hepatic glycogenesis through its actions on the insulin pathways and thus amend glucose utilization. Zinc is also additionally to keep the structure of insulin and has a part in insulin hormone biosynthesis, storage and secretion. There are several zinc transporters in pancreatic b cells like zinc transporter 8 which has a potent role in insulin secretion. In addition, zinc might

improve insulin signaling by several mechanisms, including enhance insulin receptor phosphorylation, enhancing PI3K activity and inhibition of glycogen synthase kinase-3. ZnO and Ag nanoparticles activate and significantly reduced blood sugar level, higher serum insulin, higher glucokinase activity, higher expression level of insulin, insulin receptor *GLUT-2* and glucokinase genes in diabetic rats treated with ZnO and Ag nanoparticles and insulin. In conclusion, ZnO and Ag nanoparticles act as powerful antidiabetic agents (Alkaladi *et al.,* 2014).

2.7 DIABETIC FOOT INFECTION

Diabetic foot ulcer (DFU) is one of severe complications of diabetes mellitus (DM). Patients with chronic diabetes develop foot syndrome mainly due to triad of factors like neuropathy, vascular changes and infections. Majority of diabetic foot ulcers seems to be extremely infected with microorganisms, leading to increased hospitalization, probable lower extremity amputations and increased mortality. Bacterial etiology of DFU are polymicrobial in nature, encompassing both aerobic and anaerobic bacteria (Steffy *et al.,* 2017).

Because DFU is often chronic and well-contaminated with a variety of microorganisms, it is very important to properly obtain and process the specimens from infected wounds in order to identify the actual microorganism and their antimicrobial susceptibilities and, thus, select an appropriate antimicrobial therapy. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are important causative microorganisms in diabetic foot infection (DFIs). DFIs arise mainly from skin ulceration associated with loss of protective sensation (peripheral neuropathy), altered foot architecture and some forms of trauma. Various forms of microorganisms colonize and proliferate on the injuries, which serve as a point of entry, causing tissue damage and leads to an inflammatory response that is characterized as a clinical infection. These infections may spread contiguously to beneath tissues and cause osteomyelitis if they reach bones (Kwon *et al*., 2018).

2.8 ANTIBACTERIAL ACTIVITY OF SILVER AND ZINC OXIDE NANOPARTICLES

Silver nanoparticles seem to be alternative antibacterial agents to antibiotics and have the capability to overcome the bacterial resistance against antibiotics. Therefore, it is necessary to develop as silver nanoparticles antibacterial agents. Among the several promising nanomaterials, silver nanoparticles seem to be potential antibacterial agents due

to their large surface-to-volume ratios and crystallographic surface structure. Biologically produced silver nanoparticles using culture supernatants of *Staphylococcus aureus* showed significant antimicrobial activity against methicillin-resistant *S.aureus,* followed by methicillin-resistant *Staphylococcus epidermidis* and *Streptococcus pyogenes,* whereas only moderate antimicrobial activity was observed against *Salmonella typhi* and *Klebsiella pneumoniae.* The mechanisms of silver nanoparticles stimulate cell death was observed in E. coli through the outflow of reducing sugars and proteins. Furthermore, silver nanoparticles are able to destroy the permeability of the bacterial membranes via the generation of many pits and gaps, indicating that silver nanoparticles could damage the structure of the bacterial cell membrane (Zhang *et al.,* 2016).

Zinc oxide reveals remarkable antimicrobial activities when particle size is reduced to the nanometer range, then nano-sized zinc oxide can interact with bacterial surface or with the bacterial core where it enters inside the cell, and subsequently enhance distinct bactericidal mechanisms. The interactions between these distinctive materials and bacteria are mostly toxic, which have been exploited for antimicrobial applications such as in food industry. Interestingly, zinc oxide nanoparticles are reported by several studies as non-poisionous to human cells, this aspect necessitated their usage as antibacterial agents, noxious to microorganisms, and hold good biocompatibility to human cells. The multiple antibacterial mechanisms of nanomaterials are mostly attributed to their high distinct surface area-to-volume ratios and their unique physicochemical properties (Sirelkhatim *et al.,* 2015). Impact of zinc oxide on biological functions based on its morphology, particle size, exposure time, concentration, pH and biocompatibility. They are more active versus microorganisms like *Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Pseudomonas vulgaris, Candida albicans* and *Aspergillus nige*r (Siddiq *et al.*, 2018).

2.9 MEDICINAL PLANT SELECTED FOR THE STUDY – *Boerhavia diffusa*

Boerhaavia diffusa, a perennial crawling weed found in tropics and sub-tropics may be accepted as ethno-medicinal plant. The entire plant as well as its various parts (leaves, roots and stems) and plant extracts are widely employed in numerous ancient and traditional knowledge systems of medicine for treatment of various ailments. *B. diffusa L.* is a perennial crawling weed,

prostrate or ascending herb, up to 1 m long or more, having spreading branches. The roots are grumous and fusiform with a woody root stock. The stem is prostrate, woody or succulent, cylindrical, often purplish, trichoid and thickened at the nodes. Leaves are simple, thick, fleshy, and hairy, organised in unequal pairs, green and glabrous above and usually white underneath, ovate-oblong, spherical or subcordate at the base with smooth, wavy, or undulate margins, up to 5.5×3.3 cm^2 in area. Flowers are minute, subcapitate, present 4–10 together in small bracteolate umbels, forming axillary and terminal panicles. Some of the promising effects of this plant include diuretic, hepatoprotective, anti-inflammatory, anti-fibrinolytic, anticancer, antidiabetic, immuno-modulatory, immuno-suppressive, antilymphoproliferative, analgesic properties and used in treatment of pulmonary tuberculosis. Besides some less promising effects exhibited by this plant includes non-teratogenic, antioxidant, anti-viral activity against plant viruses, anti-bacterial, anti-fungal, adaptogenic, antiamoebic, lipotropic and anticonvulsant activity (Nayak *et al.*, 2016).

EXPERIMENTAL PROCEDURE

3.0 EXPERIMENTAL PROCEDURE

3.1 COLLECTION OF PLANT SAMPLE

The leaves of *Boerhavia diffusa* were collected from Sundakkamuthur area of Coimbatore Tamil Nadu, India. The sample was identified and authenticated by Botanical Survey of India, TNAU, Coimbatore.The authentication number is BSI/SRC/5/23/2013-14/Tech/1041.The leaves of the plant were used for the further assays. Plate 1 represents and *Boerhavia diffusa* its classification.

PLATE 1

Boerhavia diffusa AND ITS CLASSIFICATION

Boerhavia diffusa(punarnava)	
Kingdom	Plantae
Division	Angioperm
Class	Eudicots
Order	Caryophyllales
Family	Nyctaginaceae
Genus	*Boerhavia*
Species	*Diffusa*

3.2 PREPARATION OF ETHANOLIC EXTRACT

The collected *B.diifusa* leave were then washed and air dried in the shade at room temperature for complete drying. The dried sample was powered. 10g of powdered sample was weighed and added to 100ml of ethanol and stored in dark with mild shaking for 7 days. The mixture was then filtered through Whatman No. 1 filter paper. The final extract was stored at 4°C for further experiments.

3.3. SYNTHESIS OF NANOPARTICLES

3.3.1 Synthesis of silver nanoparticles

The 1mM silver nitrate (Ag) was prepared using deionised water and used for the synthesis of silver nanoparticle by the method explained by Harbone, (1998). To 10 ml of ethanolic extract, 90 ml of aqueous solution of 1mM of silver nitrate was added and exposed to bright sunlight the change of color takes place within few minutes from yellowish to reddish brown color (Sulaiman *et al.,* 2013). Therefore, the AgNPs were obtained by repeated centrifugation at 12000 rpm for 20 min, and then, to remove the water-soluble biomolecules, the pellet was dispersed in deionized water, repeated thrice. The purified pellets were then dried on Petri plates at 60°C under vacuum for 24 h. Then the dried AgNPs were used for further study (He *et al.,* 2017).

3.3.2 Synthesis of zinc oxide nanoparticles

Zinc nitrate hexahydrate (2g) in aqueous plant extract (20mL) was dissolved under constant stirring using magnetic stirrer. After complete dissolution of the mixture, the solution was boiled at 70°C with continuous stirring until the formation of deep yellow colored paste. The paste was then transferred to a ceramic crucible and heated in furnace at 400°C for 2h, resulting in pale white colored powder (Rehana *et al.,* 2017).

3.4. CHARACTERIZATION OF SILVER NANOPARTICLES

The synthesized silver nanoparticles were characterized as per the methods explained below.

3.4.1 UV visible spectrum of *Boerhavia diffusa*

The formation of AgNPs was primarily observed by monitoring the change in colour of the extract after treated with $AgNO_3$ (1 mM). The bio-reduction of Ag ions in aqueous extract was monitored with the UV–visible spectra of the solutions after diluted a small aliquot (0.1 ml) of the sample to 10 times with ddH2O. UV–visible spectra were recorded with spectrophotometer from 300 to 700 nm wavelength at room temperature. Double distilled water was used as reaction blank (Premasudha *et al.,* 2015). Zinc oxide nanoparticles (100mg/ml)

dissolved in 0.1N hydro chloric acid scanned between 200-400nm using UV visible spectrometer (Analyticgena Germany).

3.4.2 Scanning Electron Microscopy (SEM) of *Boerhavia diffusa*

Silver and zinc oxide nanoparticles synthesized were characterized using high resolution scanning electron microscopy (SEM). The samples were prepared by simple drop coating of the suspension of silver nanoparticles separately on a carbon- coated copper grid, by simply dropping a very small amount of the sample on the grid and the excess solution was removed by blotting. The film on the scanning electron microscopy grid was then allowed to dry under a mercury lamp for 5 minutes. It was then subjected to SEM analysis.

3.4.3 EDAX spectrum measurements of *Boerhavia diffusa*

Synthesized silver and zinc oxide nanoparticles were dried; drop coated on to carbon film and tested using EDAX analyses

3.4.4 FOURIER–TRANSFORM IR (FT-IR) ANALYSIS of *Boerhavia diffusa*

FT-IR analysis is done to obtain the infra red spectra of absorption, emission and to ensure the formation of silver nanoparticles. It helps to identify the possible interactions between silver and zinc oxide with bioactive molecules, which may be responsible for the formation and stabilization (capping material) of silver nanoparticles. The advantage of using an FT-IR is that it simultaneously collects spectral data in a wide spectral range.

3.4.5 X-Ray diffraction (XRD) studies of *Boerhavia diffusa*

The bio reduced silver and zinc oxide nanoparticles solution was drop-coated onto glass substrate and powder X-ray diffraction measurements.The pattern was recorded by Cu Kα 1 radiation with λ of 1.5406 Å and nickel monochromator filtering the wave at tube voltage of 40 kV and tube current of 30 mA. The scanning was done in the region of 2θ from 30° to 80° at 0.02-min and the time constant was 2s. The average particle size of the silver nanoparticles formed in the bio reduction process was determined using Scherr's formula: $d = (0.9\lambda \times 180°) / \beta\cos\theta\pi$ (Priyaragini *et al.*, 2013).

3.5. *in vitro* ANTIDIABETIC ACTIVITY OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF *Boerhavia diffusa*

3.5.1 *in vitro* alpha amylase inhibitory activity of *Boerhavia diffusa*

in vitro alpha amylase inhibitory activity of silver and zinc oxide nanoparticles of *Boerhavia diffusa* was done by the method explained by Subramanian *et al.* (2008) and it is given in Appendix I.

3.5.2 Non enzymatic glycosylation of haemoglobin inhibitory activity of *Boerhavia diffusa*

Nonenzymatic glycosylation of haemoglobin inhibitory activity silver and zinc oxide nanoparticles of *Boerhavia diffusa* was measured by the method of Daksha *et al*. (2012) and detailed procedure is given in Appendix II.

3.5.3 Glucose uptake capacity by yeast cells

Glucose uptake capacity of silver and zinc oxide nanoparticles of *Boerhavia diffusa* was studied by the method of Vijayalakshimi *et al*. (2015) and detailed procedure is given in Appendix III.

3.5.4 Glucose diffusion inhibitory activity of *Boerhavia diffusa*

Glucose diffusion inhibitory activity of silver and zinc oxide nanoparticles of *Boerhavia diffusa* was studied by the method of Gallager *et al*., (2003) and detailed procedure is given in Appendix IV.

3.5.5 *in vitro* protein glycation inhibitory activity of *Boerhavia diffusa*

in vitro protein glycation inhibitory activity of silver and zinc oxide nanoparticles of *Boerhavia diffusa* was done by the method explained by Mc Pherson *et al.* (1998) and it is given in Appendix V.

3.6 DETERMINATION OF ANTIBACTERIAL ACTIVITY OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF *Boerhavia diffusa*

3.6.1. MICROORGANISMS

The bacterial strains used for assessing the antibacterial activity of include *Klebsiella pnueumoniae, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis.* The strains were obtained from Microbiology Lab, Coimbatore and were stored at 4°C.

3.6.2 AGAR WELL DIFFUSION ASSAY

The Agar Well Diffusion Assay was employed with modifications as described by Irshad *et al.* (2012). The Agar Well Diffusion medium was prepared by pouring molten Mueller-Hinton agar on petri dishes and allowing it to solidify. Afterwards, 100µl of inoculums, approximately 1.5 X 108 cells /ml was seeded into warm molten Mueller-Hinton agar and poured on the surface of the solidified agar. This was allowed to solidify and holes of 5 mm width were made into the agar using sterile Pasteur pipettes. An amount of 100 mg/ml stock of crude plant extract was prepared for each plant by dissolving 100 mg of dried plant extract in 1 ml of 10% DMSO. 100 µL of the stock extract was pipetted onto the holes to give a concentration of 10 mg per hole. 100 µL of 0.5 mg/ml ciprofloxacin was also pipetted into one of the holes to give a final concentration of 0.05 mg. This served as the positive control, while 100 µL of 10% DMSO was pipetted into one of the holes, which served as the negative control.

3.6.2 BROTH MICRODILUTION ASSAY FOR MIC

The MIC of the plant extracts was determined with some modification, as described by Eloff (1998). 100 µL of nutrient broth was added to all the wells of a 96-well microtitre plate. 100 µL of each dissolved plant extract (50mg/ml) was then added in triplicate for each bacterial plate on the first rows (row A). These were serially diluted row by row and 100 µL of the mixture was discarded from the last row, thus leaving each diluted well with a volume of 100 µL. The same procedure was carried out for ciprofloxacin (positive control), during which 100 µL of 2.5 mg/ml of dissolved ciprofloxacin was added in triplicate to row A. 100 µL of each bacterial suspension in suitable growth medium (nutrient broth) was then added to all the wells except the last column, which served as the sterile control (containing 200 µL of nutrient broth).

Wells containing bacterial suspensions and growth medium, as well as wells containing 10% DMSO, bacteria suspensions and growth medium, were used as negative control. The microtitre plates were incubated at 37°C for 24 hours and the MICs were the lowest concentration where no viability was observed after 24 hours on the basis of metabolic activity. To indicate respiratory activity, a change in colour from blue to pink would be determined after adding 20 µL of Presto blue per well in duplicate and incubating it at 37°C for 30 min.

RESULTS AND DISCUSSION

4.0 RESULTS AND DISCUSSION

"Green synthesis" or "Green Nanotechnology" is a new platform to design novel products that are benevolent to human and environment health and has huge potential to revolutionize large scale nanosynthesis procedures (Matus, 2011). These green synthesis approaches for nanomaterials are supposed to benefit environmental and biomedicine segments of nanotechnology applications in future (Preeti, 2016). This new concept can be seen as a bench mark for clean and sustainable nanomaterials. Basic pillars of green chemistry are utilization of less toxic, safe biodegradable and cost effective sources, energy efficient reactions and inherently safer chemistry. Nanotechnology is gradually being benefited by these green and ecofriendly synthesis features and witnessing a steady process. Many reports have come on nanoparticles synthesized from plants, microbes or other natural resources (Mishra *et al.*, 2011).

. The present study was formulated to synthesis silver and zinc oxide nanoparticles of *Boerhavia diffusa* and to find out their efficacy on antidiabetic and antimicrobial activities. The results obtained were furnished and discussed below.

