Comparative Study of Silver and Zinc Oxide Nanoparticles Synthesized from Ethanolic Extract of Fruits of *Helicteres isora* for their *in vitro* Antidiabetic and Antibacterial Activities

ASWATHY R

(17PBT003)

Thesis submitted to

Avinashilingam Institute for Home Science and

Higher Educationfor Women, Coimbatore - 641 043

In Partial Fulfilment of the Requirements for the Degree of

Master of Science in Biotechnology

April, 2019

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Signature of the Head of the Department

Signature of the Supervisor

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"GRATTITUDE IS THE MEMORY OF HEART"

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INTRODUCTION

INTRODUCTION

Nanotechnology is one of the most active areas of research in modern material science which involves the manipulation of matter at a molecular or atomic level in order to produce novel materials and devices with new extraordinary properties (Wang, 2018). Nanoscience involves the design and engineering of functional system at molecular level. It aims at manipulating matter at the nanoscale to create new and unique materials. Nanoscaled materials are the substance that has atleast one critical dimension less than 100nm. They are of great interest because of their extremely small size and high surface to volume ratio that alter their chemical and physical properties compared to bulk of same chemical composition. Due to their specific characteristics, nanocrystalline particles have found tremendous application in the field of electronics, photonics, catalysis, chemical sensing and imaging, biological labelling, drug delivery, cosmetics, information storage, environmental remediation, biomedical, optics, chemical industries, single electron transistors and nonlinear optical devices (Vijayaraghavan*et al*., 2017).

Nanotechnology has become a new advent in the field of medicine. The utilisation of nanotechnology in medicine offers some exciting possibilities. It helps us to improve health by enhancing the efficacy and safety of nanosystems and nanodevices. Moreover, early diagnosis, implants with improved properties, cancer treatment and minimum invasive treatments for heart disease, diabetes and other diseases are expected. It also play a key role in the medicine of tomorrow providing revolutionary opportunities for early disease detection, diagnostic and therapeutic procedures for improvement of health and enhancing human physical abilities and enabling precise and effective therapy tailored to the patient in the coming years. Though nanomedicine is in its infancy, it has the potential to change medical research dramatically in the 21st century. Nanomedical devices can be used for analytical, imaging, detection, diagnostic and therapeutic purposes and procedures, such as targeting cancer, drug delivery, improving cell-material interactions, scaffolds for tissue engineering and gene delivery systems and they can provide an innovative way to fight against incurable diseases. Many novel nanoparticles and nanodevices are anticipated to be used, with an enormous positive impact on human health. Within the next ten to twenty years nanotechnology may transform science, technology and society offering a significant opportunity to enhance human health in novel ways, especially by providing early disease detection and diagnosis, as well as precise and effective therapy to the patient (Abeer, 2012).

Diabetes mellitus is a group of metabolic disorder characterized by a high level of blood glucose due to insufficient or inefficient insulin secretory response. Diabetes mellitus has been considered as a fast growing epidemic worldwide. According to the survey, the number of people with diabetes mellitus will rise to 591.9 million in 2053. Diabetes mellitus can be grouped into two –Type 1 (insulin dependent diabetes mellitus) and type 2 (non-insulin dependent diabetes mellitus). The factors that can contribute to the development of diabetes mellitus include the genetic condition (Monogenic and polygenic mutation) and environmental factors (overweight, obesity and inactivity). The type of diabetes mellitus which is most common in people is type 2, accounted for 90-95% of the diabetic case worldwide. This occurs at the age over 40, caused either because of the deficiency in insulin secretion in the pancreatic beta cells or insulin resistance in the body. The organs that can be affected by the type 2 diabetes mellitus includes heart, blood vessels, eyes, nerves and kidneys leading life-threatening complications such as cardiac dysfunction, nephropathy and atherosclerosis. Hence, this disorder should be considered as a global problem and needs a greater emphasis on its prevention and therapeutic strategies in health system (Hajiaghaalipour*et al*., 2015).

One of the most costly and devastating complication of diabetes mellitus is diabetic foot ulcer (DFU). It is observed that about 15% of diabetic patients gets affected by DFU during their life time. People with diabetes are more likely to develop foot ulcer that fails to heal which leads to lower limb amputation and even death if properly not treated. Multiple risk factors are associated with diabetic foot ulcer which includes gender (male), duration of diabetes longer than 10 years, advanced age of patients, high Body Mass Index and otherco-morbidities such as retinopathy, diabetic peripheral neuropathy, peripheral, vascular disease, glycated haemoglobin level (HbA1C), foot deformity, high plantar pressure, infections and inappropriate foot self-care habits. The most common causes that increases the risk of developing ulcers in diabetic patients is periphery sensory motor and autonomic neuropathy that leads to high foot pressure, foot deformities and gait instability. Recent studies have also shown that elevated plantar pressure is associated with foot ulcer (Yazdanpanah*et al*., 2015).

The development and complication of diabetes mellitus such as retinopathy, nephropathy and neuropathy can be reduced by glycemic control. Self-monitoring of blood glucose and interval measurement of HbA1C can be done to assess the quality of a patient's glycemic control. Treatment option includes the use of insulin sensitizers, insulin secretagogues, alpha-glucosidase inhibitors, incretin-based therapies, SGLT-2 inhibitors,

amylinomimetics (pramlintide), dopamine receptor agonist (bromocriptine) and insulin (Skugor*et al*., 2017).

One of the major drawback of using synthetic drugs are its side effects that includes nausea, vomiting, abdominal pain, diarrhoea, head ache, abnormal weight gain, allergic reaction, low blood glucose, dark urine, fluid retention or swelling. Therefore, number of researches has been carrying out on traditional medicinal plants for the discovery of anti-diabetic drugs as an alternative for synthetically derived drugs. There are hundreds of herbal plants having antidiabetic potential and thus can be used for deriving anti-diabetic drug. The effect of these plants on the body may delay the complication of diabetes and correct the metabolic abnormalities. Due to the natural origin and less side effects, there has been a growing interest in choosing herbal medicine for care and management of diabetes in both developing and developed countries (Rashid *et al*., 2014).

Medicinal plants are those plants which can be used for treating various disorders. These are marked as a prime and effective primary health care in rural areas. Ethnomedicine is a branch of botany that deals with the utilization of plants for the medicinal purposes (Patel *et al*., 2015). Plants have been used as a source of drug for thousands of years. These were taken initially in the form of crude drugs such as tincture, poultice, powders and other herbal formulations. In the early 19th century, the use of plants as medicines has involved isolation of active compounds, for example morphine from opium (Balunas*et al.,* 2005).

Medical science has progressed to a newer height from Indian traditional medicine "Ayurveda"-up to the development of sophisticated genetic engineering technologies of its own. This lead to the development of many potent drugs from several plants which includes anticancer, antidiabetic, antibacterial and antidiuretic drugs (Swargiary*et al*., 2017).

Helicteres isora is a medicinal plant of Indian origin which belongs to Sterculiaceae family.*H.isora* is a tropical south-east Asian shrub cultivated throughout India. This is also called as Indian screw tree. The plant is a large shrub or small tree having hairy, ovate shaped leaves with serrate margins. *H.isora* is an indigenous medicinal plant possessing remarkable nutritional and therapeutic activities. This plant is found to be a rich source of bioactive compounds like polyphenol, tannins and alkaloids that exhibit therapeutic effects. The bark of the tree is used for the treatment of diabetes and diarrhoea, fruits can be used for gastrointestinal problems. Seeds are used for treating dysentery, roots are used against diabetes, diarrhoea, for curing cut and wounds. Leaves of the plant can be used for the treatment of snake bite, skin infection and scabies (Dayal *et al*., 2015).

The synthesis of nanoparticles is a milestone of nanotechnology. There are many physical and chemical methods that can be used for their synthesis. Among this biogenic reduction of metal precursors to produce corresponding nanoparticles have attracted researches because it is a eco-friendly, less expensive process and also the less chance of chemical contamination that makes them to have various medical and biological application. The green synthesis of nanoparticles utilizes natural reducing, capping and stabilizing agents without the use of toxic expensive chemicals. Rapid enterprise, urbanisation and population explosion are leading to deterioration of earth atmosphere and a large quantity of venturesome and unwanted substances are being discharged. There is a need to learn the facts that are present in the nature and its products which lead to the advancement in the synthesis process of nanoparticles. The nanoparticles are widely applied to human contact areas and there is a growing need to develop processes for synthesis that do not use harsh chemicals. Therefore, green/chemical synthesis is a possible alternative to chemical and physical methods (Imitiyaz*et al*., 2015).

Nowadays, plant mediated synthesis of nanomaterial is gaining much more importance due to their high yield, reduced cost and easy availability. Plants contain biomolecules such as flavones, terpenoids, ketones, aldehydes, proteins, amino acids, vitamins, alkaloids, tannins, phenolics, saponins and polysaccharides which plays a vital role in reduction of metals. Plant parts such as flowers, leaves, roots, stem, latex, seed and seed coat are being used for the synthesis of nanoparticles. It is simpler and productive method to use the extract of above parts than using the whole plant or plant tissue. The ability of plant part to reduce metal ion has been well known since the early 1900s (Mittal *et al*., 2013).

Zinc nanoparticles have drawn maximum attention in past two-three years because they are inexpensive to produce and safe method. ZnO nanoparticles shown to have antibacterial effect at a very low concentration of gram negative and gram positive bacteria as confirmed and also have strong antibacterial effect than the ZnO nanoparticles synthesized chemically (Agarwal*et al.,* 2017).

Silver nanoparticles have unique properties which find myriad applications such as antimicrobial, anticancer, larvicidal, catalytic and wound healing activities. Biogenic synthesis of silver nanoparticles using plants and their pharmacological and other potential applications are gaining momentum owing to its assured rewards (Firdhouse and Lalitha, 2015). The biological activity of silver nanoparticles depends on the morphology and structure of silver nanoparticles, controlled by size and form of the particles. Compared to chemical ways, biological waysaffordа lot of ease within

the management of form, size and distribution of the made nanoparticles by improvement of the synthesis ways, together with the quantity of precursors, temperature, pH and therefore the quantity of reducing and stabilising factors (Zhang *et al*., 2016).

With this above background of information, the present study "Comparative study of silver and zinc oxide nanoparticles synthesized from ethanolic extract of fruits of *Helicteres isora* for their *in vitro* antidiabetic and antibacterial activities" was undertaken with the following objectives

- To synthesis of silver and zinc oxide nanoparticles of ethanolic extract of fruits of *Helicteres isora*
- To characterize the synthesized silver and zinc oxide nanoparticles of ethanolic extract of fruits of *Helicteres isora*
- To determine the *in vitro* antidiabetic activity of silver and zinc oxide nanoparticles of ethanolic extract of fruits of *Helicteres isora*
- To determine the antibacterial potential of silver and zinc oxide nanoparticles of ethanolic extract of fruits of *Helicteres isora*

REVIEW OF LITERATURE

2.0 REVIEW OF LITERATURE

Nanotechnology is a rapidly growing field creating an impact in all spheres of human life.Nanoparticles can be defined as clusters of atoms in the size range of 1-100nm. Nanoparticles (NP) attract larger attention because of their numerous applications in various fields especially nanomedicine (Vadlapudiand Kaladhar, 2014).Nanoscience is the study of the fundamental principles of molecules and structures with atleast one dimension roughly between 1 and 100 nanometers or it is study of phenomena and manipulation of materials at atomic, molecular and macro molecular scales. Nanotechnology is the principle of manipulation of atom by controlling the structure of matter at the molecular level or can be called as a process involving design, characterization, production and application of structures, devices and system (Kumar *et al*., 2014).

2.1 History of nanotechnology
2.2 Nanomaterial
2.3 Applications of medical nanotechnology
2.4 Diabetes mellitus
2.5 Diabetic foot ulcer
2.6 Medicinal plants
2.7 Silver nanoparticles
2.8 Zinc oxide nanoparticles
2.9 Methods of synthesis of nanoparticles
2.10 Characterisation of nanoparticles
2.11 Antidiabetic activity of silver and zinc nanoparticles
2.12 Antimicrobial activity of silver and zinc oxide nanoparticles

2.1 HISTORY OF NANOTECHNOLOGY

As is the case with many other disciplines, applications of nanotechnology were in used centuries before the field was formally defined. The term 'nanotechnology' was used first by the Japanese scientists Norio Taniguchi (1912-1999) in a 1974 paper on production technology that creates objects and features on the order of a nanometer. The American engineer K. Eric Drexler in the year 1955 is credited with the development of molecular nanotechnology, leading to nanosystems machinery manufacturing. The invention of scanning tunnelling microscope in the 1980s by IBM Zurich scientists and then the atomic force microscope allowed scientists to see materials at an unprecedented atomic level. The

availability of more and more powerful computers around this time enabled large scale simulations of material systems using supercomputers. These studies provided insight into nanoscale material structures and their properties. The complementary activities of modelling and simulation, atomic scale visualization and characterization and experimental synthesis activities fueled nanoscale research activities in the year 1980s. At the year 1991 the first nano medicine book by R. Freitas "Nano medicine" was published for developing theory of nanometre-scale electronic devices and for synthesis and characterization of carbon nanotubes and nano wires. Feynman Prize in Nanotechnology was awarded for using DNA to enable the self-assembly of new structures and for advancing our ability to model molecular machine systems at the year of 2002. At 2003, Feynman Prize in Nanotechnology was awarded for modelling the molecular and electronic structures of new materials and for integrating single molecule biological motors with nano-scale silicon devices. First policy conference on advanced nanotech was held. First centre for nano mechanical systems was established, Feynman Prize in Nanotechnology was awarded at 2004 for designing stable protein structures and for constructing a novel enzyme with an altered function. 3D Nano systems like robotics, 3D networking and active nano products that change their state during use were prepared during 2005-2010. From 2011, the era of molecular nanotechnology started (Nikalje, 2015).

2.2 NANOMATERIAL

Nanomaterials can be defined as those materials which have structured components with size less than 100nm at least in one dimension. Bulk materials which are macro sized will exhibits some properties (such as optical, magnetic, electrical, mechanical) which will be entirely different when the same material are at the nanoscale level. These materials exhibit novel and significantly improved physical, chemical and biological properties, phenomena and processes because of their significant nano size. The structural features of nanomaterial fall between of those of atoms and the bulk materials. The properties of nanomaterial include the large fraction of surface atoms,high surface energy, spatial confinement**,** reduced imperfections which do not exist in the corresponding bulk materials.Nanomaterial differs from the others by two ways they have a relatively large surface area as compared to the same mass of material produced in a larger form, this makes material more chemically reactive and affects their strength as electrical properties and the quantum effects, can begin to dominate the behaviour of matter at the nanoscale affecting the optical, magnetic properties than the bulk counterpart (Singh *et al*., 2011). The use of nanoparticles for modulating immunity seems to be an obvious perspective.

Engineered nanoparticles are nonself and they overlap in size with viruses (mostly 10-200 nm size range), they fall well within the target size range to which immunity is capable to respond, and since at least some types of nanoparticles are toxic, they can be expected to result in danger signals as well.

2.2.1 TYPES AND CLASSIFICATION OF NANOMATERIAL

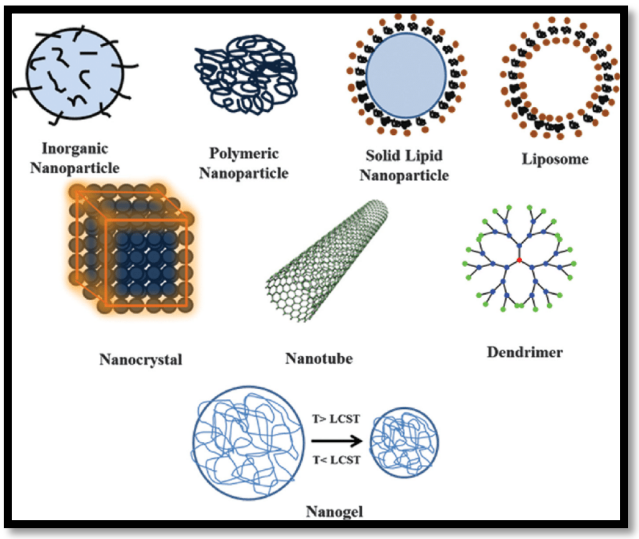
There are different types of nanoparticles which includes;

- Carbon-based nanomaterials: These contain carbon and are found in morphologies such as hollow tubes, ellipsoids or spheres. Fullerenes (C60), carbon nanotubes (CNTs), carbon nanofibers, carbon black and graphene (Gr).
- Inorganic-based nanomaterials, these are the metal and metal oxide nanoparticles and nanosystems. These nanomaterials can be synthesized into metals like Au or Ag nanoparticles, metal oxides such as TiO_2 and ZnO nanoparticles and semiconductors such as silicon and ceramics.
- Organic-based nanomaterials, these include nanomaterials made mostly from organic matter, excluding carbon-based or inorganic-based nanomaterials. The utilization of noncovalent (weak) interactions for the self-assembly and design of molecules helps to transform the organic NMs into desired structures such as dendrimers, micelles, liposomes and polymer nanoparticles.
- Composite-based nanomaterials, they combine nanoparticles with other nanoparticles or nanoparticles combined with larger or with bulk-type materials with one phase at nanometre scale to form large multiphase nanoparticle.

Nanomaterials are classified on the basis of the movement of electrons along the dimensions in the nanomaterials as 0D, 2D, 3D. All the dimensions of material are measured in the nanoscale (no dimensions are larger than 100 nm). The nanoparticles represent the zero dimensional nanomaterials.1-D materials include such as nanotubes, nanorods and nanowires wherein two of the dimensions are not confined to the nanoscale. 2-D nanomaterials exhibit plate-like shapes. Two-dimensional nanomaterials include nanofilms, nanolayers and nanocoatings. 3-D nanomaterials contain dispersions of nanoparticles, bundles of nanowires and nanotubes and also multinanolayers (Jeevanandham*et al.*, 2018).

FIGURE 1

TYPES OF NANOMATERIALS



2.3 APPLICATIONS OF MEDICAL NANOTECHNOLOGY

Applications of medical nanotechnology span across a variety of areas such as in drugs, medicines, therapeutics in diagnostics of diseases and abnormal conditions, in surgery, in medical robotics, in the general sake of increasing knowledge of the human body (Jong*et al*., 2008).

2.3.1 APPLICATIONS IN DRUGS AND MEDICINE

Nanotechnology can deliver medicine or drugs into specific parts of the human body, thereby making them more effective and less harmful to the other parts of the body. Anticancer gold nanoparticles have been found very effective. Gold “nanoshells” are useful to fight against cancer because of their ability to absorb radiation at certain wavelengths. Once the nanoshells enter tumor cells and radiation treatment is applied, they absorb the energy and heat up enough to kill the cancer cells. Not only gold but other elements can also be used (Jong*et al*., 2008).

2.3.2 APPLICATIONS IN SURGERY

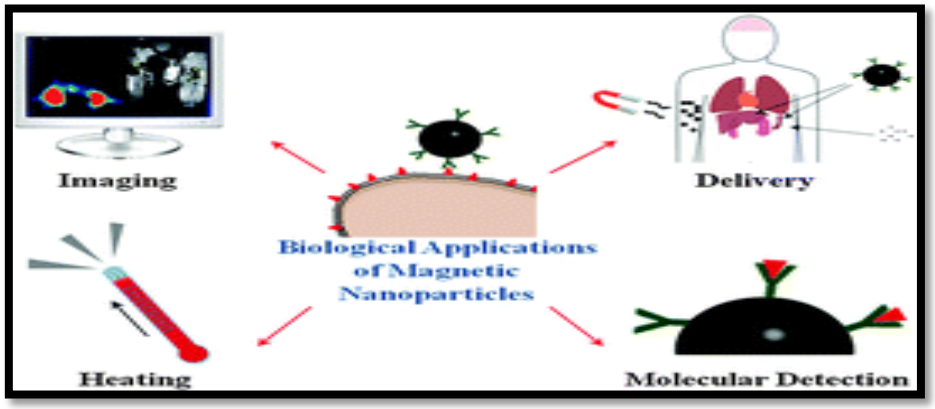
Minute surgical instruments and robots can be made which can be used to perform microsurgeries on any part of the body by using nanotechnology. Instead of damaging a large area of the body, these instruments would be precise and accurate, targeting only the area where surgery should be done. Nanotechnology can also improve the visualization of surgery. By using this technology, now computers can be used to control the nano-sized surgical instruments instead of conventional holding of instruments by the surgeons. The use of “Nanocameras” can provide close up visualization of the surgery. There is less chance of any

mistakes or faults. Surgery could also be done on tissue, genetic and cellular levels (Abeer, 2012)

- **Nano-robotics**, although having many applications in other areas, have the important uses in medical fields. Future medical nanotechnology expected to employ nanorobots injected into the patient to perform treatment on cellular level. The workings of cells, bacteria and viruses can be better explored and causes of relatively new diseases can be found and prevented (Rohit*et al*., 2014).
- **Restore vision:** Genome sequencing can be made much easier. Biological causes of mental diseases can be monitored and identified. Tissue engineering could also be done using nano-materials (Abeer, 2012).
- **Tissue engineering** makes use of artificially stimulated cell proliferation by using suitable scaffolds and growth factors based on nanomaterials. Advances in nanotechnology-based tissue engineering could also lead to life extension in humans and also in animals (Abeer, 2012).