4.1 SYNTHESIS OF SILVER AND ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*

Nanoparticles have been abundantly used in nanochemistry to enhance the immobilization and activity of catalysts in pharmaceutical nanoengineering for delivery of therapeutic agents in chronic disease diagnostics. Silver and zinc oxide nanoparticles have received considerable attention due to their good conductivity, chemical stability, catalytic property, photonics and optoelectronics, unique antibacterial, antifungal and UV filtering properties. ZnO nanomaterials are also being considered for use in next generation biological applications including antimicrobial agents, drug delivery and bioimaging probes (Chauhan *et al.*, 2015)

4.1.1 Synthesis of silver nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*

Synthesis of silver nanoparticles from natural sources has gained importance in recent days. The synthesis exhibited a notable change in color from yellow to brown and the intensity

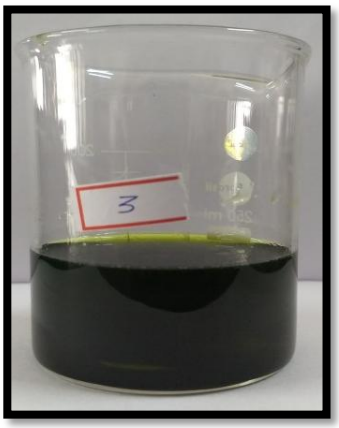
of brown colour is directly proportional to the increase in incubation period and temperature, which indicated the reduction of silver nitrate by the extract (Parveen, 2015). Plate 2 represents synthesis of silver nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*

PLATE 2

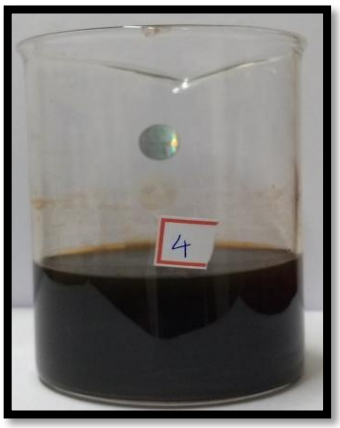
SYNTHESIS OF SILVER NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*

1mM Silver nitrate

Leaf Extract



1mM Silver nitrate + Leaves Extract



Synthesized Silver Nanoparticles

The silver nanoparticles of ethanolic extract of *Boerhavia diffusa* synthesized on exposure of sunlight for 20 minutes. *Boerhavia diffusa* was fond to act as the stabilizing agent for the reduction of $Ag^{+}$to Ag^{0}. As the silver nanoparticles are formed, the color of the solution changes from green to dark brown which is an indication of the presence of silver nanoparticles,

the variation of the color was due to the change in surface Plasmon resonance of silver nanoparticles during the formation.

According to Prathna *et al.* (2014), on the addition of the juice of lemon to silver nitrate solution, a color change from pale yellow to brown was observed on placing the reaction mixture in direct sunlight. Similar results were observed by Sumitha *et al.* (2018), that aqueous extract of durian seeds was utilized to reduce $AgNO_3$ to Ag^0 where the observed color change from colorless to yellowish brown confirms the reduction. The maximum absorbance exhibited by the reaction solution at 420 nm is the characteristic surface plasmon resonance (SPR) peak of *Durio zibethinus* silver nanoparticles.

According to Mankad *et al.* (2018), green synthesis of silver nanoparticles was carried out by mixing aqueous 1 mM $AgNO_3$ and different volume of neem leaf extract that is 5 ml, 10 ml, 15 ml and 20 ml respectively under sunlight exposure for 5, 10, 15 and 20 min. With increasing sunlight exposure period, the color changed from yellow to reddish brown. The intensity of color increases in proportion to time due to reduction of Ag^+. The change in color is due to the excitation of Surface Plasmon Resonance (SPR) in solution.

4.1.2 Synthesis of zinc oxide nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*

The green synthesis of zinc oxide nanoparticles employs simple procedures, easily available raw materials and ambiance for the synthesis process, where the precursors used are safe, with minute possibility for the production of harmful by-products (Basnet *et al.,* 2018). Plate 3 represents synthesis of zinc oxide nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*.

PLATE 3

SYNTHESIS OF ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*

Zinc nitrate+ Leaves Extract | Yellow colour paste | Zinc oxide nanoparticles

Zinc nitrate hexa chloride was dissolved in ethanolic extract of *Boerhavia diffusa.* After the solution was boiled it results in the formation of deep yellow colored paste. The paste was then transferred to a ceramic crucible and heated in furnace resulting in pale yellow colored powder which is an indication of formation of zinc oxide nanoparticles.

Similar results were observed by Ramesh *et al.* (2015) for the synthesis of nanopartilces, 50 ml of *S. nigrum* leaves extract was taken and boiled at 60–80°C by using a stirrer-heater. Then, 5 g of zinc nitrate was added to the solution as the temperatures reached at 60° C. This mixture was then boiled until it was converted to a deep yellow colored suspension. This paste was then collected in a ceramic crucible and heated in an air heated furnace at 400° C for 2 h. A light white colored powder was obtained.

According to Anvekar *et al.* (2017) for the synthesis of nanoparticles, *Adhatoda/ Lemongrass* leaves extract (50 mL) was taken and heated to 80 °C with vigorous stirring. $ZnNO_3.6H_2O$ (5 g) was added to the solution under heating. The paste thus obtained was heated in ceramic crucible at 400°C for 3 h in air. Zinc oxide nanoparticles was obtained as white powder.

According to Blessy and Gomez. (2016), for preparation of nanoparticles 50ml of *Cassia auriculata* flower extract was taken and boiled of 60-80°C. When the temperature of solution reaches at 60°C , 5g of Zinc nitrate hexahydrate Zn $(NO3)_2$. $6H_20$ was added. The mixture is

boiled until it changes to yellow color paste. Then the paste was collected in ceramic crucible and heated at 400°C for 2 hrs. Finally the yellow color powder was grinded with motor and pestle for fine particle and further used for characterization.

4.2 CHARACTERIZATION OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES *OF Boerhavia diffusa*

The characterization of silver nanoparticles synthesized from *Boerhavia diffusa* was done by UV-Visible spectroscopy, Fourier-Transform infrared spectroscopy (FT-IR), Scanning Electron Microscopy (SEM) and Energy dispersive analysis of X-rays (EDX) and X-Ray Diffraction (XRD).

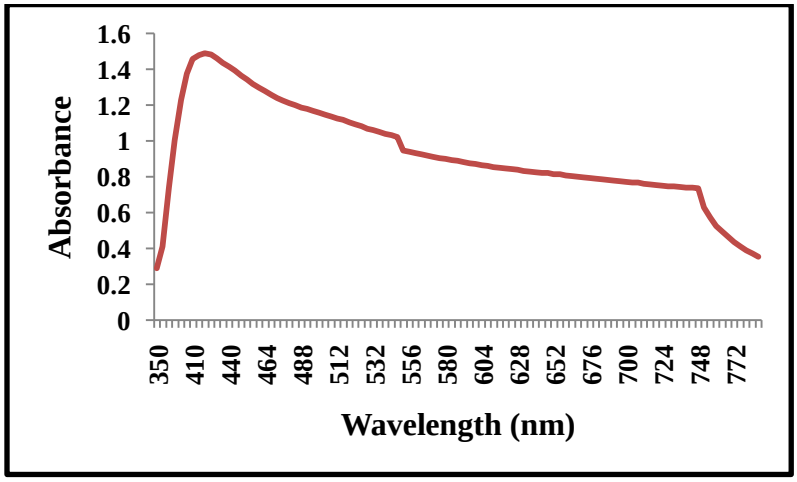
4.2.1 UV-VISIBLE SPECTROSCOPY OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*

4.2.1.1 UV-visible spectroscopy of synthesized silver nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*

The reduction of silver ions to silver nanoparticle was reflected in spectral data obtained using a UV-Visible spectrometer. The synthesis of silver nanoparticles was analyzed using UV-Visible (UV-Vis) spectrometry. The absorbance of nanoparticles was measured in the range 300-700nm. The UV-visible absorption spectrum of silver nanoarticles of the *Boerhavia diffusa* shown in the Figure 9.

FIGURE 9

UV-VISIBLE SPECTROSCOPY OF SYNTHESIZED SILVER NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*



From the above figure, it is clear that the silver nanoparticles synthesized from leaves of *Boerhavia diffusa* formed in the reaction media has absorption spectra of maximum at 400 - 440 nm and the remarkable broadening of peak indicated that the particles polydispered. The absorption peak of *Boerhavia diffusa* was found at 420nm. This clearly indicates that there is an interaction between silver nanoparticles and bimolecules present in the leaves of *Boerhavia diffusa*

Krithiga *et al.* (2015) reported that the reduction of silver nitrate using the plant leaf extract was viewed by the colour change in the reaction solutions. The UV-Vis spectra recorded for the reaction solution of reduced silver nitrate by leaf extract of *Clitoria ternatea* and *Solanum nigrum*. The maximum absorbance peak was seen at 420 and 440 nm for *Clitoria ternatea* and *Solanum nigrum*, respectively.

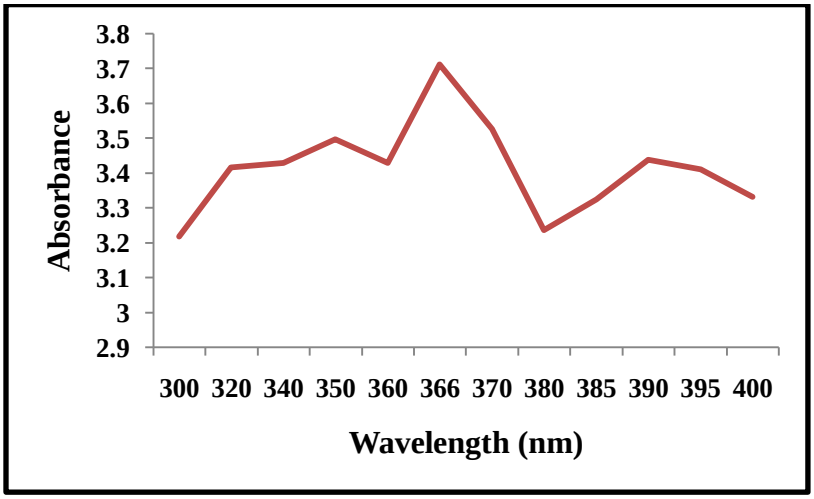
Yasir *et al.* (2017) reported that *S. podophyllum* silver nanoparticles synthesis were measured by observing surface plasmon resonance (SPR) by UV spectroscopy. The SPR peaks were observed at 455 nm for the synthesized silver nanoparticles.

4.2.1.2 UV-Visible spectroscopy of synthesized zinc oxide nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*

The zinc ions reduced to zinc oxide nanoparticles was monitored by UV- Visible spectroscopy. The absorbance of nanoparticles was measured in the range of 200-700 nm. The UV-Visible absorption spectrum of the *Boerhavia diffusa* is shown in the Figure 10.

FIGURE 10

UV-VISIBLE SPECTROSCOPY OF SYNTHESIZED ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*



From the above figure, it is clear that the absorb spectra of zinc oxide nanoparticles synthesized from leaves of *Boerhavia diffusa* formed in the reaction media has absorption maximum at 360 - 380 nm and shows intense peak at 366 nm.

According to Umar *et al.* (2019), *Albizia lebbeck* stem bark extracts show that the absorption peak for the synthesized ZnO NPs is 368 nm, and is in conformity with the range of light absorption of ZnO NPs, which is 360–380 nm. Zare *et al*. (2017), reported the biosynthesis of ZnO NPs using cumin seeds shows UV–Vis absorption peak at 370 nm.

4.2.2 FT-IR OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*

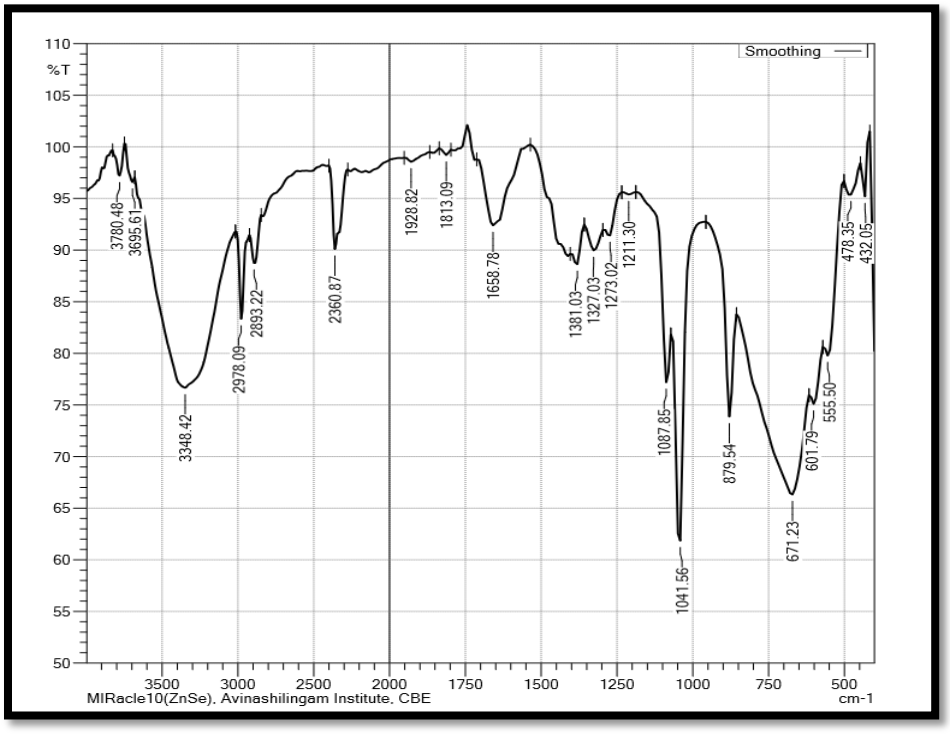
4.2.2.1 FT-IR of synthesized silver nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*

Fourier Transform Infrared Spectrophotometer (FT-IR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined (Ashokkumar and Ramaswamy, 2014).

FT-IR spectra of the silver nanoparticles were carried out to identify the functional groups and the possible interaction between the compounds and the silver nanoparticles. The results are presented in the Figure 11.

FIGURE 11

FT-IR OF SYNTHESIZED SILVER NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*



The FT-IR spectrum of synthesized silver nanoparticles is presented in the above figure. The peak 3780.48 – 3348.42 cm^{-1} reveal O-H stretching of alcohols. 2978.09 – 2893.22 cm^{-1} represents C-H stretching mode in alkenes. The distinct peaks 2356.87 cm^{-1} which is attributed to O=C=O stretching vibrations of carbon dioxide. The peak 1928.82-1658.78 cm^{-1} reveals C-H bending of aromatic compounds. 1381.03 – 1327.03 cm^{-1} represents O-H bending of phenol.1273.02- 1211.30 cm^{-1} C-O stretching of alkyl aryl ether. The peak 1087.85-1041.56 cm^{-1} reveals C-O stretching of primary alcohol. 879.54 - 671.23 cm^{-1} represents C=C bending of alkenes. 601.79-432.05 cm^{-1} represents C-Br stretching of halo compounds.

Devaraj *et al.* (2013), reported that the FT-IR spectra of cannonball leaf extract shows 3432.94 cm^{-1} N–H stretch, 2777.28 cm^{-1} single aldehyde, 2676.19 cm^{-1} C–H; O–H, 2071.75 cm^{-1} C≡C, 1637.58 cm^{-1} C=C and 1121.56 cm^{-1} C=O. The peaks near 3440 cm^{-1}, 2924 cm^{-1} and 2854 cm^{-1} assigned to OH stretching and aldehydic C–H stretching respectively. The band at 1629 cm^{-1} corresponds to amide arising due to carbonyl stretch in proteins. The peak at 1041 cm^{-1} corresponds to C–N stretching vibration of the amine. The peak near 1741 cm^{-1} corresponds to C=C stretching (non conjugated). The peak near 833 cm^{-1} assigned to $C=CH_2$ and the peaks near 677 cm^{-1} and 651.96 cm^{-1} assigned to CH out of plane bending vibrations are substituted ethylene systems CH=CH (cis). FT-IR spectra of silver nanoparticles exhibited prominent peaks at 2,927, 1,631, and 1,383 cm^{-1}. The spectra showed sharp and strong absorption band at 1,631 cm^{-1}assigned to the stretching vibration of (NH) C=O group. The band 1,383 developed for C–C and C–N stretching; presence of the sharp peak at 2,927 cm^{-1} was assigned to C–H and C–H (methoxy compounds) stretching vibration, respectively.