FIGURE 2

APPLICATIONS OF NANOTECHNOLOGY



(Columbo*et al*., 2012)

2.4 DIABETES MELLITUS

Diabetes Mellitus (DM) is a systemic metabolic disease characterized by hyperglycemia, abnormal elevated levels of lipid and fat in blood and hypoinsulinaemia which now assumed epidemic proportions in many countries of the world. India presently has been infamously known as the “diabetic capital of the world”, has the largest number of diabetic patients in the world.The symptoms of type 1 diabetes includes polyuria (frequent urination), polydipsia (increased thirst), polyphagia (increased hunger) and weight loss.Drug therapies have been in use for the treatment of diabetes and some of the standard synthetic

drugs used for the treatment of diabetes are sulfonylureas, biguanides, α-glucosidase inhibitors and glinides. These synthetic drugs cause side effects like nausea, vomiting, abdominal pain, diarrhoea, head ache, abnormal weight gain, allergic reaction, low blood glucose, darkurine, fluid retention or swelling. These drugs are also not safe for use during pregnancy. Therefore, now traditional plants has been searched and processed for the discovery of new antidiabetic drugs as an alternative to the synthetic drugs (Vijalakshmi*et al*., 2014).

2.4.1 ALPHA AMYLASE

The α–amylase (α -1, 4-glucan-4- glucanohydrolases) is one of the major secretory products of the pancreas which constitute about 5-6% and salivary glands, playing a role in digestion of starch and glycogen. They are found in microorganisms, plants and higher organisms. They belong to family of endoamylases that catalyse the initial hydrolysis of starch into shorter oligosaccharides through the cleavage of α-D-(1-4) glycosidic bonds. The end products of α-amylase action are oligosaccharides which are of varying length that have an α-configuration and α-limit dextrins, which constitute a mixture of maltose, maltotriose and branched oligosaccharides of 6–8 glucose units that contain both α-1,4 and α-1,6 linkages. Alpha amylase inhibitors have the ability to decrease the high glucose levels in animals that can occur after a meal by slowing the speed with which alpha amylase can convert starch to simple sugars. This is more importance in diabetic people where low insulin levels prevent the fast clearing of extracellular glucose from the blood. Therefore diabetics tend to have low alpha amylase levels in order to keep their glucose levels under control. Plants also use alpha amylase inhibitors as a defence mechanism as a protection from the coming insects, this inhibitors alter the digestive action of alpha amylases and proteinases present in the gut of insects and this will inhibit their normal feeding behaviour. Therefore, alpha amylase inhibitors can control blood sugar levels (Sales *et al*., 2012).

2.4.2 PROTEIN GLYCOSYLATION

Protein molecules can universally bind non-enzymatically with glucose or other sugars to form initially unstable aldimine and ketamine versions of the original protein and then, later, they become more stable structures. The degree to which this happens is proportional to the concentration of the sugar in the surrounding medium in which the protein molecules are present and to the duration of the exposure of the protein to this medium. The most important of this is the reaction with glucose and this is called as ‘glycation’ or ‘glycosylation’. Glycosylation is the only sugar adduction process to occur both intra and extracellularly. Diabetic patients with raised plasma glucose levels have proportionally more

glycation occurring. The affect this has on various protein structures and consequently functions has been implicated in the mechanisms of several diabetic complications. Haemoglobin A (HbA) β chains is one of the best known example of a glycated protein. The plasma levels of glycatedHbA are used to monitor overall glycaemic control in the preceding 2–3 months. The time period corresponds to the natural average lifespan of the HbA molecule in red blood cells (Stanway and Grill, 2000)

2.4.3 GLUCOSE UPTAKE BY YEAST CELLS

In the diabetic patients, regulation of glucose level in the blood can prevent the various complications associated with the disease. The maintenance of plasma glucose concentration for a long term under a selection of dietary conditions is one of the most significant and closely regulated processes observed in the mammalian species (Santhiya*et al*., 2012).

The characteristics of sugar transport system in yeast have been receiving renewed attention nowadays. All yeast presently known is able to utilize one or more sugars as their principle source of carbon for energy. The yeast converts the sugars into ethanol. The inhibition of such glucose uptake by yeast cell can be considered as an important tool for evaluation of antidiabetic property of traditional plant based compounds (Jijjithand Jayakumari, 2017).

2.4.4 NON-ENZYMATIC GLYCOSYLATION

Glucose reacts non-enzymatically with the NH2-terminal amino acid of the beta chain of human haemoglobin by forming ketoamine linkage, this results in the formation of haemoglobin AIc. Other minor components also appear to be adducts of glucose 6-phosphate and fructose 1,6-diphosphate. These haemoglobins are formed slowly and continuously throughout the 120-day life-span of the red blood cell, which results in a two- to threefold increase in haemoglobin A1c in the red cells of patients with diabetes mellitus. By providing an integrated measurement of blood glucose, haemoglobin A1C is useful parameter in assessing the degree of diabetic control. Furthermore, this haemoglobin is a useful model of non-enzymatic glycosylation of other proteins that may be involved in the long-term complications of diabetes mellitus (Bunn *et al*., 1978).

2.5 DIABETIC FOOT ULCER

Ulceration of the foot in diabetes is common and disabling which frequently leads to amputation of the leg. Mortality is high and healed ulcers can often recur. The pathogenesis of foot ulceration is very complex, clinical presentation variable and management requires early expert assessment. Interventions should be directed at infection, peripheral

ischaemiaand abnormal pressure loading caused by peripheral neuropathy and limited joint mobility. Despite treatment, ulcers can readily become chronic wounds. Diabetic foot ulcers have been neglected in health-care research and planning and clinical practice is based more on opinion than scientific fact (Jeffcoate and Harding, 2003).

Furthermore, the pathological processes are poorly understood and poorly taught about the DFU. In diabetes, nerve damage occurs from interacting metabolic abnormalities, worsened by disease of the vasa nervorum. The damage results in affecting the peripheral sensation, innervation of the small muscles of the foot and fine vasomotor control of the pedal circulation. Loss of protective sensation leads to lack of awareness of incipient or actual ulceration in the case of sensory neuropathy. Motor neuropathy will affect the muscles needed for normal foot movement by altering the distribution of forces during walking and causing reactive thickening of skin (callus) at sites of abnormal load. Next, ischaemic necrosis of tissues beneath the callus leads to breakdown of skin and subcutaneous tissue, which results in a neuropathic ulcer with a punched out appearance (Jeffcoate and Harding, 2003).

2.6 MEDICINAL PLANTS

Medicinal plants have been used in healthcare since time immemorial healing with medicinal plants is as old as mankind itself. The connection between man and his search for drugs in nature starts from the far past, of which there are several evidence from various sources which includes written documents, preserved monuments and even original plant medicines. Awareness of medicinal plants usage may be as a result of the many years of struggles against various diseases due to which man learned to pursue drugs from barks, seeds and other parts of the plants. Contemporary science has acknowledged their active action and it has included in modern pharmacotherapy a range of drugs of plant origin, known by ancient civilizations and are using throughout the millennia. The knowledge of development of ideas associated with the usage of medicinal plants as well as the evolution of awareness has increased the ability of pharmacists and physicians to respond to the challenges that have emerged with the spreading of professional services in facilitation of man's life (Kumar and Singh, 2014).

As a source of medicine, medicinal plants have always been at forefront virtually of all cultures of civilizations. Medicinal plants are regarded as rich sources of traditional medicines and from these medicinal plants many of the modern medicines are produced. For thousands of years medicinal plants have been used for many purposes like to treat health

disorders, to add flavour and conserve food and to prevent diseases epidemics. The secondary metabolites such as flavonoids that are produced by the plants are usually responsible for the biological characteristics of plant species used throughout the world. The microbial growth in diverse situations can also be controlled by plant derived products.There is a promising future of medicinal plants as there are about half million plants around the world and most of them are not investigated yet for their medical activities and their hidden potential of medical activities could be decisive in the treatment of present and for future studies (Sofowara, 2013)

2.6.1*Helictere isora*

Helicteres isora Linn. (*H. isora*), Avartani is a medicinal plant which is used in treating several diseases. *Helicteres isora* iscommonly known as Marodphali, Marorphali and Enthani due to screw like appearance of its fruit. It is not described broadly in old text of Ayurveda, *i.e.* Samhitas and Nighantu. Avartani is found distributed in dry forests throughout Central and Western India, from Bihar as far West as Jammu and Western Peninsula. It is sub-deciduous small tree or shrub of about 1.5-3.0 m height. Young branches are rough with scattered stellate hairs. The leaves are serrate, obliquely cordate or ovate, shortly acuminate and rough above and pubescent beneath. The flowers are solitary or in sparse clusters with red reflexed petals, become pale-blue when old. The fruits are 5.0 cm long, greenish-brown, beaked and cylindrical with 5 spirally twisted carpels. The seeds are tubercled. Fruits, seeds, bark and roots of the plant are used. The flowering time of *H. isora* is from April to December and the fruiting time is from October to June (Kumar *et al*., 2016).

Helicteres isora is used as antigastrospasmodic, anthelmintic, antispasmodic, antipyretic, antidiarrheal, antidysenteric and as a tonic after childbirth. Stems of this plant are used as anthelmintic, colic and aphtha, while fruits are used as colic, anticonvulsant and abdominalgia. Traditionally, the root juice is claimed to be useful in diabetes, emphysema and snakebite. From the roots, betulic acid, daucosterol, sitosterol, isorin were isolated. Cucurbitacin B and isocucurbitacin B were isolated and reported to possess cytotoxic activity (Sutradhar and Saha, 2016).

2.7 SILVER NANOPARTICLES

Silver nanoparticles (AgNPs) are used in many fields, including medical, food, health care, consumer and industrial purposes, because of their unique physical and chemical properties. These include their optical, electrical and thermal, high electrical conductivity and applications, including as antibacterial agent, household, industries and healthcare-related products, in consumer products, medical device coatings, optical sensors and cosmetics,

pharmaceutical industry, the food industry, diagnostics, orthopaedics, drug delivery, as anticancer agents and have increased the tumour-killing effects of anticancer drugs.

The biological activity of silver nanoparticles depends on factors such as its surface chemistry, various size, size distribution, shape, particle morphology, particle composition, coating or capping, agglomeration and dissolution rate, particle reactivity in solution, efficiency of ion release and cell type and the type of reducing agents used for their synthesis which decides it cytotoxic effect (Zhang *et al*., 2016).

2.8 ZINC OXIDE NANOPARTICLES

Zinc oxide is an inorganic compound whose chemical formula is ZnO. It is a white powder which is nearly insoluble in water. Zinc oxide (ZnO) nanoparticles have received a remarkable consideration due to their extra ordinary UV filtering properties, photochemical antifungal, high catalytic and antibacterial activities. Zinc Oxide (ZnO) is an exasperating material for applications in electronics, photonics, acoustics and sensing. Zinc Oxide nanoparticle is a unique material that exhibits itself as semiconductor and piezo-electromaterial properties (Uikey and Vishwakarma, 2016).

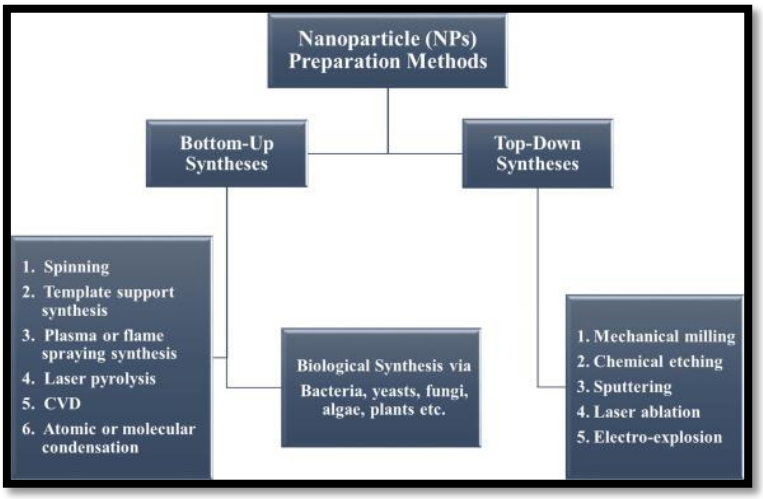
Zinc oxide is used as an additive in numerous materials and products such as ceramics, glass, cement, rubber (car tyres), lubricants, paints, ointments, adhesives, plastics, pigments, foods (source of Zn nutrient), batteries, ferrites and fire retardants (Sabir*et al*., 2014).

2.9 METHODS OF SYNTHESIS OF NANOPARTICLES

Synthesis of nanomaterial is a major milestone in the modern science. Material scientists and engineers have significantly marked in the development of synthesis methods of nanoparticles. Nanotechnology enables us to synthesis nanomaterials of needed size and characteristics. Synthesis of nanomaterials can be classified into bottom-up and top-down approach. Bottom up approach involves building up of the atom or molecular constituents whereas top down method involves making smaller and smaller structures to nanomaterial through etching from the bulk material as exemplified by the semiconductor industry (Rajput, 2015).

FIGURE 3

SYNTHESIS METHODS OF NANOPARTICLES



(Khan *et al*., 2017)

Nanoparticles can be synthesised by three ways which includes chemical, physical and biological methods. Some of the commonly used physical and chemical methods include chemical reduction, sol-gel method, inert gas condensation, laser ablation, solvothermal synthesis (Singh *et al*., 2011).

Due to high cost procedures and other adverse effects, green synthesis is preferred over chemical and physical methods. Biological synthesis using microorganism, enzymes, fungus and plant or plant extracts is an eco-friendly approach. Microorganisms both prokaryotes and eukaryotes can be used for metallic nanoparticle synthesis (Hasan, 2015).

2.9.1 GREEN SYNTHESIS OF NANOPARTICLES

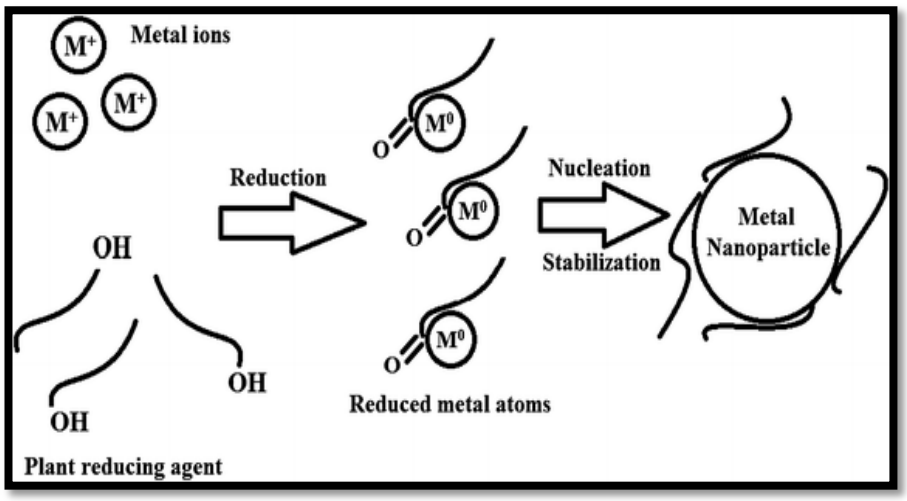
Biological methods of nanoparticle synthesis using have been suggested as possible eco-friendly alternatives to chemical and physical methods. For this microorganism, enzymes, fungus and plants or plant extracts can be used. The synthesis of nanoparticles using plants or parts of plants is more advantageous over other biological processes by eliminating the elaborate processes of maintaining microbial cultures (Ponarulselvam*et al*., 2012).

In order to combat the toxicity of metals and maintain homeostasis plants have their own several cellular structures and physiological processes. They also possess the ability to detoxify metals, the methods of detoxification includes immobilization, exclusion, chelation and compartmentalization of the metals ions and the expression of more general stress

response mechanisms, such as ethylene and stress proteins. The plant kingdom has ability to tolerate inimical concentrations of toxic metals which is known for ages. Plants have the ability to accumulate high concentrations of metals, observed for both essential nutrients including copper (Cu), iron (Fe), zinc (Zn), and selenium (Se), as well as non-essential metals, including cadmium (Cd), mercury (Hg), lead (Pb), aluminium (Al) and arsenic (As) (Keat*et al*., 2015).

FIGURE 4

MECHANISM OF PLANT MEDIATED SYNTHESIS OF METAL NANOPARTICLES



(Keat*et al*., 2015)

2.10 CHARACTERISATION OF NANOPARTICLES

The physicochemical properties of nanoparticles decide its behaviour, bio-distribution, safety and efficacy. Therefore, characterization of nanoparticles is important inorder to evaluate the functional aspects of the synthesized particles. Characterization is performed using a variety of analytical techniques, including UV-Visible spectroscopy, X-ray diffractrometry (XRD), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM) (Zhang *et al*., 2016).

2.10.1UV-VISBLE SPECTROSCOPY

UV-Vis spectroscopy (UV-Vis) is a relatively facile and low-cost characterization method that is often used for the study of nanoscale materials. It measures the intensity of light reflected from a sample and compares it to the intensity of light reflected from a reference material. The optical properties of nanoparticle that are sensitive to size, shape, concentration, agglomeration state and refractive index of the nanoparticle surface, makes

UV-Visible spectroscopy an important tool for the identification, characterization and investigation of the nanoparticles and evaluate the stability of nanoparticle colloidal solution (Suri*et al.*, 2017).

The principle of Ultraviolet-visible spectroscopy or ultraviolet-visible spectrophotometer (UV- Vis) involves the spectroscopy of photons in the UV-Visible region. It uses light in the visible and adjacent near ultraviolet (UV) region and near infrared (NIR) ranges. Molecules undergo electronic transitions in this region of the electromagnetic spectrum. UV-Vis spectrophotometers are mainly used to measure transmission or absorption in liquids and transparent or opaque solids (Suri*et al.,* 2017).

2.10.2 X-RAY DIFFRACTION (XRD) ANALYSIS

X-ray diffraction technique is avery useful characterization tool to study, non-destructively, the crystallographic structure, chemical composition and physical properties of materials and thin films. They can also be used to measure various structural properties of these crystalline phases such as strain, grain size, phase composition and defect structure. XRD determines the thickness of thin films, as well as the atomic arrangements in amorphous materials such as polymers. The diffraction pattern obtained from XRD is like a finger print of the crystal structure. It is a powerful and rapid technique which can be used for identification of an unknown material. In most cases, it provides an unambiguous phase determination. One of the major advantages is that it requires minimal sample preparation and also XRD units are widely available. The data interpretation is relatively straight forward (Sharma *et al*., 2012).

2.10.3 SCANNING ELECTRON MICROSCOPY (SEM)

Scanning electron microscopy is another characterisation imaging technique which provides information on the morphology, size, shape and the inner structure of nanoparticles. In addition to this, advanced Energy dispersive analysis of X-rays (EDAX) spectrum imaging has become possible which provides essential chemical characterisation of nanoparticulate materials. Scanning electron microscopy, produces images by recording various signals resulting from interactions of an electron beam with the sample as it is scans the sample surface. A fine electron probe, with a spot size from a few angstroms to several hundred nanometers, is generated by focusing electrons produced from an electron source. SEM in transmission mode (T-SEM) gives us a metrological tool for dimensional (lateral) measurements of nanoparticles, i.e., particle size distribution, but it is also well suited for "in-depth" observation of structured nanoparticles with high resolution (Hodoroaba*et al*., 2016).

2.10.4 FOURIER TRANSFORM INFRARED (FT-IR) SPECTRUM

Fourier Transform Infrared (FT-IR) spectroscopy is used to study the chemical composition of the mixture. The modern advance of infrared spectroscopic technique determines it as a reliable method, which is easy to implement for characterization of nanomaterials and investigation of phenomena that occur on the interface of the nanosized particles. IR spectroscopy can be successfully applied to investigate metallic nanomaterials (nanoparticles and nanowires). It is possible to characterize the surface state of nanosized objects by IR spectroscopy with Fourier transformation in the range of 4000 cm^{-1} - 400 cm^{-1} (Zaharie*v et al*., 2017).

2.10.5 ENERGY DISPERSIVE ANALYSIS OF X-RAYS (EDAX)

EDAX is a characterisation technique which gives an overall map of sample by analysing near surface elements and estimates their proportion at different position. This technique is usually used in conjunction with SEM. The energy of the beam is in the range of 10-20KeV and caused X-rays to be emitted from the material. The energy of the X-rays emitted depends on the sample we are using. EDAX is not a truly surface science technique because X-rays are generated only from a depth of 2 microns. It takes many hours to acquire because of low intensity. The composition or the amount of nanoparticles can be estimated by using EDAX (Joshi *et al*., 2008).