According to Jyoti *et al.* (2016), leaves of *Uritica dioica* shows the bands at 3422 cm^{-1} in the spectra corresponds to O–H stretching vibration indicating the presence of alcohol and phenol. Bands at 2921 and 2856 cm^{-1} region arising from C–H stretching of aromatic compound were observed. The band at 1743 cm^{-1} was assigned for C–C stretching (non-conjugated). The band at 1631 cm^{-1} in the spectra corresponds to C–N and C–C stretching indicating the presence of proteins. The band at 1450 cm^{-1} was assigned for N–H stretch vibration present in the amide linkages of the proteins. The bands at 1450 cm^{-1} and 1043 cm^{-1} were assigned for N–H and C–N (amines) stretch vibration of the proteins respectively. The band at

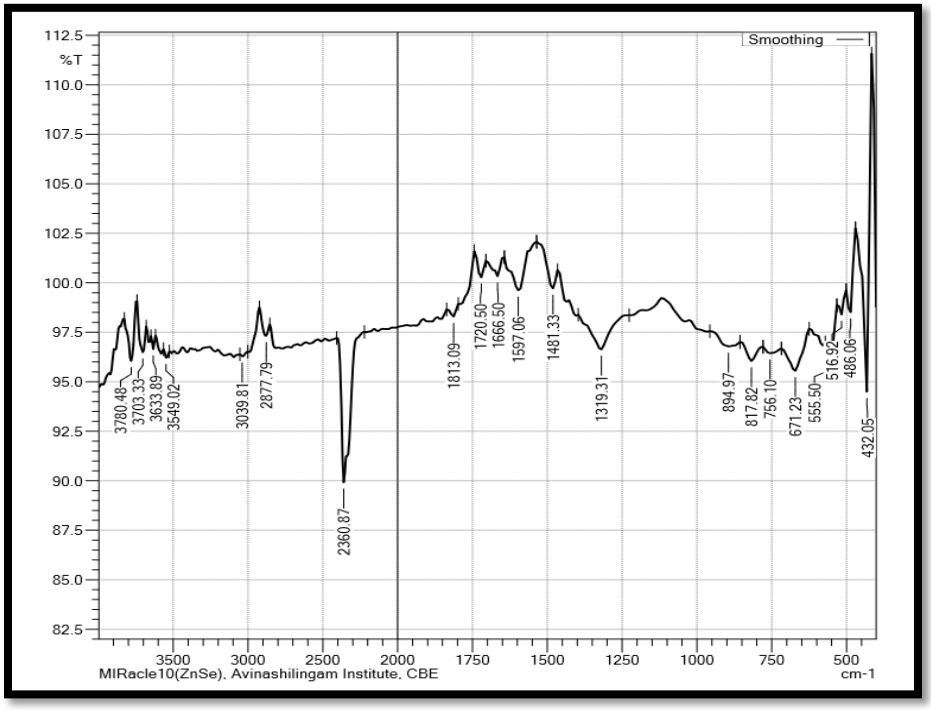
1377 cm^{-1} exemplifies the N=O symmetry stretching typical of the nitro compound. The band at 1240 cm^{-1} corresponds to C–N stretching of amines. The band at 596 cm^{-1} region could be attributed to C–Br stretching, which is characteristic of alkyl halides.

4.2.2.2 FT-IR of synthesized zinc oxide nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*

To identify the possible biomolecules responsible for capping and efficient stabilization of zinc oxide nanoparticles synthesized by leaf extract of *Boerhavia diffusa*. FT-IR spectrum was carried out. The results are presented in the Figure 12.

FIGURE 12

FT-IR OF SYNTHESIZED ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*



The FT-IR spectrum of synthesized nanoparticles are represented in the above figure. The peak 3780.48 – 3549.02 cm^{-1} reveal O-H stretching of alcohols. 3039.81 cm^{-1} ,2877.79 cm^{-1}, 2360.87 cm^{-1}, 1813.09 cm^{-1}, 1666.50 cm^{-1} represents N-H stretching of alcohols, C-H stretching of alkane, O=C=O stretching vibrations of carbon dioxide, C=O stretching of acid halide respectively. The peak 1597-1481.33 cm^{-1} represents N-O stretching nitro compounds. The

distinct peaks 1319.31 cm $^{-1}$ and 894.97 which is attributed to C-O stretching of aromatic compounds and C=C bending of alkenes respectively. The peak 817.82 - 432.06 cm $^{-1}$ represents C-Br stretching of halo compounds.

Datta *et al.* (2017), reported FT-IR analysis of leaves of *Parthenium hysterophorus* ZnONP revealed the presence of peaks at 3458, 2451, 2270, 2245, 1643 and 424 cm-1. The appearance of an intense broadened band at 3458.48 cm-1 denotes the presence of stretched OH bond. The bands at 1643.51 cm-1 confirm the presence of primary amines and the peak at 2245.22cm-1 denotes the presence of nitriles (C (triple bond) N stretch) and alkyls (C (triple bond) C– stretch). FT-IR analysis of the zinc oxide nanoparticles (ZnO NPs) reveals the appearance of bonds that confirms the presence of certain enzymes that are responsible for the reduction, capping and stability of the nanoparticles. It can be stated that the nanoparticles are shielded by the enzymes and the proteins present in the extract so as to help to prevent the formation of clusters.

According to Barrios *et al.* (2018), leaf extract of *Bauhinia tomentosa* shows the absorption band appeared at 3278 and 1339 cm^{-1} was attributed to O–H group of alcohols or plant phenolics. The alkane (C–H) bond in stretching mode was identified by the peaks observed at 2917 and 2850 cm−1. The peaks 1655 and 1543 cm^{-1} were corresponded to the amine (N–H) group of proteins or enzymes present in the *B. tomentosa* leaf extract. The absorption peaks observed in the lower wave numbers 400–600 cm^{-1} confirmed the presence of Zn–O bond in the synthesized NPs. The peaks at 448 and 406 cm^{-1} were corresponded to the stretching frequency of Zn–O bond. The peaks at 1238 and 1080 cm^{-1} represent the C–N stretching mode of aromatic or aliphatic amines. The presence of N–H and O–H bonds in the FT-IR spectrum revealed that the proteins or phenolic compounds in the *B. tomentosa* leaf extract involved in the bioreduction of $Zn2^{+}$ ions to ZnO NPs.

4.2.3 SCANNING ELECTRON MICROSCOPE (SEM) AND ENERGY DISPERSIVE SPECTROSCOPY OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*

4.2.3.1 Scanning electron microscope (SEM) and energy dispersive spectroscopy of synthesized silver nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*

The shape and size of the silver nanoparticles synthesized from leaves of *Boerhavia diffusa* was identified using SEM and the elemental components present in the nanoparticles was confirmed by the EDX analysis. The SEM and EDX images are presented Figure 13 and 14.

FIGURE 13

SEM IMAGES OF SYNTHESIZED SILVER NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*

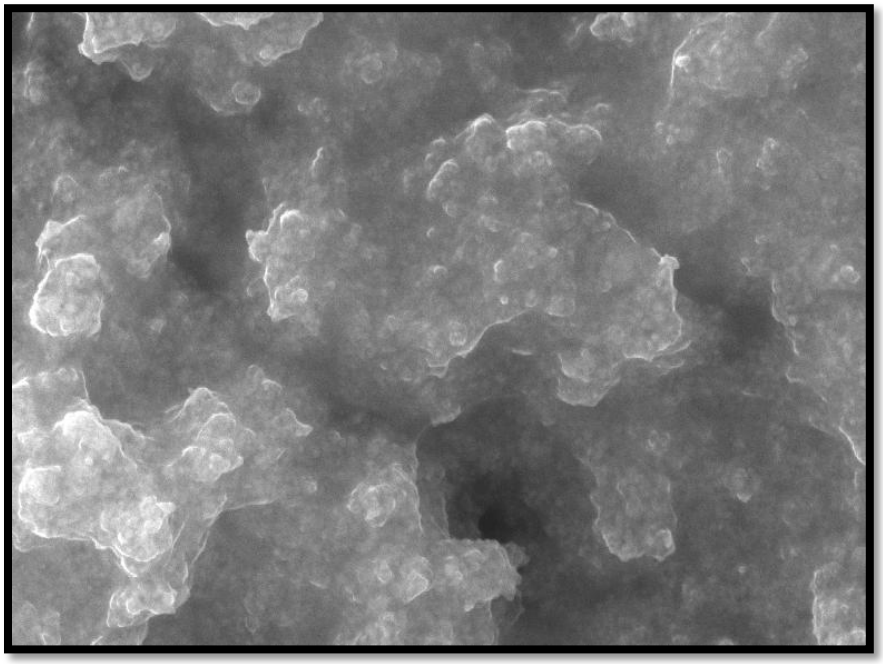
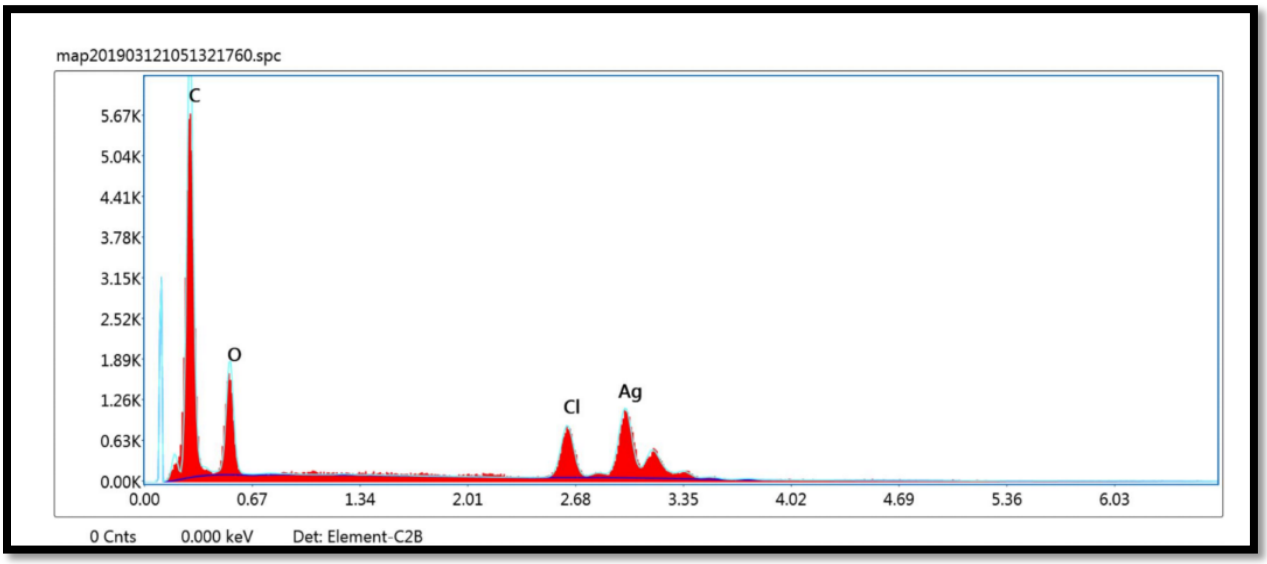


FIGURE 14

EDX IMAGES OF SYNTHESIZED SILVER NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*



The SEM images (figure 13) revealed that the synthesized silver nanoparticle were in the range of 26.80 - 44.40 µm. It shows a clear image of highly dense silver nanoparticles of leaf extracts of *Boerhavia diffusa*. A spherical shape was observed as well as a variation of the nanoparticle size with variation of the silver nitrate volume in the colloidal solution. The figure 14 shows the EDX quantitative analysis graph, which confirms the nanostructure of the silver nanoparticles of leaf extracts of *Boerhavia diffusa*. The silver nanoparticles synthesized from leaf extracts of *Boerhavia diffusa* contains about 36.32 weight per cent Ag, 38.18 weight per cent carbon, 9.36 weight per cent Cl and about weight per cent of oxygen.

According to Srirangam *et al.* (2017), the formation of silver NPs, as well as their morphological dimensions in the SEM study, demonstrated that the average size was 30- 35 nm with inter-particle distance. It is seen that AgNPs of different shapes were obtained in leaf extract being used as reducing and capping agents *Malachra capitata(L.)* leaf extract formed approximately tubular and cuboidal AgNPs, respectively. This may be due to the availability of different quantity and nature of capping agents present in the leaf extract. From EDX spectra, it is clear that silver nanoparticles reduced by *M.capitata(L.)* have the weight percentage of silver as 70.36 per cent.

Rautela *et al.* (2019) reported that the SEM analysis of seeds of *Tectona grandis* clearly shows the silver nanoparticles were oval, spherical in shape. Most of the nanoparticles were aggregated and few individual particles were also observed. Elemental mapping of AgNPs by FESEM-EDX shows the presence of 94 per cent of Ag and 6 per cent of oxides.

4.2.3.2 Scanning electron microscope (SEM) and energy dispersive spectroscopy of synthesized zinc oxide nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*

The size and morphology of the synthesized zinc oxide nanoparticles of leaves of *Boerhavia diffusa* was determined by the Scanning Electron Microscopy (SEM) and elemental composition of it was further confirmed by the Energy Dispersive X-ray (EDX) analysis. The SEM and EDX images are shown in Figure 15 and 16.

FIGURE 15

SEM IMAGES OF SYNTHESIZED ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*

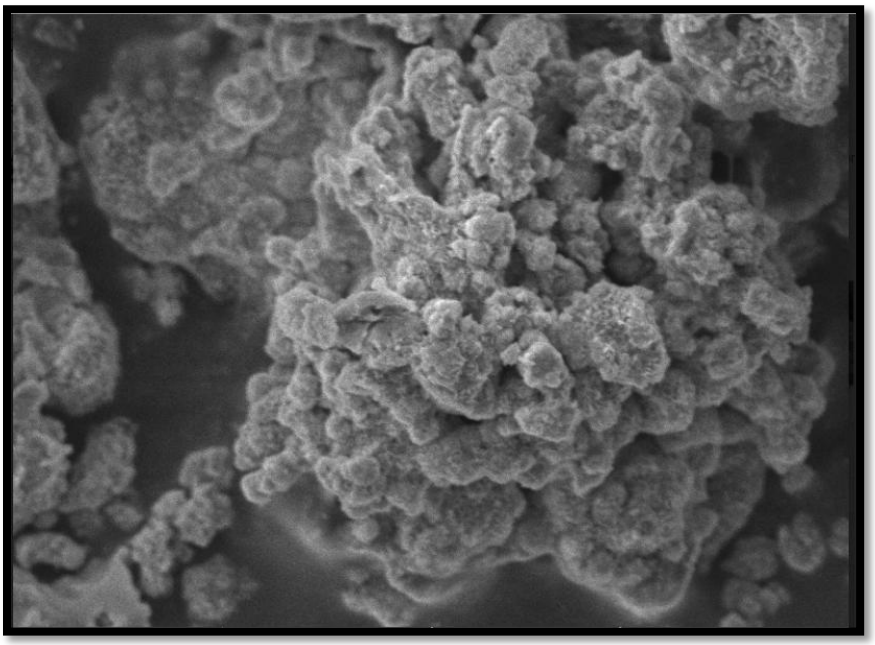
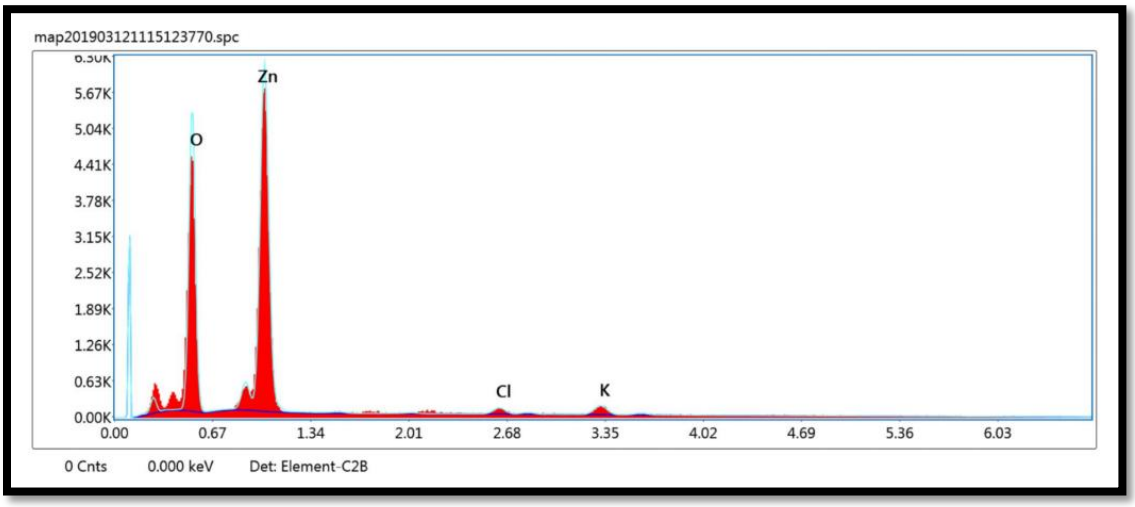


FIGURE 16

EDX IMAGES OF SYNTHESIZED ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*



The SEM images (Figure 15) revealed that the synthesized zinc oxide nanoparticle were found to in the range of 2.50 – 56.70 µm. It shows a clear image of highly dense silver nanoparticles of leaf extracts of *Boerhavia diffusa*. SEM images revealed the agglomeration of nanoparticles and individual nanoparticles are more or less spherical and granular in shape. The figure 16 shows the EDX quantitative analysis graph, which confirms the nanostructure of the zinc oxide nanoparticles of leaf extracts of *Boerhavia diffusa*. The zinc oxide nanoparticles synthesized from leaf extracts of *Boerhavia diffusa* contains about 64.33 weight per cent Zn, 4.14 weight per cent K, 1.90 weight per cent Cl and about 29.63 weight per cent of oxygen.