2.11 ANTIDIABETIC ACTIVITY OF SILVER AND ZINC OXIDE NANOPARTICLES

Medicinal plants and green synthesis of silver and zinc oxide nanoparticle have proven to be good sources of agents effective in the treatment of diabetes mellitus. Zinc is an essential metal which is an activator for more than three hundred enzymes in the body and they also plays a key role in different metabolic pathways including glucose metabolism. Zinc promotes hepatic glycogenesis through its actions on the insulin pathways and improves glucose utilization. Zinc is also known for their ability to keep the structure of insulin and has a role in insulin biosynthesis, storage and secretion. There are several zinc transporters in pancreatic beta cells like zinc transporter which can play a potent role in insulin secretion. In addition to this, zinc could improve insulin signalling by several mechanisms, which includes the increased insulin receptor phosphorylation, enhancing PI3K activity and inhibition of glycogen synthase kinase-3. Although silver is an important metal in a huge number of metabolic processes, there is no that much information about the effective power of silver or

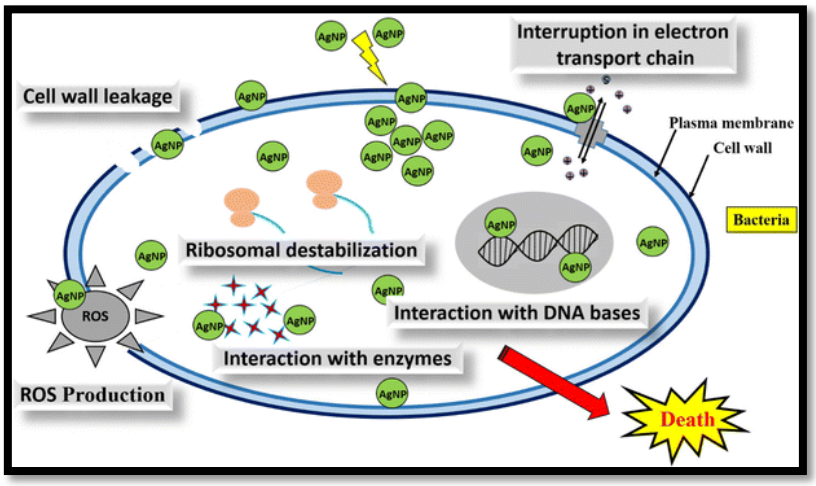
silver nanoparticles on the glucose status. Therefore, the effect of silver nanoparticles on the diabetics is more subjected to studies (Alkaladi*et al*., 2014).

2.12 ANTIMICROBIAL ACTIVITY OF SILVER AND ZINC OXIDE NANOPARTICLES

Silver ions and silver based compounds are highly toxic to several microorganisms, which make them interesting candidates for multiple applications in the medical field. The mechanism of antibacterial activity of silver nanoparticle is given in Figure 5.

FIGURE 5

ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES

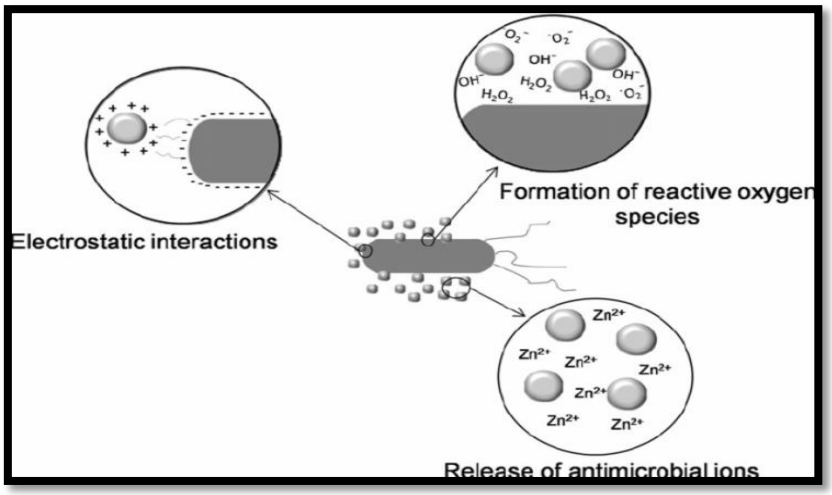


Silver is generally used as nitrate salt, but in the form of their nanoparticle form (Ag-NPs) the surface area is increased and thereby antimicrobial efficacy is greatly enhanced. Even though silver nanoparticles have use in many antibacterial applications, the action of this metal on microbes is not fully known. It has been hypothesized that silver nanoparticles can cause cell lysis or inhibit the growth via various mechanisms (Salem *et al*., 2015).

Inorganic antibacterial agents such as metal and metal oxides are advantageous compared to organic compound because of their stability. Zinc oxide has attracted a special attention as antibacterial agent among metal oxides. The mechanism of antibacterial activity of silver nanoparticle is given in following figure

FIGURE 6

ANTIBACTERIAL ACTIVITY OF ZINC OXIDE NANOPARTICLES



ZnO inhibits the adhesion and internalization of enterotoxigenic *bacteria* into enterocytes. In addition, ZnO nanoparticles (ZnO-NPs) exhibit antibacterial activity and have the ability to reduce the attachment and viability of microbes on biomedical surfaces. Several mechanisms have been reported for the antibacterial activity of ZnO-Nanoparticles, for example these nanoparticles can interact with membrane lipids and disorganize the membrane structure, which leads to loss of membrane integrity, malfunction and finally to bacterial death. ZnO can penetrate into bacterial cells at a nanoscale level and result in the production of toxic oxygen radicals, which damage DNA, cell membranes or cell proteins and this will finally lead to the inhibition of bacterial growth and eventually to bacterial death (Espitia*et al*., 2016).

EXPERIMENTAL PROCEDURES

3.0 EXPERIMENTAL PROCEDURES

Nanotechnology is a multidisciplinary field that involves the design and engineering of materials at nanoscale. Biological synthesis of nanoparticle has been portrayed as an efficient, low cost and environmentally. Silver and zinc oxide nanoparticle are gaining much interest because of their potent antibacterial property. Medicinal are used for treating many disorders one such plant is *Helicteres isora* showing multifarious medicinal properties (Vijayaraghavan*et al*., 2017).

The present study was aimed to compare the effect of silver and zinc oxide nanoparticles conjugated with *Helicteres isora* fruit extracts for their antidiabetic and antibacterial activity. The experimental procedure employed in the present study is discussed below.

3.1 COLLECTION OF PLANT SAMPLE

The fruits of *Helicteres isora* were collected from the local market of Coimbatore. The sample was identified and authenticated by Botanical survey of India, TNAU, Coimbatore. Plate 1 represents *Helicteres isora* and its classification.

PLATE 1

***Helicteres isora* AND ITS SCIENTIFIC CLASSIFICATION**

Helicteres isora

Scientific Classification

CLASSSIFICATION	
Kingdom	Plantae
Class	Eudicots
Order	Malvales
Family	Malvalceae
Genus	Helicteres
Species	*H.isora*

3.2 PREPARATION OF ETHANOLIC EXTRACT OF FRUITS OF *Helicteres isora*

The collected *H.isora* fruits 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 of fruits of *H.isora*, 90 ml of aqueous solution of 1mM of silver nitrate was added and exposed to bright sunlight the change of colour takes place within few minutes from yellowish to reddish brown (Sulaiman*et al.,* 2013). Therefore, the silver nanoparticles (AgNP) were obtained by repeated centrifugation at 12000 rpm for 20 min and then removed 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 silver nanoparticles of ethanol extract of *H.isora* 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 coloured powder (Rehana*et al.,* 2017).

3.4 CHARACTERIZATION OF SILVER AND ZINC OXIDE NANOPARTICLES

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

3.4.1 UV-VISIBLE SPECTRA OF *Helicteres isora*

The UV spectroscopy is a useful study to confirm the bio-reduction of zinc oxide and silver nanoparticles. Bio-reduction of zinc nitrate into zinc oxide nanoparticles and reduction of silver ions in the presence of fruit extract of *H.isora* was confirmed from UV–Vis spectral measurements. These nanoparticles contain free electrons, these free electrons have the

probability to give rise to a SPR (Surface Plasmon Resonance) absorption band(Sathish and Satyanarayana, 2018)

3.4.2 SCANNING ELECTRON MICROSCOPY (SEM) OF *Helicteres isora*

The synthesized silver and zinc oxide nanoparticles were monitored morphologically 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 (Abhishekh*et al*., 2014)

3.4.3 EDAX SPECTRUM MEASUREMENTS OF *Helicteres isora*

The silver and zinc oxide nanoparticle is subjected to EDAX analysis in order to visualize shape, size and purity. The dried powders of the both nanoparticles were drop coated on to carbon film and tested using EDAX analyses.

3.4.4 FOURIER–TRANSFORM IR (FT-IR) ANALYSIS OF *Helicteres isora*

The infrared spectra for the green synthesized silver and zinc nanoparticles were attained for the identification of functional groups in a (Perkin Elmer Spectrum 2, Germany) spectrophotometer IR affinity-1 by employing KBr pellet technique and registering amplitude waves ranging from 450 to 4000 cm−1 .

3.4.5 X-RAY DIFFRACTION (XRD) STUDIES OF *Helicteres isora*

The phase variety and grain size of synthesized silver nanoparticles was determined by X-ray diffraction spectroscopy (Philips PAN analytical). The synthesized silver nanoparticles were studies with CUKα radiation at voltage of 30 kV and current of 20 MA with scan rate of 0.030/s. Different phases present in the synthesized samples were determined by X′ pert high score software with search and match facility (Mohan and Rajanadevi, 2016)

3.5 *IN VITRO* ANTIDIABETIC ACTIVITY OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF *Helicteres isora*

3.5.1 *IN VITRO* ALPHA AMYLASE INHIBITORY ACTIVITY OF *Helicteres isora*

In vitro alpha amylase inhibitory activity of silver and zinc oxide nanoparticles of *Helicteres isora* 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 *Helicteres isora*

Non-enzymatic glycosylation of haemoglobin inhibitory activity silver and zinc oxide nanoparticles of *Helicteres isora* was measured by the method of Chandrasekar*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 *Helicteres isora* 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 *Helicteres Isora*

Glucose diffusion inhibitory activity of silver and zinc oxide nanoparticles of *Helicteres isora* was studied by the method of Gallagher *et al*. (2002) and detailed procedure is given in Appendix IV.