Similar findings of the SEM and EDX characterization revealed that the zinc oxide nanoparticles synthesised from *Passifloraceae caerulea* having nearly spherical shaped particles of 2 µm–200 nm and the composition obtained from EDX analysis was Zinc (75.36 per cent), Oxygen (22.36 per cent), and Carbon (2.29 per cent) (Santhoshkumar *et al.*, 2017).

Datta *et al.* (2017) reported SEM analysis of leaves of *Parthenium hysterophorus* revealed that the zinc oxide particles were quasi-spherical, radial and cylindrical in shape with different sizes. They were also seen to be present in small aggregated/ clustered forms. The EDX analysis of ZnONP's with 97.6 per cent of Zinc and 2.4 per cent of oxides which confirms the

elemental composition of zinc oxide nanoparticles. EDX spectrum shows four peaks which were identified as Zinc (97.6 per cent) and Oxygen (2.4 per cent).

4.2.4 X-RAY DIFFRACTION (XRD) PATTERN OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*

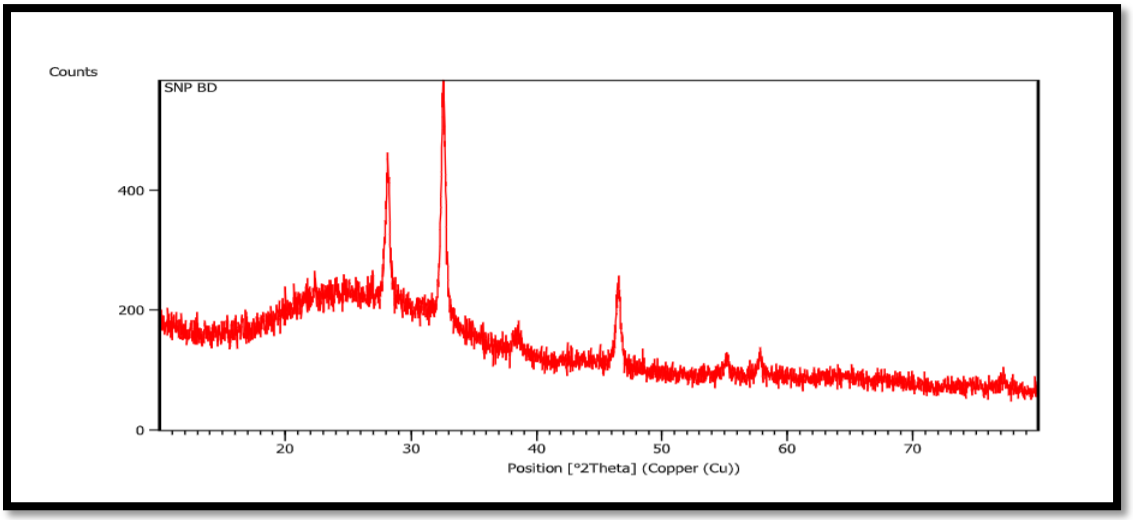
4.2.4.1 X-ray diffraction (XRD) pattern of synthesized silver nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*

The particle shape, morphology and control particles are very much important in nanoparticles preparation. The important tool to study the nano materials is X –ray diffraction. The size, shape, lattice parameter determination and phase fraction analysis of any compound cane be determined easily by XRD.

The silver nanoparticles synthesized from the leaves of *Boerhavia diffusa* was dried and small amount of dry silver nanoparticles can be obtained for XRD analysis. XRD pattern was analysed to confirm the crystalline nature of silver nanoparticles of *Boerhavia diffusa* and the results are given in Figure 17.

FIGURE 17

XRD PATTERN OF SYNTHESIZED SILVER NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*



The XRD pattern (figure 17) of silver nanoparticles synthesized from the leaves of *Boerhavia diffusa* indicated that the structure of nanoparticles may be simple cubic. The XRD spectrum showed eleven different peaks. These diffraction peaks observed at 2θ =28.16 º, 32.52 º and 46.56º respectively have been indexed as (111), (200) and (222).

According to Jyoti *et al.,* (2016), leaves of *Uritica dioica* shows the diffracted intensities were recorded from 20 to 80. Four strong Bragg reflections at 38.45 , 46.35 , 64.75 and 78.05 corresponds to the planes of (1 1 1), (2 0 0), (2 2 0) and (3 1 1) respectively which can be indexed according to the facets of face centered cubic crystal structure of silver.

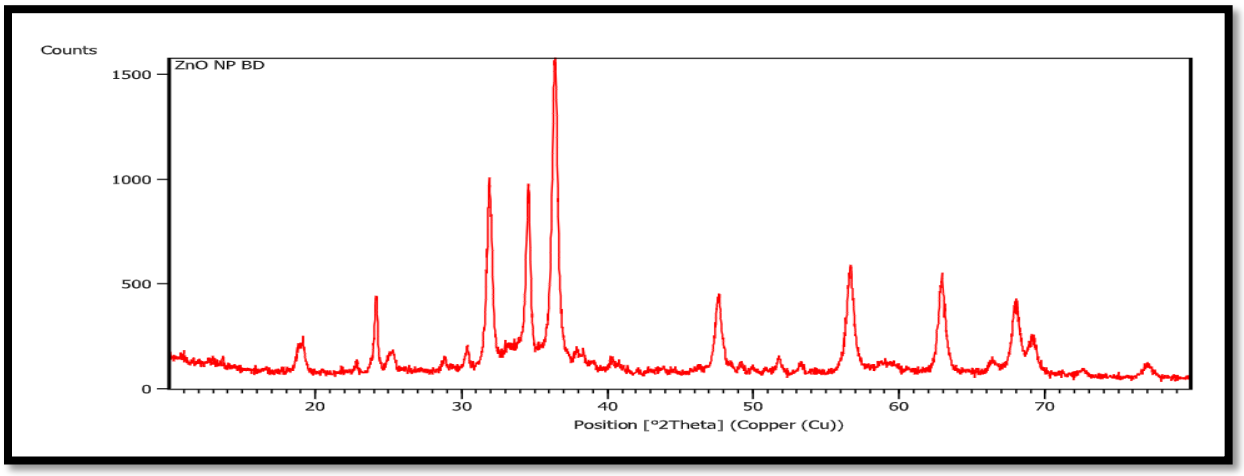
Shameli *et al.* (2012) observed that the XRD patterns of vacuum-dried Ag-NPs synthesized using *Curcuma longa* indicated that the structure of Ag-NPs is face-centered cubic. In addition, all the Ag-NPs had a similar diffraction profile and XRD peaks at 2θ of 38.18°, 44.25°, 64.72° and 77.40° could be attributed to the 111, 200, 220, and 311 crystallographic planes of the face-centered cubic silver crystals respectively.

4.2.4.2 X-ray diffraction (XRD) pattern synthesized zinc oxide nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*

The zinc oxide nanoparticles synthesized from the leaves of *Boerhavia diffusa***.** The crystalline nature of zinc oxide nanoparticles of *Boerhavia diffusa* was confirmed by the analysis of XRD pattern and the results are presented in Figure 18.

FIGURE 18

XRD PATTERN OF SYNTHESIZED ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*



The XRD pattern (figure 19) of zinc nanoparticles synthesized from the leaves of *Boerhavia diffusa* indicated that the structure of nanoparticles may be face centered cubic (FCC). The XRD spectrum showed eleven different peaks. These diffraction peaks observed at 2θ = 31.89º, 34.59º, 36.42 º, 47.72º, 56.66º, 62.92º, 67.95 º, 69.13º and 77.01 º respectively have been indexed as (100), (002), (101), (102), (110), (103), (112), (201) and (202).

According to Suresh *et al.* (2018), leaves of *Costus pictus D.* shows the x-ray diffraction (XRD) spectrum of the biosynthesized zinc oxide nanoparticles. The main peaks were found to be Bragg reflections with 2θ values of 31.74°, 34.38° 36.21°, 47.49°, 56.54°, 62.86°, 66.43°, 67.89°, 68.99°, 72.76°, 76.94°, 81.32° and 89.56°. Locations of the characteristic Bragg reflections were indexed to (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (1 0 3), (2 0 0), (1 1 2), (2 0 1), (0 0 4), (2 0 2), (1 0 4) and (2 0 3) planes of ZnO hexagonal phase structures, respectively and this confirms the presence of zinc oxide nanoparticles.

Santhoshkumar *et al.* (2017) observed that the zinc oxide nanoparticles synthesized using *Passifloraceae caerulea* fresh leaf extract was confirmed by the X-ray diffraction peaks obtained at 31.8°, 34.44°, 36.29°, 47.57°, 56.61°, 67.96° and 69.07° corresponded to the lattice plane of (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (1 1 2), (2 0 1) suggesting the face-centered cubic (fcc) crystal structure of the nanoparticle.

4.3 *in vitro* ANTIDIABETIC ACTIVITY OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*

Diabetes mellitus is one of the common metabolic disorders with micro and macro vascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world. In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus. There is increasing demand by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents (Ahmed *et al.,* 2016)

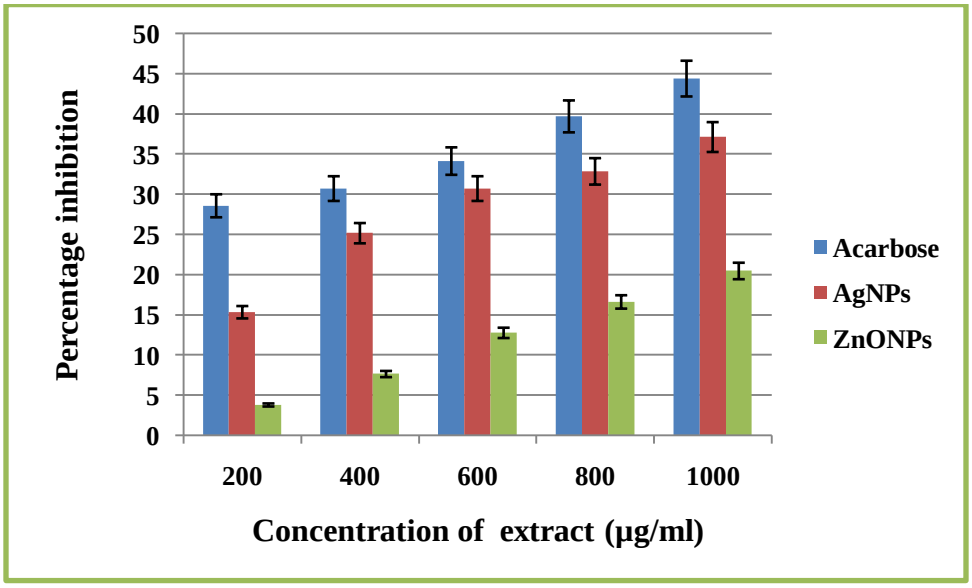
In the present study, the inhibitory activity of different concentations (200 – 1000 µg/ml) of silver and zinc oxide nanoparticles synthesized from ethanolic extract leaves of *Boerhavia diffusa* was investigated for its alpha amylase activity, Nonenzymatic glycosylation, Glucose uptake capacity, Glucose diffusion inhibitory activity and *in vitro* protein glycation inhibitory activities and the results are given below.

4.3.1 Inhibition of Alpha amylase activity of synthesized silver and zinc oxide nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*

The inhibition of alpha-amylase which involved in the digestion of carbohydrates can significantly reduce the post-prandial blood glucose level and therefore can be an important strategy in the management of blood glucose level in type 2 diabetic and borderline patients (Tundis *et al.,* 2010). Figure 19 represents alpha amylase activity of synthesized silver and zinc oxide nanoparticles of ethanolic extract leaves of *Boerhavia diffusa*.

FIGURE 19

ALPHA AMYLASE ACTIVITY OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*



From the above figure, it is clear that the alpha amylase activity of silver and zinc oxide nanoparticles from leaves of *Boerhavia diffusa* was found to be increased with increase in concentration from 200-1000 µg/ml. At a concentration of 1000 µg/ml the silver nanoparticles from leaves of *Boerhavia diffusa* showed 37.17±1.28 percentage inhibition and zinc oxide nanoparticles from leaves of *Boerhavia diffusa* showed 20.51±1.28 percentage inhibition. Thus the silver nanoparticles synthesized showed potent inhibitory activity against alpha amylase when compared with zinc oxide nanoparticles. The positive control, acarbose has exerted the highest potent inhibitory action against alpha amylase (44.44±1.96 per cent).

According to Kiran and Murugesan (2013), the silver nanoparticles of *Halymenia poryphyroides* showed a dose dependent significantly, the increase in percentage inhibitory activity against α–amylase enzyme, at a concentration of 0.2 ml 26.20 ± 0.02% inhibition was noted and at 1.0 ml 91.30 ± 0.02 per cent inhibition was observed.