3.5.5 *IN VITRO* PROTEIN GLYCATION INHIBITORY ACTIVITY OF *Helicteres Isora*

In vitro protein glycation inhibitory activity of silver and zinc oxide nanoparticles of *Helicteres isora* was done by the method explained by McPherson*et al.* (1998) and it is given in Appendix V.

3.6 DETERMINATION OF ANTIBACTERIAL ACTIVITY OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF FRUIT EXTRACT OF *Helicteres isora*

3.6.1. MICROORGANISMS

The bacterial strains used for assessing the antibacterial activity of include *Klebsiellapnueumoniae, 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 100mg/ml stock of crude plant extract was prepared for each plant by dissolving 100mg of

dried plant extract in 1 ml of 10% DMSO. 100µL of the stock extract was pipetted onto the holes to give a concentration of 10mg per hole. 100µL of 0.5mg/ml ciprofloxacin was also pipetted into one of the holes to give a final concentration of 0.05mg. This served as the positive control, while 100µL of 10% DMSO was pipetted into one of the holes, which served as the negative control.

3.6.3 MINIMUM INHIBITORY CONCENTRATON OF *Helicteres isora*

The MIC of the plant extracts was determined with some modification, as described by Eloff (1998). 100µL of nutrient broth was added to all the wells of a 96-well microtitre plate. 100µL 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 100µL of the mixture was discarded from the last row, thus leaving each diluted well with a volume of 100µL. The same procedure was carried out for ciprofloxacin (positive control), during which 100µL 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 20µL of Presto blue per well in duplicate and incubating it at 37°C for 30 min.

4.0 RESULTS AND DISCUSSION

The foundations of typical traditional systems of medication for thousands of years that exists have shaped from plants. The plants remain offer humans with new medicines. Some of the useful properties ascribed to plants have recognised to be flawed and medicinal plant treatment is predicated on the experimental findings of hundreds to thousands of years (Dar *et al.*, 2017).The planet is fertile with natural and medicinal plants. Medicative plants are now more focused than ever because they have the potential of producing many benefits to society indeed to mankind, particularly within the line of medicine and pharmacology. The medicinal power of these plants lies in phytochemical constituents that cause definite pharmacologic action on human body. Some of the most significant bioactive phytochemicals the plants contain are alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds and many more. These natural compounds form the foundation of modern prescription drugs (Ullah*et al*., 2014).

The convergence of nanometre size scale technologies and biological technologies has created the new field of nanobiotechnology. This field is concentrated on the creation, manipulation and use of materials at the nanometre scale for advanced biotechnology, at the forefront of this field is the synthesis of nanometre size scale particles via biological entities. Recent studies have shown that green biologically based methods using plants to synthesize nanoparticles are safe, cheap and an environment-friendly alternative (Shah *et al*., 2015).

In the present study, the ethanolic extract of fruits of *Helicteres isora* were used to synthesize silver and zinc nanoparticles. The study includes the characterisation of synthesised silver and zinc nanoparticles of *Helicteres isora* and comparing their efficacy for its antidiabetic and antimicrobial activities. The results obtained were furnished and discussed below.

4.1 SYNTHESIS OF SILVER NANOPARTICLES OF FRUIT EXTRACTS OF *Helicteres isora* (AgNP*Hi*)

Silver nanoparticle of fruit extract of *Helicteres isora* (AgNP*Hi*) were synthesized after 30 minutes of exposure to sunlight as shown in Plate 2.

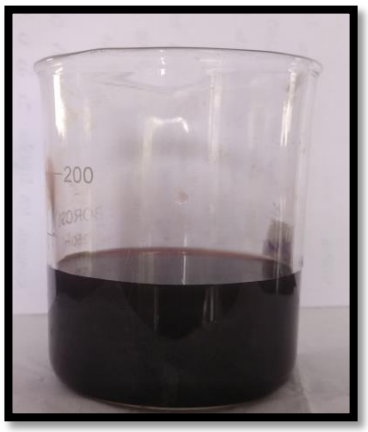
PLATE 2

SYNTHESIS OF SILVER NANOPARTICLES OF FRUIT EXTRACTS OF *Helicteres isora*

1mM Silver Nitrate

Fruit Extract

1mM Silver Nitrate+ Fruit Extract



Synthesized AgNP*Hi*

The silver nanoparticles of extracts of *Helicteres isora* synthesized on exposure to sunlight for 30 minutes resulted in a change in colour from pale yellow to brown. The plant extract acts as the stabilizing agent for the reduction of Ag^{+} to Ag^{o}. As the silver nanoparticles are formed, the colour of the solution changes from pale yellow to dark brown which is an indication of the presence of silver nanoparticles, the variation of the colour was due to the change in surface plasmon resonance of silver nanoparticles during the formation. The synthesized silver nanoparticle were centrifuged at 8000 rpm for 20 minutes and then dried to recover the silver nanoparticles in powdered form.

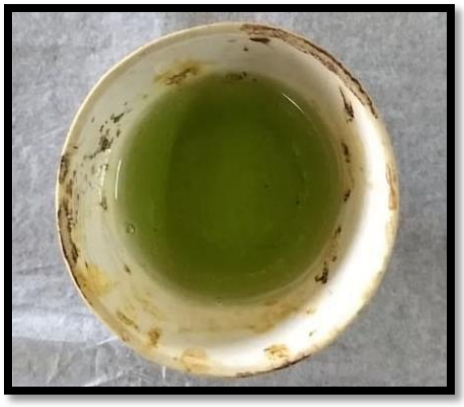
The samples mixture solution (silver nitrate solution and crude bio-surfactant) when exposed to sunlight changed from colourless to brown after 15-20 minutes, indicating the synthesis of silver nanoparticles (Das *et al*., 2016).Our findings were similar with that of work done by Krishnadhas*et al*. (2017) who reported that the synthesis of nanoparticle from *Volkameriainermis* exhibited a notable change in colour from yellow to brown and the intensity of brown colour is directly proportional to the increase in incubation period and temperature, which indicates the reduction of silver nitrate by the extract. Silver nanoparticle were produced by the addition of $AgNO_3$ to *M. oleifera* leaf extract samples which produced an instantaneous colour change from an initial yellow solution to brown colour (Moodley*et al*., 2018).

4.2 SYNTHESIS OF ZINC OXIDE NANOPARTICLES OF FRUIT EXTRACTS OF *Helicteres isora*

Zinc nanoparticles of ethanolic extract of *Helicteres isora* (ZnONP*Hi*) were synthesized and the result is shown in Plate 3.

PLATE 3

SYNTHESIS OF ZINC OXIDE NANOPARTICLES OF *Helicteres isora*



Plant extract

Plant extract + zinc nitrate

Zinc oxide nanoparticles

When the fruit extract of *Helicteres isora* was incubated with zinc nitrate, the colour changed from pale green to yellow after one hour, the precipitate was heated in furnace at 400°C for 2h, resulting in pale white coloured powder. Preliminary confirmation of nanoparticles synthesis was indicated by the visual change in the colour of the colloidal solution from brownish green to white. This change is caused due to coherent oscillation of electron gas at the surface of nanoparticles which results in Surface Plasmon Resonance

Manokari*et al*. (2016) synthesized zinc oxide nanoparticles from zinc nitrate using the aqueous plant extracts of *M. mercurialis* as a capping agent. On incubation at normal room temperature and pressure, yellow colour solution (reaction mixture) was obtained. According to the study of Shah *et al*. (2015) the zinc oxide nanoparticles are synthesized as the colour changed from pale yellow to pale brown when the leaf extract of *Camellia sinesis* was incubated with Zinc nitrate for one hour at room temperature. The change in colour indicates the formation of zinc oxide nanoparticles.

4.2 CHARACTERIZATION OF SILVER AND ZINC NANOPARTICLES OF FRUIT EXTRACTS OF *Helicteres isora*

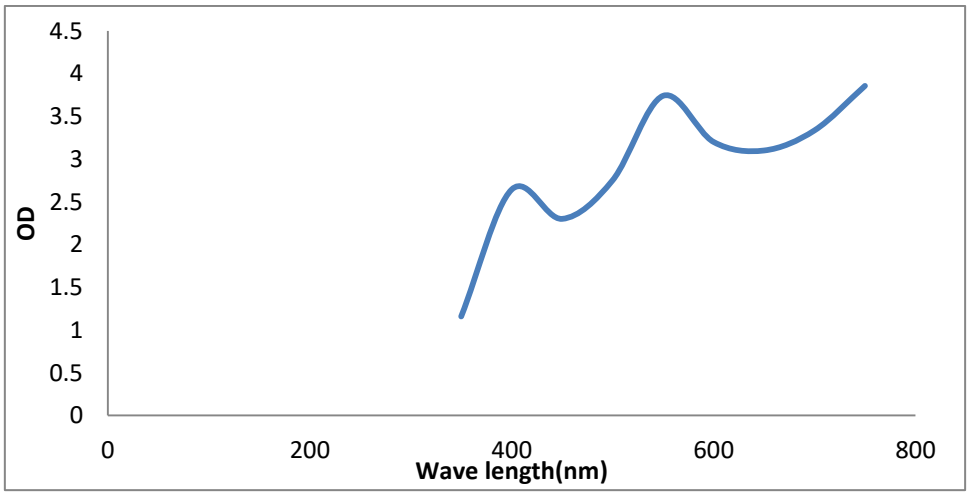
The characterization of silver nanoparticles synthesized from *Helicteres isora* was done by UV-Visible spectroscopy, Scanning Electron Microscopy (SEM) and Energy dispersive analysis of X-rays (EDX), Fourier transform infrared spectroscopy (FT-IR) and X-Ray Diffaction (XRD).

4.2.1 UV-VISIBLE SPECTROSCOPY OF SYNTHESIZED SILVER AND NANOPARTICLES OF FRUIT EXTRACTS OF *Helicteres isora*

Absorbance spectroscopy is used to determine the optical properties of a solution. A light is sent through the sample solution and the amount of absorbed light is measured. When the wavelength is varied and the absorbance is measured at each wavelength, the absorbance can be used to measure the concentration of a solution by using Beer-Lamberts Law. The UV-visible absorption spectrum of the *Helicteres isora* is shown in the Figure 7 and Figure 8.