According to Rehana *et al.* (2017), the *in vitro* α-amylase inhibitory studies showed that the nanoparticles synthesized using *Tamarindus indica* exhibited higher α-amylase inhibition

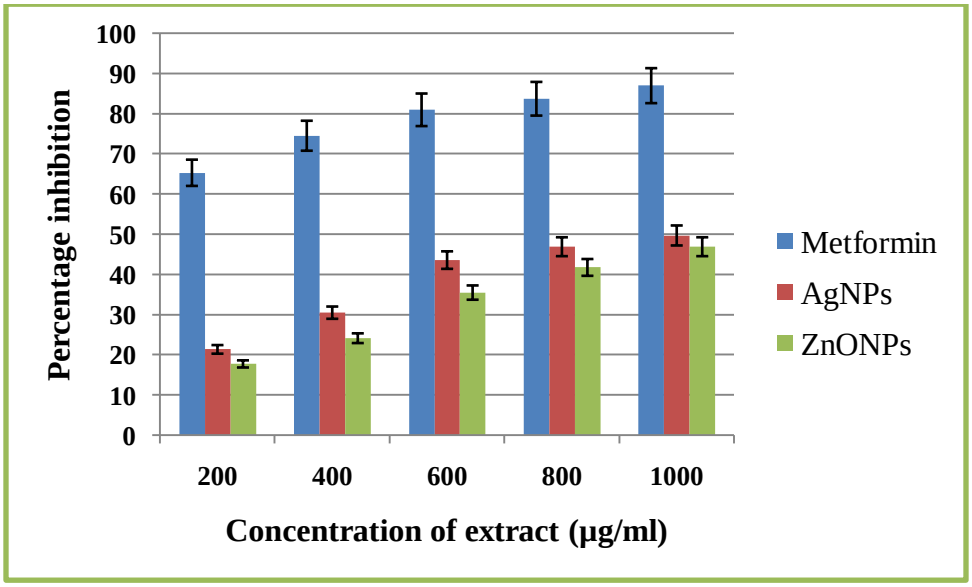
activity when compared with ZnO nanoparticles synthesized using *Azadirachta indica, Hibiscus rosa-sinensis, Murraya koenigii, Moringa oleifera* and *Tamarindus indica*

4.3.2 Inhibition of non-enzymatic glycosylation of synthesized silver and zinc oxide nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*

Nonenzymatic glycation describes a common post-translational process by which D-glucose interacts slowly with intracellular and extracellular proteins, resulting in glucose being covalently bound to the protein. Glycated human hemoglobin (HbA1C) is the first example of an *in vivo* glycated protein. HbA1C is proportionately increased with persistent hyperglycemia and the measurement of HbA1C has been a cornerstone in the monitoring and management of diabetes mellitus (Clark *et al.*, 2013). Figure 20 represents non-enzymatic glycosylation of synthesized silver and zinc oxide nanoparticles of ethanolic extract leaves of *Boerhavia diffusa.*

FIGURE 20

NON-ENZYMATIC GLYCOSYLATION OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*



From the above figure, it is clear that the non enzymic glycosylation activity of silver and zinc oxide nanoparticles from leaves of *Boerhavia diffusa* was found to be increased with

increase in concentration from 200-1000 µg/ml. At a concentration 1000 µg/ml, the silver nanoparticles from leaves of *Boerhavia diffusa* showed 49.76±4.20 percentage inhibition and zinc oxide nanoparticles from leaves of *Boerhavia diffusa* showed 46.96±2.62 percentage inhibition. Thus the silver nanoparticles synthesized showed potent inhibitory activity against non enzymic glycosylation when compared with zinc oxide nanoparticles. The positive control, metformin has exerted the highest potent inhibitory action against non enzymic glycosylation (87.03±0.42 per cent).

According to Wilson *et al.* (2015), with regard to diabetes mellitus, glycated haemoglobin levels was found to be more than the baseline value which have been associated with nephropathy, retinopathy and cardiovascular diseases. *Centella asiatica* silver nanoparticles showed a good antidiabetic activity. The percentage inhibition of glycosylation is dependent to dose, because as the concentration of drug increases the formation of glucose-haemoglobin complex decreases and free hemoglobin increases and shows the inhibition of glycosylated hemoglobin.

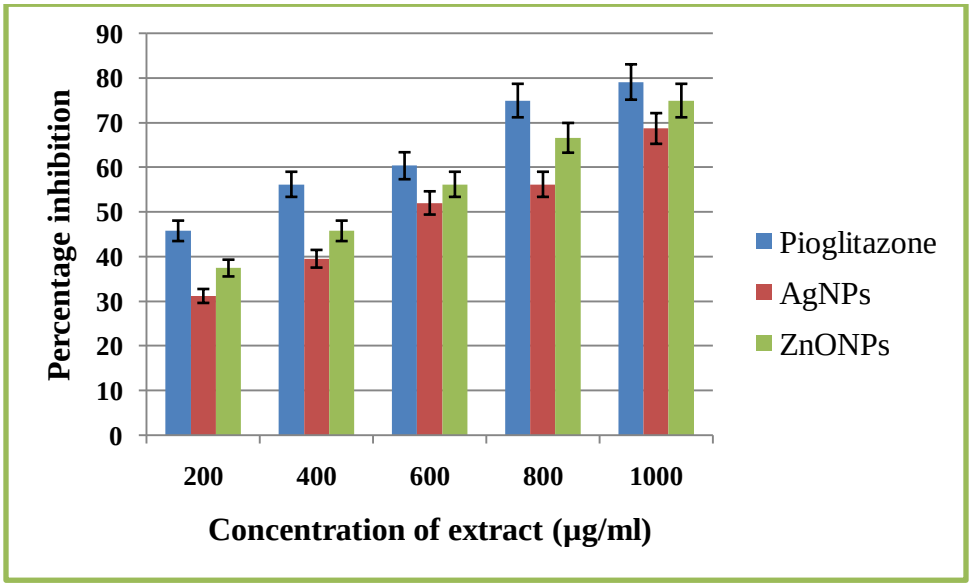
Singh and Kumar (2015) observed that at the concentration of 0.2 mg/ml, phyto chemical compound 1 and compound 2 isolated from *Pithecellobium dulce* showed a percentage inhibition of 26.1percent and 7.2 per cent for non-enzymatic glycosylation of haemoglobin assay. In the case of 1.0 mg/ml concentration inhibition level was 73.7 percent and 53.9 per cent for non-enzymatic glycosylation of haemoglobin assay.

4.3.3 Inhibition of protein glycation of synthesized silver and zinc oxide of nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*

During long standing hyperglycemic state in diabetes mellitus, glucose forms covalent adducts with the plasma proteins through a non-enzymatic process known as glycation. Protein glycation and formation of advanced glycation end products (AGEs) play an important role in the pathogenesis of diabetic complications like retinopathy, nephropathy, neuropathy, cardiomyopathy along with some other diseases such as rheumatoid arthritis, osteoporosis and aging (Singh *et al.,* 2014). Figure 21 represents protein glycation of synthesized silver and zinc oxide of leaves extract of *Boerhavia diffusa*.

FIGURE 21

PROTEIN GLYCATION OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*



From the above figure, it is clear that the protein glycation activity of silver and zinc oxide nanoparticles of leaves of *Boerhavia diffusa* was found to be increased with increase in concentration from 200-1000 µg/ml. At a concentration 1000 µg/ml the silver nanoparticles of leaves of *Boerhavia diffusa* showed 68.75±6.25 percentage inhibition and zinc oxide nanoparticles from leaves of *Boerhavia diffusa* showed 75.00±6.25 percentage inhibition. Thus the zinc oxide nanoparticles synthesized showed potent inhibitory activity against protein glycation when compared with silver nanoparticles. The positive control, piolitazone has exerted the highest potent inhibitory action against non enzymic glycosylation (79.16±3.60 per cent).

According to Thrikawala *et al.* (2018), the fluorescent intensity increased considerably throughout the period, and the introduction of *Flueggea leucopyrus Willd* (15.6–250 µg/mL) to the reaction mixtures demonstrated a drastic reduction in the fluorescent intensity of the mixtures. It is significant that the *Flueggea leucopyrus Willd* showed 98 percent inhibitory potential toward AGE formation at a concentration of 250 µg/mL . Similar to the effect of *Flueggea leucopyrus Willd* aminoguanidine (250 µg/mL and 750 µg/mL) also exhibited a significant reduction in fluorescent AGEs formation when introduced to BSA-fructose medium.

Percentage inhibition of AGE at 250 µg/mL and 750 µg/mL was 95percent and 99 percent respectively.

4.3.4 Glucose uptake assay by yeast cells of synthesized silver and zinc oxide nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*

The movement of glucose across the membrane of the yeast cells was a behavior in an *in vitro* system, which involves the yeast cells suspended in the varying concentrations. The rate of glucose uptake into yeast cells are linear in the (5mM, 10mM, 25 mM) of glucose solution including the extracts. After incubation the glucose uptake of the yeast cells was determined by the amount of glucose which is present in the solution. The results are presented in Table 1.

TABLE 1

GLUCOSE UPTAKE ASSAY BY YEAST CELLS OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*

Glucose uptake at 5mM glucose concentration			
Concentration (µg/ml)	**Standard (%)**	**Silver nanoparticles(%)**	**Zinc Oxide nanoparticles(%)**
200	6.89±1.15	2.29±1.15	1.52±0.66
400	9.50±2.39	6.89±1.15	5.35±0.66
600	15.70±0.66	9.19±1.15	6.89±1.15
800	23.74±1.75	15.32±1.75	11.10±0.66
1000	27.19±0.66	17.62±0.66	14.17±0.66
Glucose uptake at 10mM glucose concentration			
Concentration (µg/ml)	**Standard (%)**	**Silver nanoparticles(%)**	**Zinc Oxide nanoparticles(%)**
200	13.81±0.51	4.50±0.90	2.70±0.90
400	14.41±0.90	10.20±0.52	6.00±0.51
600	23.72±1.37	13.51±0.90	7.80±0.51
800	25.52±0.51	17.40±0.51	14.41±0.90
1000	29.42±0.51	19.21±1.03	18.01±0.90
Glucose uptake at 25 mM glucose concentration			
Concentration (µg/ml)	**Standard (%)**	**Silver nanoparticles(%)**	**Zinc Oxide nanoparticles(%)**
200	14.64±0.36	6.37±0.36	4.66±0.36
400	16.77±0.36	12.73±0.63	7.64±0.64
600	26.53±0.36	15.49±0.36	10.61±0.36
800	28.44±0.97	19.53±0.97	16.34±0.36
1000	31.43±0.39	22.28±0.63	19.95±0.36

The silver and zinc oxide nanoparticles of ethanolic extract of *Boerhavia diffusa* promoted the uptake of glucose across the plasma membrane of yeast cells (Table 2). The glucose uptake at an initial concentration of 5 mM, 10 mM and 25mM was comparable to that of silver and zinc oxide nanoparticels of *Boerhavia diffusa*. However, the effect of silver nanoparticles of ethanolic extract of *Boerhavia diffusa* on glucose uptake by the yeast cells at 5mM, 10mM and 25 mM glucose concentration was a bit higher as compared to that of zinc oxide nanoparticles of ethanolic extract of *Boerhavia diffusa*. The effect standard metronidazole on glucose uptake by the yeast cells at 5Mm, 10Mm and 25 mM glucose concentration was higher as compared to that of silver and zinc oxide nanoparticles of ethanolic extract of *Boerhavia diffusa*.

According to Wilson *et al.* (2015), five different concentrations 40, 80,120, 160 & 200 µg/ml of *Centella asiatica* silver nanoparticles (CANPs) and acarbose showed a concentration-dependent reduction. The highest concentration 200µg/ml of CANPs and Acarbose showed a maximum inhibition of 65.12±0.64 and 76.34±0.29 per cent.

Nair *et al.* (2013) reported that the treatment of the yeast cells with the methanolic plant extracts such as *Cinnamomum zeylanicum, Piper betle*, *Artocarpus heterophyllus* and *Artocarpus altilis*, the glucose uptake was found to increase in a dose dependent manner. The per cent increase in glucose uptake by the yeast cell at different glucose concentrations that is 25mM, 10mM and 5mM respectively.

4.3.5 Inhibition of glucose diffusion of synthesized silver and zinc oxide nanoparticles of ethanolic extract of leaves of *Boerhavia diffusa*

The blood sugar level in hypergycemic patients tends to rise enormously due to the cell membrane's inability to retain the glucose molecules. Certain viscous components present in plant extracts have shown to decrease glucose diffusion across the membrane. The plant extracts showed a great potential in inhibiting the extent of glucose diffusion across the dialysis membrane; therefore, they will act as a possible barrier in lowering the blood glucose level by inhibiting the movement of glucose molecule across the plasma membrane into the blood vessel (Akhtar *et al.,* 2016). Synthesized silver and zinc oxide nanoparticles of leaves extract of

Boerhavia diffusa were subjected to find out their glucose diffusion across the dialysis membrane and the results obtained are shown in Table 2.

TABLE 2

INHIBITION OF GLUCOSE DIFFUSION OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT OF LEAVES OF *Boerhavia diffusa*

Glucose diffusion at 1st hour			
Concentration (µg/ml)	**Standard (%)**	**Silver nanoparticles(%)**	**Zinc Oxide nanoparticles(%)**
200	24.68±2.13	22.21±3.70	16.04±2.13
400	32.09±4.27	24.68±2.13	22.21±3.70
600	35.79±5.66	32.09±2.14	29.62±3.70
800	39.50±2.14	34.56±2.13	32.09±2.14
1000	44.44±3.70	39.50±2.14	38.00±2.60
Glucose diffusion at 2nd hour			
Concentration (µg/ml)	**Standard (%)**	**Silver nanoparticles(%)**	**Zinc Oxide nanoparticles(%)**
200	26.68±3.36	24.44±1.92	18.88±1.92
400	34.44±1.92	26.68±3.36	25.55±1.92
600	39.99±3.33	36.66±3.33	32.22±1.92
800	43.33±3.33	41.11±1.92	36.66±3.33
1000	49.99±3.33	43.35±3.29	41.00±1.73
Glucose diffusion at 3rd hour			
Concentration (µg/ml)	**Standard (%)**	**Silver nanoparticles(%)**	**Zinc Oxide nanoparticles(%)**
200	27.95±1.86	26.87±1.86	25.80±3.22
400	39.77±1.86	32.25±3.22	33.32±1.86
600	44.08±4.92	38.70±3.22	37.62±1.85
800	50.53±1.86	44.08±1.86	45.15±3.22
1000	55.90±1.86	48.38±3.22	43.35±3.29

The silver and zinc oxide nanoparticles of ethanolic extract of *Boerhavia diffusa* inhibit glucose diffusion across the dialysis membrane (Table 2). The glucose diffusion inhibition was measured at different time interval like first, second and third hour and glucose diffusion inhibition was comparable to that of silver and zinc oxide nanoparticels of *Boerhavia diffusa*. However, the effect of silver nanoparticles of ethanolic extract of *Boerhavia diffusa* on glucose diffusion measurement at different time interval was a bit higher as compared to that of zinc oxide nanoparticles of ethanolic extract of *Boerhavia diffusa***.** The effect of standard acarbose on glucose diffusion measurement at different time interval was a bit higher as compared to that of zinc oxide nanoparticles of ethanolic extract of *Boerhavia diffusa*.

According to Das and Devi (2015), the ethanolic extract of fruits, leaves, and bark of *T. bellirica* exhibit higher inhibitory effects on movement of glucose into external solution across the dialysis membrane compared to other extracts and control. The higher glucose dialysis retardation index is found in the ethanolic extracts of fruits (72.9 percent), leaves (72.4 percent), and bark (42.9percent) respectively at 30 minutes.

Vijayalakshmi *et al.* (2015) observed that the level of inhibition of glucose movement by the plant extract at various intervals of time which was assayed and compared with the control in the absence of plant extract. Methanol extract of *S. brevistigma* significantly decreased the glucose movement across the membrane when compared to the control.

4.4 ANTIBACTERIAL ACTIVITY OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACTS OF LEAVES OF *Boerhavia diffusa* AGAINST BACTERIA

The antibacterial activity of synthesized silver and zinc oxide nanoparticles was investigated against various pathogenic bacteria of gram positive (*Bacillus subtilis, Staphylococcus aureus*) and gram negative strains (*Klebsiella pneumoniae, Pseudomonas aeruginosa*) using well diffusion technique. The diameter of zone of inhibition is presented in Table 3 and Plate 4 and 5.