FIGURE 7

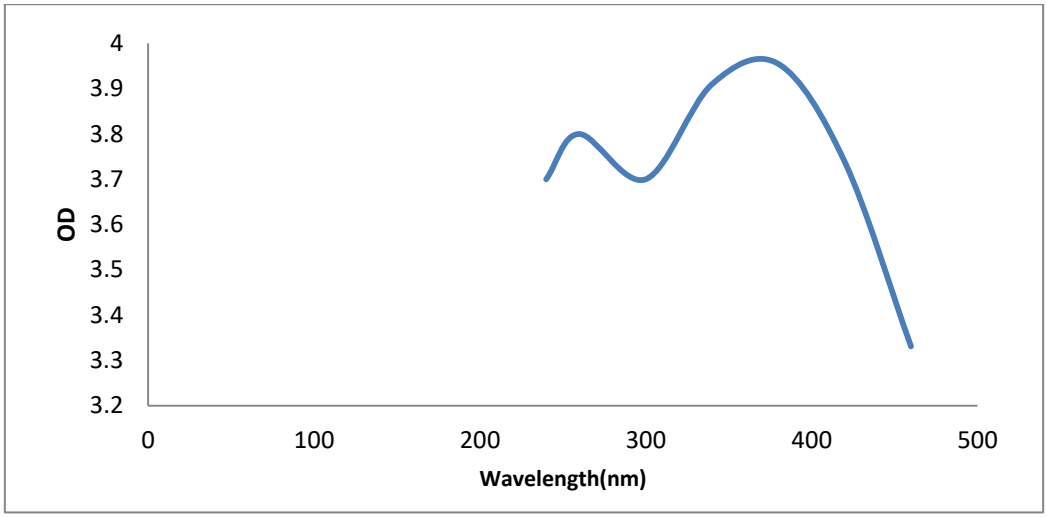
UV-VISIBLE SPECTRA OF SYNTHESIZED SILVER NANOPARTICLES OF *Helicteres isora*



From the above figure, it is clear that the absorb spectra of silver nanoparticles synthesized from fruits of *Helicteres isora* formed in the reaction media has absorption maxima at 400 - 600nm and the remarkable broadening of peak indicated that the particles polydispered. The absorption peak of *Helicteres isora* was found at 400nm and 550nm. This clearly indicates that there is an interaction between silver nanoparticles and biomolecules present in the fruits of *Helicteres isora.*

FIGURE 8

UV-VISIBLE SPECTRA OF SYNTHESIZED ZINC OXIDE NANOPARTICLES OF *Helicteres isora*



The optical measurement of UV-visible spectrophotometer has different absorbance peak like 410nm when treated with the *Nerium Oleander* plant extract after addition of aqueous 1mM Silver nitrate solution (Subbaiya*et al*., 2014). In case of *Azadirachtaindica* get synthesized with Iron nanoparticles by the indication of suitable surface Plasmon resonance with high band intensities and peaks was found through UV-visible spectroscopy at the range of 216- 265 nm. The UV-Visible characterisation of zinc oxide nanoparticle of *Nigella sativa* seed has obtained absorption spectrum of at 402 nm (Shahib*et al*., 2016).

4.2.1 SCANNING ELECTRON MICROSCOPY (SEM) and ENERGY DISPERSIVE SPECTROSCOPY (EDX) OF SYNTHESIZED SILVER AND ZINC NANOPARTICLES OF FRUIT EXTRACT OF *Helicteres isora*

The size and morphology of the synthesized silver nanoparticles of fruit extracts *Helicteres isora* 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 scanning electron microscope (SEM) and energy dispersive (EDX) images of silver nanoparticles of fruit extract of *Helicteres isora* (AgNP*Hi*) are depicted in the following figure 9 and 10.

FIGURE 9

SCANNING ELECTRON MICROSCOPY (SEM) IMAGES OF SILVER NANOPARTICLE OF *Helicteres isora*

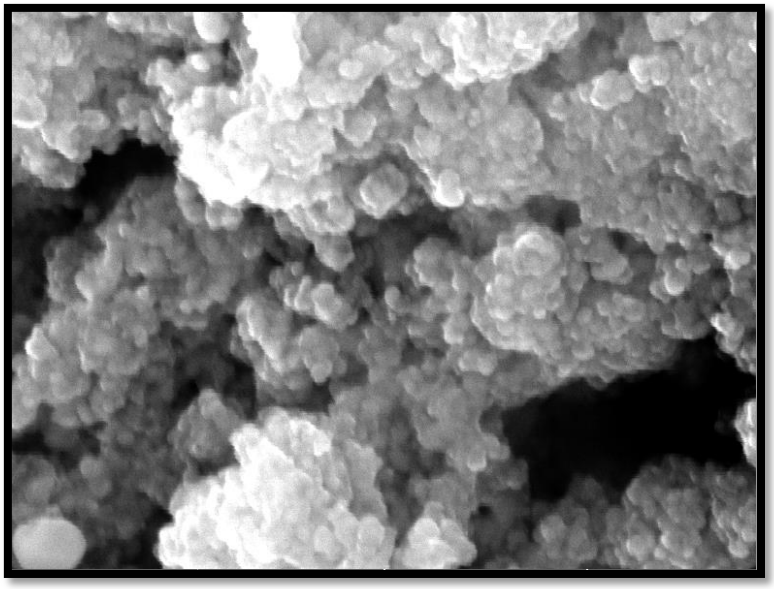
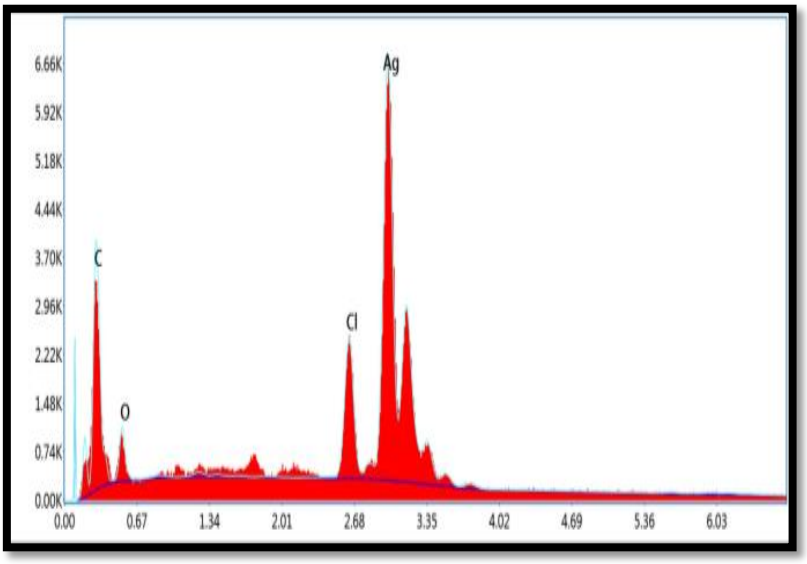


FIGURE 10

ENERGY DISPERSIVE X-RAY (EDX) OF SILVER NANOPARTICLE OF *Helicteres isora*



The SEM image revealed the presence of highly dense silver nanoparticle. The synthesized silver nanoparticle was found to be in the range of 1-10 µm with clearly observed spherical shapes. SEM- EDAX analysis confirmed that the nanoparticles are primarily composed of silver particles. EDAX quantitative analysis graph was obtained with a sharp peak between 2.68-3.35 KeV and confirms the nanostructure of the silver nanoparticles of fruit extracts of *Helicteres isora*. The silver nanoparticles synthesized from the fruits of *Helicteres isora* contains about 81.54 weight percentage Ag, 6.09 weight percentage carbon, 8.95 weight percentage Cl and about 3.42 weight percentage of oxygen.This result strongly confirms that *Helicteres isora* extracts might act as a reducing and capping agent in the production of silver nanoparticles.

Similar SEM analysis of nanoparticles synthesized using plant extract of *Kedrostisfoeditissima (jacq)*. Linn was carried out to understand the topology of silver nanoparticles, which showed the synthesis of monodisperse spherical Ag nanoparticles, with the size ranging from 20 to 25 nm (Nirmalaand Pandian, 2015).

SEM image showing the high density silver nanoparticles synthesized by the *Padinatetrastromatica* further confirmed the development of silver nanostructures. The SEM micrographs of nanoparticle obtained in the filtrate showed that the silver nanoparticles are spherical shaped, well distributed without aggregation in solution and their EDX spectrum

recorded showing sharp peak between 2.7 and 4 keV confirming the presence of silver (Jagadeeswaran*et al*., 2012).

A scanning electron microscopy employed to analyse the structure of the nanoparticles showed that the silver nanoparticles were spherical and cuboid in shape, with a size range of about 5-50nm. Polydispersed silver nanoparticles were also observed to be synthesized by *Cassia auriculata* flower (Paavani*et al*., 2013).

An EDAX study was used to confirm the formation of silver nanoparticles. The EDAX recorded showed strong signal of silver from 3 keV with weak signals from oxygen. Weak signals of oxygen are due to X-ray emission from carbohydrates or proteins or enzymes present within the leaves of *C. aromaticus*. Throughout the scanning range of binding energies, there is no peak to detect the impurity. This result indicated that the product was composed of high purity silver nanoparticles (Vanajaand Annadurai, 2013).

The scanning electron microscope (SEM) and energy dispersive (EDX) images of zinc oxide nanoparticles of fruit extract of *Helicteres isora* (ZnONP*Hi*) are depicted in the following figures 11 and 12.