TABLE 3

ZONE OF INHIBITION (mm) OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACTS OF LEAVES OF *Boerhavia diffusa* AGAINST BACTERIA

MICROORGANISM	**ZONE OF INHIBITION (mm) OF SYNTHESIZED SILVER NANOPARTICLES OF *Boerhavia diffusa***				
	200 (µg/ml)	**400 (µg/ml)**	**600 (µg/ml)**	**800 (µg/ml)**	**1000 (µg/ml)**
K.pneumoniae	10	16	18	19	20
S.aureus	10	13	13	15	15
B.subtilis	17	19	22	23	24
P.aeruginosa	12	14	14	16	17
MICROORGANISM	**ZONE OF INHIBITION (mm) OF SYNTHESIZED ZINC OXIDE NANOPARTICLES OF *Boerhavia diffusa***				
	200 µg/ml	**400 µg/ml**	**600 µg/ml**	**800 µg/ml**	**1000 µg/ml**
K.pneumoniae	15	18	19	19	20
S.aureus	11	12	14	15	16
B.subtilis	12	14	15	16	18
P.aeruginosa	10	11	13	17	18

PLATE 4

ANTIBACTERIAL ACTIVITY OF SYNTHESIZED SILVER NANOPARTICLES OF ETHANOLIC EXTRACTS OF LEAVES OF *Boerhavia diffusa*

Klebsiella pnueumoniae

Staphylococcus aureus

Bacillus subtilis

Pseudomonas aeruginosa

PLATE 5

ANTIBACTERIAL ACTIVITY OF SYNTHESIZED ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACTS OF LEAVES OF *Boerhavia diffusa*

Klebsiella pnueumoniae

Staphylococcus aureus

Pseudomonas aeruginosa

Bacillus subtilis

The above Table 3 and Plate 4 proves that the different concentrations of silver and zinc oxide nanoparticles synthesized from *Boerhavia diffusa* leaf extract have potent antimicrobial activity against gram positive and gram negative bacteria. From the results, it can be inferred that the zone of inhibition was increased when the concentration of silver and zinc oxide nanoparticles increased. However the highest zone of inhibition was found to be *B.subtilis*

(24 mm) while compared with other bacterial strains at 1000 µg/ml concentration of silver nanoparticles synthesized from *Boerhavia diffusa* leave followed by *K.pneumoniae* (20 mm), *P.aeruginosa* (17 mm) and *S.aureus* (15 mm). The highest zone of inhibition was found *K.pneumoniae* (20 mm) while compared with other bacterial strains at 1000 µg/ml concentration of zinc oxide nanoparticles synthesized from *Boerhavia diffusa* leaf extract followed by *B.subtilis* (18 mm), *P.aeruginosa* (18 mm) and *S.aureus* (15 mm)

According to Vanaja. (2014), antibacterial activity of synthesized silver nanoparticles of *Solanum trilobatum* was performed against *Streptococcus species, Serratia species, Bacillus subtilis, K.pneumonia, K.planticola* and *E.coli* by well diffusion method. The antibacterial activity of synthesized silver nanoparticles was compared with plant extract, silver nitrate, and commercial antibiotic disc. The zone of inhibition was measured and denoted in millimeter (mm) in diameter. The zone of inhibition in diameter was tabulated by performing triplicate experiments. Among the four antibacterial agents, silver nanoparticles highly inhibit the growth of pathogenic bacteria. Highest inhibition was noted against *E. coli, Streptococcus spices* and *K. pneumoniae*.

According to Gupta *et al.* (2018), the antimicrobial activity of ZnO NPs of *Catharanthus roseus* was demonstrated with the help of the disk diffusion method. The different concentration of nanoparticles were tested against *S.aureus, S.pyogenes, B.cereus, P.aeruginosa, P.mirabilis* and *E. coli* bacteria. The Zinc oxide NPs at 1500 µg/ml displayed good antibacterial activity against all the six pathogenic bacteria included in the study, as indicated by the diameter of inhibition zones of 11.09–11.74 mm when compared to streptomycin.

4.4.2 MINIMUM INHIBITORY CONCENTRATION OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACTS OF LEAVES OF *Boerhavia diffusa* AGAINST BACTERIA

The AgNPs and ZnONPs synthesized from leaves *Boerhavia diffusa* of exhibited maximum zone of inhibition for all the organisms studied. Therefore it was used for minimum inhibitory concentration (MIC) assay. The MIC was determined as the lowest concentration that inhibited the visible growth of the used bacterium. The result of MIC is shown in Table 4.

TABLE 4

MINIMUM INHIBITORY CONCENTRATION OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACTS OF LEAVES OF *Boerhavia diffusa* AGAINST BACTERIA

MICROORGANISM	MINIMUM INHIBITORY CONCENTRATION OF SYNTHESIZED SILVER NANOPARTICLES *Boerhavia diffusa* (µg/ml)							
	0	180	90	45	22.5	11.25	5.62	2.81
K.pneumoniae	+	+	+	+	+	+	-	-
S.aureus	+	+	+	+	+	-	-	-
B.subtilis	+	+	+	+	+	+	-	-
P.aeruginosa	+	+	+	+	-	-	-	-
MICROORGANISM	**MINIMUM INHIBITORY CONCENTRATION OF SYNTHESIZED ZINC OXIDE NANOPARTICLES *Boerhavia diffusa* (µg/ml)**							
	0	**180**	**90**	**45**	**22.5**	**11.25**	**5.62**	**2.81**
K.pneumoniae	+	+	+	+	+	+	+	-
S.aureus	+	+	+	+	+	+	-	-
B.subtilis	+	+	+	+	+	+	+	-
P.aeruginosa	+	+	+	+	+	-	-	-

+ No growth - growth

The synthesized silver nanoparticles of the leaves of *Boerhavia diffusa* was inoculated against *K.pneumoniae, P.aeruginosa, S.aureus* and *B.subtilis* in the concentration of 180-2.81 µg / 100µl. The growth of *S.aureus P.aeruginosa K.pneumoniae* and *B.subtilis* were inhibited by synthesized silver nanoparticles of *Boerhavia diffusa* of 180-2.81µg/100µl concentration. *K.pneumoniae* and *B.subtilis* was found to have significant killing effect at 180-11.25 µg / 100µl whereas *S.aureus* have the killing effect at 180- 22.5 µg /100µl concentration. *P.aeruginosa* found to have killing effect only at 180-45 µg / 100µl.

The growth of *S.aureus P.aeruginosa K.pneumoniae and B.subtilis* were inhibited by synthesized zinc oxide nanoparticles of *Boerhavia diffusa* of 180-2.81µg / 100µl concentration. *K.pneumoniae* and *B.subtilis* was found to have significant killing effect at 180-5.62 µg / 100µl whereas *S.aureus* have the killing effect at 180-11.25 µg / 100µl concentration. *P.aeruginosa* found to have killing effect only at 180-22.5 µg / 100µl.

According to Keshari *et al.* (2017) the MIC value of AgNPs *Cestrum nocturnum* against selected bacteria was varied and this variability depends upon the bacterial strains. The MIC values of AgNPs against bacteria were 16 µg/ml (*Citrobacter*), 4 µg/ml (*E. faecalis*), 16 µg/ml (*S. typhi*), 16 µg/ml (*E. coli*), 8 µg/ml (*P. vulgaris*) and *8* µg/ml (*V. cholerae*).

According to Manyasree *et al.* (2018), the ZnONPs showed significant MIC values between 2mg/ml to 8mg/ml concentration. *E. coli* and *Proteus vulgaris* showed MIC at 6 mg/ml, *Streptococcus mutans* showed MIC at 8 mg/ml and *Staphylococcus aureus* showed MIC at 4 mg/ml for zinc oxide nanoparticles.

SUMMARY AND CONCLUSION

5.0 SUMMARY AND CONCLUSION

Nanotechnology is one of the emerging field in the medical developments over the coming years will have a wide variety of uses and could potentially save a great number of lives. Nanotechnology is already moving from being used in passive structures to active structures, through more targeted drug therapies or "smart drugs". These new drugs therapies have already been shown to cause fewer side effects and more effective than traditional therapies. One area of nanotechnology application that holds the promise of providing great benefits for society in the future is in the realm of medicine. Nanotechnology is already being used as the basis for new, more effective drug delivery system (Moghimi *et al.,* 2005).

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Metabolic abnormalities in carbohydrates, lipids and proteins result from the importance of insulin as an anabolic hormone. The severity of symptoms is due to the type and duration of diabetes. Some of the diabetic patients are asymptomatic especially those with type 2 diabetes during the early years of the disease, others with marked hyperglycemia and especially in children with absolute insulin deficiency may suffer from polyuria, polydipsia, polyphagia, weight loss and blurred vision. Uncontrolled diabetes may lead to stupor, coma and if not treated death, due to ketoacidosis. Diabetic foot infections (DFIs) are associated with significant mortality and morbidity and are the leading cause of non-traumatic lower extremity amputations. Foot infections are caused by microorganisms namely, *Klebsiella pnueumoniae, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis* (Ahmed *et al.,* 2016)

The new era in diabetes treatment facilitates the rapid green synthesis of multifunctional, biocompatible and eco-friendly metal colloidal nanoparticles, as effective nanomedicine against the emerging threat of diabetes mellitus and as a result the harsh environmental impact of its associated complications (Kharroubi and Darwish, 2015). Hence, the study was formulated to synthesis silver and zinc oxide nanoparticles of *Boerhavia diffusa* and to find out and compare the efficacy of its antidiabetic and antimicrobial potential.

The silver and zinc oxide nanoparticles are synthesised from ethanolic extract of leave of *Boerhavia diffusa*. The synthesis of nanoparticles was noticed by change in colour and absorbance. The color of the solution changes from green to dark brown which is an indication of the presence of silver nanoparticles and the formation of pale yellow colored powder which is an indication of formation of zinc oxide nanoparticles. The variation of the colour was due to the change in surface plasmon resonance of silver nanoparticles during the formation.

UV-Visible spectroscopy is used determine the particles formation and properties. The absorption spectrum of silver and zinc oxide nanopartilces of ethanolic extract of leaves of *Boerhavia diffusa* shows intense peak at 420 nm and 366 nm respectively. This clearly indicates that there is an interaction between nanoparticles and bimolecules present in the leaves of *Boerhavia diffusa.*

Functional groups of the plant secondary metabolites involved in the reduction and capping of nanoparticles were identified by FT-IR technique. The FT-IR spectrum of synthesized silver nanoparticles of *Boerhavia diffusa* showed the peaks assigned for O-H stretching of alcohols, C-H stretching mode in alkenes, O=C=O stretching vibrations of carbon dioxide,C-H bending of aromatic compounds. O-H bending of phenol, C-O stretching of alkyl aryl ether. C-O stretching of primary alcohol, C=C bending of alkenes and C-Br stretching of halo compounds.
The FT-IR spectrum of synthesized zinc oxide nanoparticles of *Boerhavia diffusa* showed the peaks assigned for O-H stretching of alcohols, N-H stretching of alcohols, C-H stretching of alkane, O=C=O stretching vibrations of carbon dioxide, C=O stretching of acid halide, N-O stretching nitro compounds, C-O stretching of aromatic compounds, C=C bending of alkenes and C-Br stretching of halo compounds.

The size and morphology of the synthesized silver and zinc oxide nanoparticles of *Boerhavia diffusa* was determined by the Scanning Electron Microscopy (SEM) and elemental composition of it was further confirmed by the Energy Dispersive X-ray (EDX) analysis. The SEM images revealed that the synthesized silver nanoparticle were in the range of 26.80 - 44.40 µm. It shows a clear image of highly dense silver nanoparticles of leave extracts of *Boerhavia diffusa*. A spherical shape was observed as well as a variation of the nanoparticle size with variation of the silver nitrate volume in the colloidal solution. EDX quantitative analysis confirms the nanostructure of the silver nanoparticles of leave extracts of *Boerhavia diffusa*.The

silver nanoparticles synthesized from leave extracts of *Boerhavia diffusa* contains about 36.32 weight per cent Ag, 38.18 weight per cent carbon, 9.36 weight per cent Cl and about 16.15 weight per cent of oxygen. The SEM images revealed that the synthesized zinc oxide nanoparticle were in the range of 2.50 – 56.70 µm. It shows a clear image of highly dense silver nanoparticles of leave extracts of *Boerhavia diffusa*. SEM images revealed the agglomeration of nanoparticles and individual nanoparticles are more or less spherical and granular in shape. EDX quantitative analysis confirms the nanostructure of the Zinc oxide nanoparticles of leave extract of *Boerhavia diffusa*. The zinc oxide nanoparticles synthesized from leave extracts of *Boerhavia diffusa* contains about 64.33 weight per cent Zn, 4.14 weight per cent K, 1.90 weight per cent Cl and about 29.63 weight per cent of oxygen.

The XRD pattern of silver nanoparticles synthesized from the leaves of *Boerhavia diffusa* indicated that the structure of nanoparticles may be simple cubic. The XRD spectrum showed eleven different peaks. These diffraction peaks observed at 2θ =28.16 º, 32.52 º and 46.56º respectively have been indexed as (111), (200) and (222). The XRD pattern of zinc nanoparticles synthesized from the leaves of *Boerhavia diffusa* indicated that the structure of nanoparticles may be face centered cubic (FCC). The XRD spectrum showed eleven different peaks. These diffraction peaks observed at 2θ = 31.89º, 34.59º, 36.42 º, 47.72º, 56.66º, 62.92º, 67.95 º, 69.13º and 77.01 º respectively have been indexed as (100), (002), (101), (102), (110), (103), (112), (201) and (202).

In the present, the inhibitory activity of different concentations (200 – 1000 µg/ml) of silver and zinc oxide nanoparticles synthesized from ethanolic of extract leaves of *Boerhavia diffusa* was investigated for its alpha amylase activity, Nonenzymatic glycosylation, Glucose uptake capacity, Glucose diffusion inhibitory activity and *in vitro* protein glycation inhibitory activities. The silver nanoparticles synthesized showed potent inhibitory activity against alpha amylase when compared with zinc oxide nanoparticles. The positive control, acarbose has exerted the highest potent inhibitory action against alpha amylase (44.44±1.96 percent). The silver nanoparticles synthesized showed potent inhibitory activity against non enzymic glycosylation when compared with zinc oxide nanoparticles. The positive control, metformin has exerted the highest potent inhibitory action against non enzymic glycosylation. The zinc oxide nanoparticles synthesized showed potent inhibitory activity against protein glycation when

compared with silver nanoparticles. The positive control, metronidazole has exerted the highest potent inhibitory action against non enzymic glycosylation

The silver and zinc oxide nanoparticles of ethanolic extract of *Boerhavia diffusa* promoted the uptake of glucose promoted the uptake of glucose across the plasma membrane of yeast cells. The glucose uptake at an initial concentration of 5 mM, 10 mM and 25Mm was comparable to that of silver and zinc oxide nanoparticels of *Boerhavia diffusa*. However, the effect of silver nanoparticles of ethanolic extract of *Boerhavia diffusa* on glucose uptake by the yeast cells at 5Mm, 10Mm and 25 mM glucose concentration was higher as compared to that of zinc oxide nanoparticles of ethanolic extract of *Boerhavia diffusa*. The effect of silver standard metronidazole on glucose uptake by the yeast cells at 5Mm, 10Mm and 25 mM glucose concentration was higher as compared to that of silver and zinc oxide nanoparticles of ethanolic extract of *Boerhavia diffusa*.

The silver and zinc oxide nanoparticles of ethanolic extract of *Boerhavia diffusa* inhibit glucose diffusion across the dialysis membrane. However, the effect of silver nanoparticles of ethanolic extract of *Boerhavia diffusa* on glucose diffusion measurement at different time interval was higher as compared to that of zinc oxide nanoparticles of ethanolic extract of *Boerhavia diffusa*. The effect of standard acarbose on glucose diffusion measurement at different time interval was higher as compared to that of zinc oxide nanoparticles of ethanolic extract of *Boerhavia diffusa*.

The antibacterial activity of synthesized silver and zinc oxide nanoparticles was investigated against various pathogenic bacteria of gram positive (*Bacillus subtilis, Staphylococcus aureus*) and gram negative strains (*Klebsiella pneumoniae, Pseudomonas aeruginosa*).

It was observed that the zone of inhibition was increased when the concentration of silver and zinc oxide nanoparticles increased. However the highest zone of inhibition was found *B.subtilis* (24 mm) while compared with other bacterial strains at 1000 µg/ml concentration of silver nanoparticles synthesized from *Boerhavia diffusa* leave followed by *K.pneumoniae* (20 mm), *P.aeruginosa* (17 mm), *S.aureus* (15 mm). But the highest zone of inhibition was found *K.pneumoniae* (20 mm) while compared with other bacterial strains at 1000 µg/ml concentration

zinc oxide nanoparticles synthesized from *Boerhavia diffusa* leave followed by *B.subtilis* (18 mm), *P.aeruginosa* (18 mm), *S.aureus* (15 mm).

The synthesized silver nanoparticles from leaves of *Boerhavia diffusa* was inoculated against *K.pneumoniae, P.aeruginosa, S.aureus* and *B.subtilis* in the concentration of 180-2.81 µg / 100µl. The growth of *S.aureus P.aeruginosa K.pneumoniae and B.subtilis* were inhibited by synthesized silver nanoparticles of *Boerhavia diffusa* of 180-2.81µg / 100µl concentration. *K.pneumoniae* and *B.subtilis* was found to have significant killing effect at 180-11.25 µg / 100µl whereas *S.aureus* have the killing effect at 180- 22.5 µg / 100µl concentration. *P.aeruginosa* found to have killing effect only at 180-45 µg / 100µl.

The growth of *S.aureus P.aeruginosa K.pneumoniae and B.subtilis* were inhibited by synthesized zinc oxide nanoparticles of *Boerhavia diffusa* of 180-2.81µg / 100µl concentration. *K.pneumoniae* and *B.subtilis* was found to have significant killing effect at 180-5.62 µg / 100µl whereas *S.aureus* have the killing effect at 180-11.25 µg / 100µl concentration. *P.aeruginosa* found to have killing effect only at 180-22.5 µg / 100µl.

Altogether, the present study demonstrated that the silver and zinc oxide nanoparticles synthesized from leaves extracts of *Boerhavia diffusa* are possess effective antidiabetic and antibacterial activity. But the silver nanoparticles of *Boerhavia diffusa* possess better antidiabetic activity than that of zinc oxide nanoparticles of *Boerhavia diffusa.* Whereas antibacterial activity silver and zinc oxide nanoparticles of *Boerhavia diffusa* found to be good antibacterial activity. Hence, these nanoparticles can be exploited in future for medicinal use. However, future studies have to be carried out to find out the efficacy of *in vivo* antidiabetic activity using animal models.

BIBLIOGRAPHY

BIBLIOGRAPHY

- Abeer, S. (2012), Future medicine: nanomedicine. Journal International Medical Sciences Academy, 25(3):187-192.
- Agarwal, P. and Gupta, R. (2016), Alpha-amylase inhibition can treat diabetes mellitus, Research and Reviews Journal of Medical and Health Sciences, 5: 1-8.
- Ahmed, S., Ahmad, M., Swami, B. L. and Ikram, S. (2016), A review on plant extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise, Journal of Advanced Research, *7*(1): 17-28.
- Akhtar, M.N. and Gayathri, M. (2016), Anti-oxidant, anti-microbial and glucose diffusion inhibition activities of the aqueous and chloroform extract of *Phyllanthus urinaria*, International Journal of Pharmacy and Pharmaceutical Sciences, 8 (4): 278-280.
- Alkaladi, A., Abdelazim, A. and Afifi, M. (2014), Antidiabetic activity of zinc oxide and silver nanoparticles on streptozotocin-induced diabetic rats, International Journal of Molecular Sciences, 15(2): 2015-2023.
- Anvekar, T. S., Chari, V. R. and Kadam, H. (2017), Green Synthesis of ZnO Nanoparticles, its Characterization and Application, Material Science Research India, 14(2): 153-157.
- Ashokkumar, R. and Ramaswamy, M. (2014), Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian medicinal plants, Journal of Current Microbiology and Applied Sciences, 3(1): 395-406.
- Aslam, M.S. and Ahmad, M. S. (2016), Worldwide importance of medicinal plants: current and historical perspectives, Recent Advances in Biology and Medicine, 2: 88-93.
- Barrios, V. A. E., Mendez, J. R. R., Aguilar, N. V. P., Espinosa, G. A. and Rodríguez, J. L. D. (2012), FTIR-An Essential Characterization Technique for Polymeric Materials. In Infrared Spectroscopy-Materials Science, Engineering and Technology. Intech Open, 196-211.
- Basnet, P., Chanu, T. I., Samanta, D. and Chatterjee, S. (2018), A review on bio-synthesized zinc oxide nanoparticles using plant extracts as reductants and stabilizing agents, Journal of Photochemistry and Photobiology B, Biology, 183: 201-221.

- Bhowmik, D., Sampath Kumar, K.P., Srivastava, S., Paswan, A.S. and Dutta, S. (2012), Traditional Indian Herbs Punarnava and Its Medicinal Importance, Journal of Pharmacognosy and Phytochemistry, 1:46-51.
- Blessy. J., and Gomez, L.A. (2016), Biosynthesis and Characterization of ZnO Nanoparticle using *Cassia auriculata* Flower Extract and their Antibacterial, Photocatalytic Activity, International Journal of Innovative Research in Science, Engineering and Technology 5(4): 6437-6445.
- Campoy, A. H. G., Gutierrez, R. M. P., Manriquez-Alvirde, G. and Ramirez, A. M. (2018), Protection of silver nanoparticles using *Eysenhardtia polystachya* in peroxide-induced pancreatic β-cell damage and their antidiabetic properties in zebrafish, International Journal of Nanomedicine, 13: 2601–2612.
- Chauhan, R., Reddy, A. and Abraham, J. (2015), Biosynthesis of silver and zinc oxide nanoparticles using *Pichia fermentans* JA2 and their antimicrobial property, Applied Nanoscience, *5*(1): 63-71.
- Chintamani, R. B., Salunkhe, K. S. and Chavan, M. J. (2018), Emerging use of green synthesis silver nanoparticle: an updated review, International Journal of Pharmaceutical Sciences and Research, 9(10): 4029-4055.
- Chokkareddy, R. and Redhi, G. G. (2018), Green synthesis of metal nanoparticles and its reaction mechanisms, Green Metal Nanoparticles: Synthesis, Characterization and their Application, 113-139.
- Clark, S. L., Santin, A. E., Bryant, P. A., Holman, R. W. and Rodnick, K. J. (2013), The initial noncovalent binding of glucose to human hemoglobin in nonenzymatic glycation, Glycobiology, 23(11): 1250-1259.
- Daksha, G., Lobo, R., Nayak, Y. and Nilesh, G. (2012), In-vitroAntidiabetic activity of stem bark of *Bauhinia purpurea Linn*, Der Pharmacia Lettre, 4(2): 614-619.
- Das M.S. and Devi, G.(2015), *in Vitro* Glucose Binding Activity Of *Terminalia bellirica,* International Journal of Applied Pharmaceutics, 8(2) :320-323.
- Datta, A., Patra, C., Bharadwaj, H., Kaur, S., Dimri, N. and Khajuria, R. (2017), Green synthesis of zinc oxide nanoparticles using *Parthenium hysterophorus* leaf extract and evaluation of their antibacterial properties, Journal of Biotechnology and Biomaterials, 7: 271-275.

- Devaraj, P., Kumari, P., Aarti, C. and Renganathan, A. (2013), Synthesis and characterization of silver nanoparticles using cannonball leaves and their cytotoxic activity against MCF-7 cell line, Journal of nanotechnology, 2013: 1-5.
- Di Santo, R. M., Subramanian, V. and Gu, Z. (2015), Recent advances in nanotechnology for diabetes treatment, Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, 7(4): 548-564.
- El-Nour, K. M. A., Eftaiha, A. A., Al-Warthan, A. and Ammar, R. A. (2010), Synthesis and applications of silver nanoparticles, Arabian Journal of Chemistry, 3(3):135-140.
- Eloff, J.N. (1998), A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria, Planta Med., 64: 711-713
- Gallagher A.M, Flatt P.R, Duffy G. and Abdel Y.H.A.(2002), The effects of traditional antidiabetic plants on *in vitro* glucose diffusion, Nutrition Research, 23(3): 413–424.
- Gupta, M., Tomar, R. S., Kaushik, S., Sharma, D. and Mishra, R. K. (2018), Effective antimicrobial activity of green ZnO nano particles of *Catharanthus roseus*, Frontiers in microbiology, 9: 2030.
- Gutierrez, P.R. M. (2012). Inhibition of advanced glycation end-product formation by *Origanum majorana* L. *in vitro* and in streptozotocin-induced diabetic rats, Evidence-Based Complementary and Alternative Medicine, 2012: 1-8.
- Harborne J. B. (1998), Phytochemical methods – A guide to modern techniques of plant analysis, Chapman and Hall Ltd, London.
- He, Y., Wei, F., Ma, Z., Zhang, H., Yang, Q., Yao, B., and Zhang, Q. (2017), Green synthesis of silver nanoparticles using seed extract of *Alpinia katsumadai* and their antioxidant, cytotoxicity and antibacterial activities, RSC Advances, 7(63): 39842-39851.
- Hodoroaba, V. D., Rades, S., Salge, T., Mielke, J., Ortel, E. and Schmidt, R. (2016). Characterisation of nanoparticles by means of high-resolution SEM/EDS in transmission mode, In IOP Conference Series: Materials Science and Engineering, 109(1): 012006.
- Hulla, J. E., Sahu, S. C. and Hayes, A. W. (2015), Nanotechnology: History and future. Human and experimental toxicology, 34(12): 1318-1321.

- Irshad, S., Mahmood, M. and Perveen, F. (2012), *in vitro* antibacterial activities of three medicinal plants using agar well diffusion method, Research journal of Biology, 2(1): 1-8.
- Jiang, J., Pi, J. and Cai, J. (2018), The advancing of zinc oxide nanoparticles for biomedical applications, Bioinorganic Chemistry and Applications, 2018: 1-18
- Joladarashi, D., Chilkunda, N. D. and Salimath, P. V. (2014), Glucose uptake-stimulatory activity of *Tinospora cordifolia* stem extracts in Ehrlich ascites tumor cell model system, Journal of Food Science and Technology, 51(1): 178-182.
- Jyoti, K., Baunthiyal, M. and Singh, A. (2016), Characterization of silver nanoparticles synthesized using *Urtica dioica Linn*. leaves and their synergistic effects with antibiotics, Journal of Radiation Research and Applied Sciences, 9(3): 217-227.
- Kamal, R., Khan, K., Khan, M.F. and Dawar, F.U (2015), Application of Nanotechnology in medical, The Journal of Zoology Studies, 2(3): 22-34.
- Keshari, A. K., Srivastava, R., Singh, P., Yadav, V. B. and Nath, G. (2018), Antioxidant and antibacterial activity of silver nanoparticles synthesized by *Cestrum nocturnum*, Journal of Ayurveda and integrative medicine.1-8.
- Khan, M. A. (2016), Introduction and importance of medicinal plants and herbs, National Health Portal India.
- Kharroubi, A. T. and Darwish, H. M. (2015), Diabetes mellitus: The epidemic of the century, World journal of diabetes, 6(6): 850.
- Kiran, V.M. and Murugesan, S. (2013). Biogenic silver nanoparticles by *Halymenia poryphyroides* and its *in vitro* anti-diabetic efficacy. Journal of Chemical and Pharmaceutical Research, 5(12), 1001-1008.
- Krithiga, N., Rajalakshmi, A. and Jayachitra, A. (2015), Green synthesis of silver nanoparticles using leaf extracts of *Clitoria ternatea* and *Solanum nigrum* and study of its antibacterial effect against common nosocomial pathogens, Journal of Nanoscience, 2015: 1-8.
- Kwon, K. T. and Armstrong, D. G. (2018), Microbiology and antimicrobial therapy for diabetic foot infections, Infection and Chemotherapy, 50(1): 11-20.

- Lanka, S. (2017), A review on mangrove vegetation in diviseema and machilipatnam regions and potential applications of these plants in nanoparticle synthesis, World Journal of Pharmaceutical Research, 6(15): 185-197.
- Lin, P. C., Lin, S., Wang, P. C. and Sridhar, R. (2014), Techniques for physicochemical characterization of nanomaterials, Biotechnology Advances, *32*(4): 711-726.
- Logothetidis, S. (2012), Nanotechnology: Principles and applications, In Nanostructured Materials and Their Applications, Springer Science and Business Media, 1-22.
- Luby, S., Lubyova, M., Siffalovic, P., Jergel, M. and Majkova, E. (2015), A brief History of Nanoscience and Foresight in Nanotechnology, In Nanomaterials and Nanoarchitectures, 63-86.
- Malaikozhundan, B. (2018), Pharmaceutical Applications of Zinc Oxide Nanoparticles-A Review, Acta Scientific Pharmaceutical Sciences, *2*(1): 11-12.
- Mankad, M., Patil, G., Patel, D., Patel, P. and Patel, A. (2018), Comparative studies of sunlight mediated green synthesis of silver nanoparticles from *Azadirachta indica* leaf extract and its antibacterial effect on *Xanthomonas oryzae pv. Oryzae*, Arabian Journal of Chemistry, 1-8.
- Manyasree D., Kiranmayi P. and Venkata R. (2018), Characterization and antibacterial activity of zno nanoparticles synthesized by co precipitation method, International Journal of Applied Pharmaceutics, 10(6): 224-228.
- Matsuura, N., Aradate, T., Kurosaka, C., Ubukata, M., Kittaka, S., Nakaminami, Y. and Ohara, M. (2014), Potent protein glycation inhibition of plantagoside in Plantago major seeds, BioMed Research International, 2014: 1-5
- Matus, K. J., Hutchison, J. E., Peoples, R., Rung, S. and Tanguay, R. (2011), Green nanotechnology challenges and opportunities, 1-32.
- McPherson, I. D., Shilton, B. H and Walton, P. J.(1998), Role of fructose in glycation and cross-linking of proteins, Biochemistry, 27(6): 1901–1907.
- Midha, K., Singh, G., Nagpal, M. and Arora, S. (2016), Potential Application of Silver Nanoparticles in Medicine, Nanoscience and Nanotechnology Asia, 6(2):82-91.
- Mishra, P. K., Mishra, H., Ekielski, A., Talegaonkar, S. and Vaidya, B. (2017), Zinc oxide nanoparticles: a promising nanomaterial for Biomedical applications, Drug Discovery Today, 22(12): 1825-1834.

- Mishra, V., Sharma, R., Jasuja, N. D. and Gupta, D. K. (2014), A review on Green synthesis of nanoparticles and evaluation of antimicrobial activity, International Journal of Green and Herbal Chemistry, 3: 081-094.
- Moghimi, S. M., Hunter, A. C. and Murray, J. C. (2005), Nanomedicine: current status and future prospects, The FASEB journal, 19(3): 311-330.
- Mohanraj, V. J. and Chen, Y. (2006). Nanoparticles-a review, Tropical journal of Pharmaceutical Research, 5(1): 561-573.
- Mourdikoudis, S., Pallares, R. M. and Thanh, N. T. (2018), Characterization techniques for nanoparticles: Comparison and complementarity upon studying nanoparticle properties, Nanoscale, 10(27): 12871-12934.
- Mukhopadhyay, A. and Mondal, P. (2018), Application of Nano-biotechnology for Improvement in Therapeutic Approaches for the Treatment of Diabetes, Journal of Clinical and Molecular Endocrinology, 3:1-9
- Nair, S. S., Kavrekar, V. and Mishra, A. (2013), Evaluation of in vitro antidiabetic activity of selected plant extracts, International Journal of Pharmaceutical Science Invention *2*(4): 12-19.
- Nayak, P. and Thirunavoukkarasu, M. (2016), A review of the plant *Boerhaavia diffusa*: Its chemistry, pharmacology and therapeutical potential. The Journal of Phytopharmacology, 5(2), 83-92.
- Nurani, S. J., Saha, C. K., Khan, M. R. and Sunny, S. M. H, (2015) Silver Nanoparticles Synthesis, Properties, Applications and Future Perspectives: A Short Review, Journal of Electrical and Electronics Engineering, 10(6): 117-126.
- Oladeji.O (2016), The Characteristics and Roles of Medicinal Plants: Some Important Medicinal Plants in Nigeria, Natural Products: An Indian Journal, 12(3):102.
- Oscar, L.F., Vismaya, S., Arunkumar, M., Thajuddin, N., Dhanasekaran, D. and Nithya, C. (2016), Algal nanoparticles: synthesis and biotechnological potentials. In Algae-Organisms for Imminent Biotechnology, IntechOpen, 157-188
- Parthasarathy, G., Saroja, M., Venkatachalam, M., Shankar, S. and Evangeline, V. K. (2016), Green synthesis of zinc oxide nanoparticles-review paper, World Journal of Pharmacy and Pharmaceutical Sciences, 5(4): 922-931.

- Parveen, A. and Rao, S. (2015), Mechanistic Approach of Functionalized Noble Metal Nanoparticles Synthesis from *Cassia auriculata* L, Journal of Cluster Science, 26(4), 1295-1303.
- Parveen, K., Banse, V. and Ledwani, L. (2016), Green synthesis of nanoparticles: Their advantages and disadvantages, In AIP Conference Proceedings, AIP Publishing, 1724(1): 020048
- Pourmand, A., Pourmand, M. R., Wang, J. and Shesser, R. (2012), Application of nanomedicine in emergency medicine; point-of-care testing and drug delivery in twenty-first century, DARU Journal of Pharmaceutical Sciences, 1-3.
- Prabhu, S., Vinodhini, S., Elanchezhiyan, C. and Rajeswari, D. (2018), Evaluation of antidiabetic activity of biologically synthesized silver nanoparticles using *Pouteria sapota* in streptozotocin-induced diabetic rats, Journal of Diabetes 10: 28–42
- Prathna, T. C., Raichur, A. M., Chandrasekaran, N. and Mukherjee, A. (2014), Sunlight irradiation induced green synthesis of stable silver nanoparticles using *Citrus limon* extract. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 84(1): 65-70.
- Preeti, N. (2016), Green Chemistry for Nanotechnology: Opportunities and Future Challenges. Research and Reviews: Journal of Chemistry, 5(1).
- Premasudha, P., Venkataramana, M., Abirami, M., Vanathi, P., Krishna, K. and Rajendran, R. (2015), Biological synthesis and characterization of silver nanoparticles using *Eclipta alba* leaf extract and evaluation of its cytotoxic and antimicrobial potential. Bulletin of Materials Science, 38(4): 965-973.
- Priyaragini, S., Sathishkumar, S.R. and Bhaskararao, K.V. (2013), Biosynthesis of silver nanoparticles using actinobacteria and evaluating its antimicrobial and cytotoxicity activity, International Journal of Pharmacy and Pharmaceutical Sciences,5(2): 709-712.
- Ramesh, M., Anbuvannan, M. and Viruthagiri, G. (2015), Green synthesis of ZnO nanoparticles using *Solanum nigrum* leaf extract and their antibacterial activity, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 136: 864-870.

- Rautela, A., Rani, J. and Das, M. D. (2019), Green synthesis of silver nanoparticles from *Tectona grandis* seeds extract: characterization and mechanism of antimicrobial action on different microorganisms, Journal of Analytical Science and Technology, 10(1): 5.
- Rehana, D., Mahendiran, D., Kumar, R. S. and Rahiman, A. K. (2017), *In vitro* antioxidant and antidiabetic activities of zinc oxide nanoparticles synthesized using different plant extracts, Bioprocess and biosystems engineering, 40(6): 943-957.
- Roy, A., Bulut, O., Some, S., Mandal, A. K. and Yilmaz, M. D. (2019), Green synthesis of silver nanoparticles: biomolecule-nanoparticle organizations targeting antimicrobial activity, RSC advances, 9(5): 2673-2702.
- Saji, V. S., Choe, H. C. and Yeung, K. W. (2010). Nanotechnology in biomedical applications: a review, International Journal of Nano and Biomaterials, 3(2), 119-139.
- Salata, O. V. (2004), Applications of nanoparticles in biology and medicine. Journal of nanobiotechnology, 2(1): 1-6.
- Santhoshkumar, J., Kumar, S. V. and Rajeshkumar, S. (2017), Synthesis of zinc oxide nanoparticles using plant leaf extract against urinary tract infection pathogen, Resource Efficient Technologies, 3(4): 459-465.
- Scimeca, M., Bischetti, S., Lamsira, H. K., Bonfiglio, R. and Bonanno, E. (2018), Energy Dispersive X-ray (EDX) microanalysis: A powerful tool in biomedical research and diagnosis, European Journal of Histochemistry, 62(1).
- Sekhar, S. M., Vyas, N., Unnikrishnan, M. K., Rodrigues, G. S., and Mukhopadhyay, C. (2014). Antimicrobial susceptibility pattern in diabetic foot ulcer: A pilot study, Annals of Medical and Health Sciences Research, 4(5), 742-745.
- Shah, M., Fawcett, D., Sharma, S., Tripathy, S. and Poinern, G. (2015), Green synthesis of metallic nanoparticles via biological entities, Materials, 8(11): 7278-7308.
- Shameli, K., Ahmad, M. B., Zamanian, A., Sangpour, P., Shabanzadeh, P., Abdollahi, Y. and Zargar, M. (2012), Green biosynthesis of silver nanoparticles using *Curcuma longa* tuber powder. International Journal of Nanomedicine, 7: 5603.
- Sharma, R., Bisen, D. P., Shukla, U. and Sharma, B. G. (2012), X-ray diffraction: a powerful method of characterizing nanomaterials, Recent Research in Science and Technology, 4(8).

- Siddiqi, K. S., Rahman, A. and Husen, A. (2018), Properties of zinc oxide nanoparticles and their activity against microbes, Nanoscale research letters, 13(1): 141.
- Singh, A., Singh, N. B., Afzal, S., Singh, T. and Hussain, I. (2018), Zinc oxide nanoparticles: a review of their biological synthesis, antimicrobial activity, uptake, translocation and biotransformation in plants, Journal of Materials Science, 53(1): 185-201.
- Singh, S. P. and Kumar, S. P. (2015), *In vitro* antidiabetic activity of compounds from *Pithecellobium dulce* fruit peel, International Journal of Pharmaceutical Chemistry *5*(4): 124-127.
- Singh, V. P., Bali, A., Singh, N. and Jaggi, A. S. (2014), Advanced glycation end products and diabetic complications, The Korean Journal of Physiology and Pharmacology, 18(1), 1-14.
- Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N. H. M., Ann, L. C., Bakhori, S. K. M. and Mohamad, D. (2015), Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism, Nano-Micro Letters, 7(3): 219-242.
- Soliman, H., Elsayed, A. and Dyaa, A. (2018), Antimicrobial activity of silver nanoparticles biosynthesised by *Rhodotorula sp.* strain ATL72, Egyptian Journal of Basic and Applied Sciences, 5(3): 228-233
- Srirangam, G. M. and Rao, K. P. (2017), Synthesis and charcterization of silver nanoparticles from the leaf extract of *Malachra capitata (L.)*, Rasayan Journal Chemistry 10: 46-53.
- Steffy, K., Shanthi, G. and Natarajan V. (2017), Chemical composition and antibacterial activity of Pajanelia *longifolia* (willd.) K.schum against multi drug resistant clinical isolates from diabetic foot ulcer, International Journal of Advanced Research, 5(4): 907-915.
- Subramanian, R., Zaini Asmawi, and Amirin Sadikun (2008), *In vitro* α-glucosidase and α-amylase enzyme inhibitory effects of *Andrographis paniculata* extract and andrographolide, Acta Biochim Pol, *55*(2): 391-398.
- Sulaiman, G.M., Mohammed, W.H., Marzoog, T.R., Al-Amiery, A.A.A., Kadhum, A.A.H. and Mohamad, A.B. (2013), Green synthesis, antimicrobial and cytotoxic effects

of silver nanoparticles using *Eucalyptus chapmaniana* leaves extract, Asian Pacific Journal of Tropical Biomedicine, 3: 58-63.

- Sumitha, S., Vasanthi, S., Shalini, S., Chinni, S. V., Gopinath, S. C., Anbu, P. and Ravichandran, V. (2018), Phyto-Mediated Photo Catalysed Green Synthesis of Silver Nanoparticles Using *Durio zibethinus* Seed Extract: Antimicrobial and Cytotoxic Activity and Photocatalytic Applications, Molecules, 23(12): 3311.
- Surendiran, A., Sandhiya, S., Pradhan, S. C. and Adithan, C. (2009), Novel applications of nanotechnology in medicine, Indian Journal of Medical Research, 130(6).
- Suresh, J., Pradheesh, G., Alexramani, V., Sundrarajan, M. and Hong, S. I. (2018), Green synthesis and characterization of zinc oxide nanoparticle using insulin plant (*Costus pictus D. Don*) and investigation of its antimicrobial as well as anticancer activities, Advances in Natural Sciences: Nanoscience and Nanotechnology, 9(1), 015008.
- Thrikawala, V. S., Deraniyagala, S. A., Dilanka Fernando, C. and Udukala, D. N. (2018), *In Vitro* α-Amylase and Protein Glycation Inhibitory Activity of the Aqueous Extract of *Flueggea leucopyrus Willd*, Journal of Chemistry, 1-7.
- Tundis, R., Loizzo, M. R. and Menichini, F. (2010), Natural products as α-amylase and α-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update, Mini reviews in medicinal chemistry, *10*(4): 315-331.
- Umar, H., Kavaz, D. and Rizaner, N. (2019), Biosynthesis of zinc oxide nanoparticles using *Albizia lebbeck* stem bark and evaluation of its antimicrobial, antioxidant, and cytotoxic activities on human breast cancer cell lines, International journal of nanomedicine, 14:87-100.
- Vanaja, M., Paulkumar, K., Gnanajobitha, G., Rajeshkumar, S., Malarkodi, C. and Annadurai, G. (2014). Herbal plant synthesis of antibacterial silver nanoparticles by *Solanum trilobatum* and its characterization. International Journal of Metals.
- Vasundhara, C. C. S. and Devi, G.S. (2014), In vitro antidiabetic activity of leaves and seeds of Boerhavia diffusa, Journal of Pharmaceutical and Biological Research, 2: 184-9.

- Vijayalakshmi, K., Selvaraj, I. C., Sindhu, S., and Arumugam, P. (2015). *In vitro* investigation of antidiabetic potential of selected traditional medicinal plants, International Journal of Pharmacognosy and Phytochemical Research, 6(4); 856-861,
- Virk, P. (2018), Antidiabetic Activity of Green Gold-Silver Nanocomposite with *Trigonella foenum graecum L.* Seeds Extract on Streptozotocin-Induced Diabetic Rats. Pakistan Journal of Zoology, 50(2).
- Wilson, S., Cholan, S., Vishnu, U., Sannan, M., Jananiya, R., Vinodhini, S. and Rajeswari, D. V. (2015), *In vitro* assessment of the efficacy of free-standing silver nanoparticles isolated from *Centella asiatica* against oxidative stress and its antidiabetic activity, *Der Pharmacia Lettre*, *7*(12), 194-205.
- Woldu, M. A. and Lenjisa, J. L. (2014), Nanoparticles and the new era in diabetes management, International Journal of Basic and Clinical Pharmacology, 3(2):277-284.
- Yasir, M., Singh, J., Tripathi, M. K., Singh, P. and Shrivastava, R. (2017). Green synthesis of silver nanoparticles using leaf extract of common arrow head houseplant and its anticandidal activity, Pharmacognosy magazine, *13*(4), 840-844.
- Zare, E., Pourseyedi, S., Khatami, M. and Darezereshki, E. (2017), Simple biosynthesis of zinc oxide nanoparticles using nature's source, and it's *in vitro* bio-activity, Journal of Molecular Structure, 1146, 96-103.
- Zdrojewicz, Z., Waracki, M., Bugaj, B., Pypno, D. and Cabała, K. (2015) Medical applications of nanotechnology, Postepy Hig Med Dosw (online), 69: 1196-1204.
- Zhang, X. F., Liu, Z. G., Shen, W. and Gurunathan, S. (2016), Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches, International Journal of Molecular Sciences, 17(9): 1534.

APPENDICES

APPENDICES

APPENDIX I

IN VITRO ALPHA AMYLASE INHIBITION STUDY

(Subramanian *et al.*, 2008)

PROCEDURE:

α -amylase inhibition method, the enzyme solution was prepared by dissolving α - amylase in 20mM phosphate buffer (6.9) at the concentration of 0.5mg/ml. 1ml of the extract of various concentrations (250, 500, 750, 1000 µg/ml) and 1ml of enzyme solutions were mixed together and incubated at 25°C for 10min. After incubation, 1ml of starch (0.5%) solution was added to the mixture and further incubated at 25°C for 10min. The reaction was then stopped by adding 2ml of dinitrosalicylic acid (DNS, color reagent), heating the reaction mixture in a boiling water bath (5min). After cooling, the absorbance was measured colorimetrically at 565 nm. The inhibition percentage was calculated using the given formula,

$$\text{per cent inhibition} = \frac{\text{Abs Control} - \text{Abs Sample} \times 100}{\text{Abs Control}}$$

where Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample.

APPENDIX II

NON ENZYMATIC GLYCOSYLATION OF HAEMOGLOBIN METHOD

(Daksha *et al.*, 2012)

PROCEDURE:

Glucose (2 percent), haemoglobin (0.06 percent) and gentamycin (0.02 percent) solutions were prepared in phosphate buffer 0.01 M, pH 7.4. 1 ml each of the above solutions was mixed and 1ml of the methanol extract of varying concentrations was added to it, respectively. The reaction mixture was incubated in dark at room temperature for 72hrs and then the degree of glycosylation of haemoglobin was measured colorimetrically at 520nm. Metformin was used as a standard drug for the assay and percentage inhibition was calculated using the formula,

$$\text{per cent inhibition} = \frac{\text{Absorbance Sample} - \text{Absorbance Control} \times 100}{\text{Absorbance Sample}}$$

where Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample

APPENDIX III

GLUCOSE UPTAKE BY YEAST CELL

(Vijayalakshimi *et al.*, 2014)

PROCEDURE:

Yeast suspension was prepared by repeated washing (by centrifugation 3,000×g; 5 min) in distilled water until the supernatant fluids were clear. A 10% (v/v) suspension was prepared with the supernatant fluid. 1mL of the glucose solution (5, 10, and 25 mM) was added to various concentrations of methanol extract (250, 500, 750, and 1000 µg) and incubated for 10 min at 37 °C. Reaction was started by adding 100 µl of yeast suspension, vortexed and further incubated at 37 °C for 60 min. After 60 min, the reaction mixture was centrifuged (2,500g, 5 min) and the glucose content was estimated in the supernatant. Metronidazole was taken as a standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

$$\text{per cent inhibition} = \frac{\text{Absorbance Sample} - \text{Absorbance Control} \times 100}{\text{Absorbance Sample}}$$

Where Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample.

APPENDIX IV

GLUCOSE DIFFUSION ASSAY

(Gallagher *et al.*, 2003)

PROCEDURE:

2ml of 0.15 M NaCl containing 0.22mM D-glucose was loaded into a dialysis tube containing plant extract (50g/L) and the dialysis tube was sealed. The sealed tube was then

placed in a centrifuge tube containing 45 ml of 0.15 M NaCl and kept in an orbital shaker at a room temperature. The diffusion of glucose into the external solution was monitored by measuring the glucose concentration in the external solution every 60min.

APPENDIX V

IN VITRO PROTEIN GLYCATION INHIBITION

(McPherson *et al.*, 1998)

PROCEDURE:

Fructose (1000 mM, in 200 mM, phosphate buffer pH 7.4) 4.0 mL was incubated with 5.0 mL of BSA (20 mg/mL, in 200 mM phosphate buffer, pH 7.4), 1.0 mL of FLAE (final concentration: 15.6–500 µg/mL), and 10 mL of phosphate buffer (200 mM, pH 7.4) at room temperature for one week. A control was prepared using only BSA and fructose in order to induce the formation of the AGEs and to compare the inhibitory activity of the extract. Control blank was prepared using only BSA, whereas sample blanks were prepared only with the plant extract with respective concentrations. The total volume of tubes brought up to 20.0ml with buffer. The fluorescence emission of each mixture was measured with the excitation and emission wavelengths at 355 nm and 440 nm, respectively, using fluorescence spectrometer (Hitachi-Japan F-2700). The percentage inhibition of fluorescent AGE formation was calculated using the following equation,

$$\text{per cent inhibition} = \frac{(F_C - F_B) - (F_S - F_{SB}) \times 100}{(F_C - F_B)}$$

where FC is the fluorescence intensity of the control, FCB is the fluorescence intensity of the control blank, FS is the fluorescence intensity of the sample and FSB is the fluorescence intensity of the sample blank. Aminoguanidine (1.25, 0.75 and 0.25 mg/mL) was used as the positive control.