FIGURE 11

SCANNING ELECTRON MICROSCOPY (SEM) IMAGES OF ZINC OXIDE NANOPARTICLES OF *Helicteres isora*

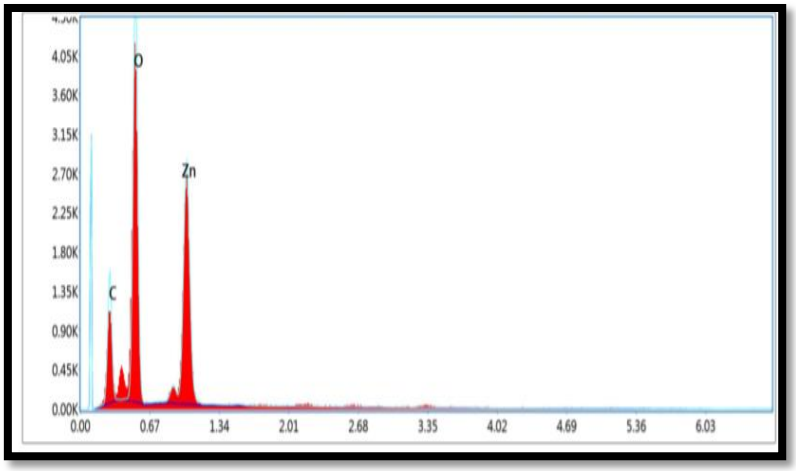
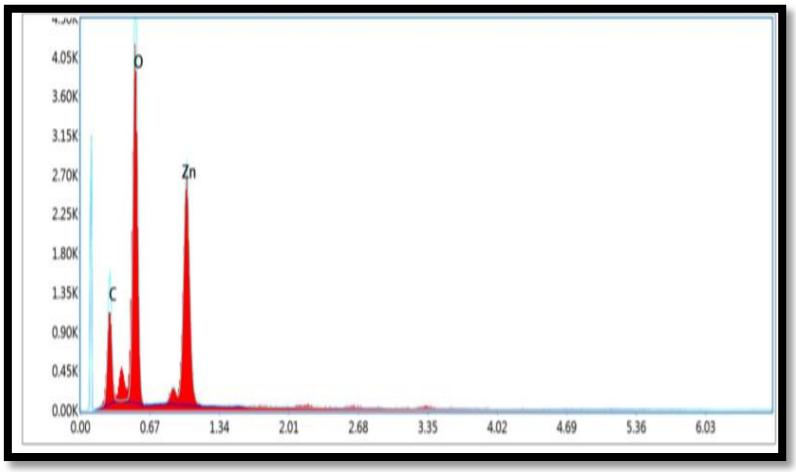


FIGURE 12

ENERGY DISPERSIVE X-RAY (EDX) OF ZINC OXIDE NANOPARTICLES OF *Helicteres isora*



The SEM images shows the structure of synthesized zinc oxide nanoparticles are flakes of size 50- 100 µm. EDAX quantitative analysis graph was obtained with a three sharp peak between 0.50-1.34 KeV and confirms the nanostructure of the zinc nanoparticles of fruit extracts of *Helicters isora*. The zinc nanoparticles synthesized from the fruits of *Helicters isora* contains about 37 weight percent Zn, 50 weight percent oxygen and 14 weight percent carbon.This result strongly confirms that *Helicteres isora* extracts might act as a reducing and capping agent in the production of zinc oxide nanoparticles.

Typical SEM micrographs of the zinc oxide nanoparticles of *P.hysterophorus* obtained by the biosynthesis method 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 or clustered forms shows the EDAX analysis of ZnONP's with 97.6 percent of zinc and 2.4 percent of oxides which confirms the elemental composition of zinc oxide nanoparticles. EDX spectrum shows peaks which were identified as zinc (97.6 percent) and oxygen (2.4 percent). The presence of copper peaks in the spectrum can be attributed to the use of copper grids for sample loading or to the fact that *Partheniumhysterophorus* is known to bioaccumulate copper (Datta*et al*., 2017).

Our results were similar to Sutradhar and Saha, (2015) that SEM image of zinc oxide nanoparticles using tomato *(Lycopersiconesculentum)* extract formed at 540W, clearly indicated their spherical shape and the size between 50 and 90 nm.

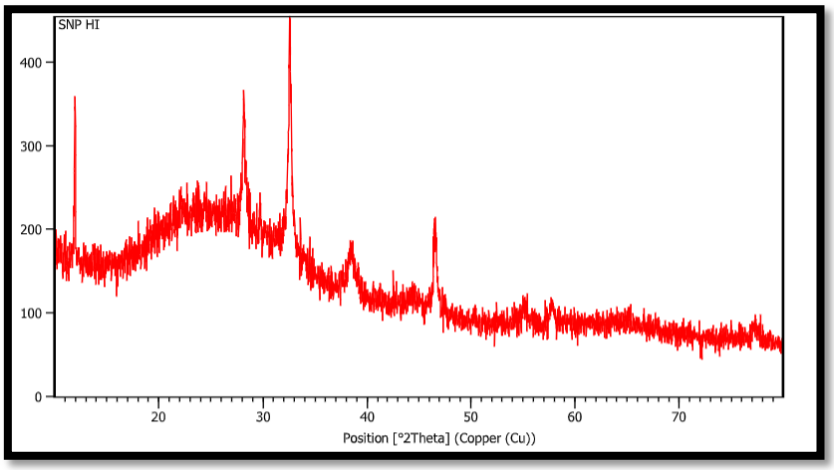
4.2.2 X-RAY DIFFRACTION (XRD) PATTERN OF SYNTHESIZED SILVER AND ZINC NANOPARTICLES OF FRUIT EXTRACT OF *Helicteres isora*

X-ray diffraction is a conventional technique for determination of crystallographic structure and morphology. There is an increase or decrease in intensity with the amount of constituent. This technique is used to establish the metallic nature of particles gives information on translational symmetry size and shape of the unit cell from peak positions and information on electron density inside the unit cell, namely where the atoms are located from peak intensities.

X-Ray Diffraction (XRD) images of silver nanoparticles of fruit extract of *Helicteres isora* (AgNP*Hi*) is depicted in the following figure 13.

FIGURE 13

X-RAY DIFFRACTION (XRD) OF SILVER NANOPARTICLES OF *Helicteres isora*



The nanoparticles synthesised in this method are characterized using XRD to confirm the particles as silver and to know the structural information. The above figure clearly shows the main peaks at (2θ) 38.1627, 32.5259, 46.5682 corresponding to the (57.35), (100.00) and (33.57) planes, respectively. By comparing Joint Committee on Powder Diffraction Standards (JCPDS) (file no: 89-3722), the typical pattern of green-synthesized silver nanoparticles may be found to possess an fcc structure.

The XRD pattern of biosynthesized silver nanoparticles using *Eclipta alba* leaf extract was confirmed by the characteristics peak observed in XRD image, four prominent diffraction peaks were observed at 2θ = 37.85◦, 44.0◦, 64.2◦ and 77.2◦, which correspond to (111), (200), (220) and (311) Bragg's reflections of the face-centred cubic (fcc) structure of metallic silver, respectively (Premasudha*et al*., 2015).

The XRD spectrum was recorded to confirm the crystalline structure of synthesized silver nanoparticles from *Chamaemelumnobile* extract. The diffraction peaks were observed at 38.1°, 44.2°, 64.4° and 77.4° in the 2θ range, the peaks can be indexed to the (111), (200),

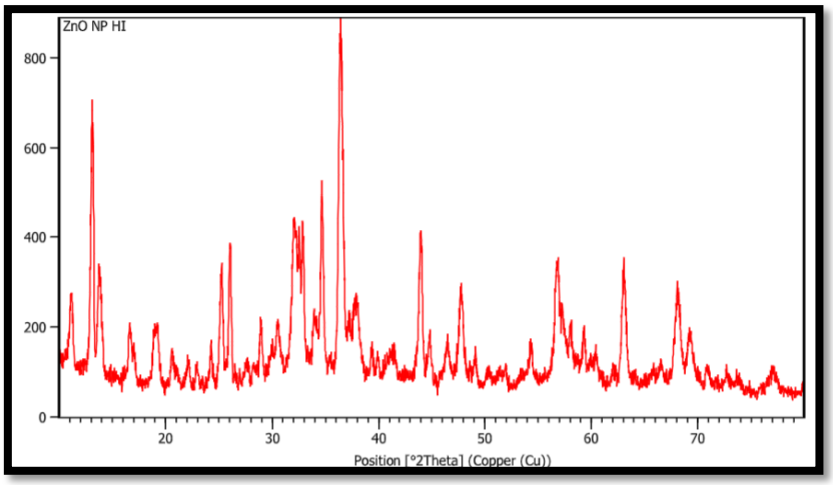
(220) and (311) reflections of face centered cubic structure of metallic silver (Erjaee*et al*., 2016). Similar results were observed in the XRD pattern of synthesized silver nanoparticles using *Coriandrumsativum* leaf extract done by Khan *et al*. (2018). The resulted peaks were found at 37.90°, 44.05°, 64.25° and 77.20° representing (111), (200), (220) and (311) which suggested the face centered cubic structure of silver.

Ganesan*et al*. (2014) suggest that the sharp bands of Bragg's peak authenticate that the particles are in the nanoregime and are stabilized by the reducing agents present in the leaf broth. The peaks for additional and yet unassigned bio-organic phase present on the surface of the silver nanoparticles are also indicated in the XRD pattern.

X-Ray Diffraction (XRD) images of zinc nanoparticles of fruit extract of *Helicteres isora* (ZnONP*Hi*) is depicted in the following figure 14.

FIGURE 14

X-RAY DIFFRACTION (XRD) OF ZINC OXIDE NANOPARTICLE OF *Helicteres isora*



The above figure is the XRD pattern recorded to know about crystalline nature of zinc nanoparticles. The peaks were obtained at 11.1662, 13.2061, 13.7745, 16.6317, 19.2419, 20.643, 22.091, 24.2526, 25.295, 26.0451, 28.941, 30.507, 3.0081, 32.8822, 33.9253, 34.6375, 36.3089, 37.8952, 44.0210, 44.8346, 46.4898, 47.7989, 49.1110, 54.3611, 56.7813, 59.3068, 63.1097, 68.1208, 69.2636, and 76.9978 at in the 2θ range, the peaks can be indexed to the 30.68, 78.80, 38.23, 19.59, 19.55, 10.59, 7.14, 10.40, 35.03, 45.91, 18.18, 17.48, 49.86, 47.08, 19.30, 60.50, 100.00, 21.97, 48.70, 15.03, 12.48, 29.68, 9.57, 11.02, 37.86, 14.54, 33.75, 28.37, 28.37, 14.09, 7.18 crystal planes. The various peaks in the XRD pattern may be assigned to the crystalline zinc oxide phase with the hexagonal wurzite structure.

Similar results of characterisation of zinc oxide nanoparticles synthesized using *Citrus sinensis* done by Luque*et al*. (2018) showed the presence of several diffraction peaks at 31.78, 34.44, 36.28, 47.55, 56.62, 62.83 and 67.96, that can be indexed to the (100), (002), (101), (102), (110), (103) and (112) crystal planes, respectively. They also indicated that this corresponds towurtzite crystalline phase.

Zinc oxide nanoparticles synthesized using *Corymbiacitriodora* leaf extract showed the peaks at 32.1°, 34.6°, 36.1°,47.7°, 56.4°, 63.1° and 68.1° and indexed to hexagonal wurtzite ZnO (Zheng*et al*., 2015).

Anvekar*et al*. (2017) has showed the XRD pattern of zinc oxide nanoparticles obtained from two plants *Adulsa* and *Lemongrass*. The peaks obtained in XRD pattern of ZnO nanoparticles prepared using *Adulsa* are indexed as 34.45º (100), 31.78º (002), 36.235º (101), 56.55º (102), 47.51º (110), 62.84º (103), 66.06° (200), 67.95º (112), 69.01° (201) and 78.82° (202). These peaks correlate to the hexagonal wurtzite structure of ZnO without any impurity peaks. XRD pattern for ZnO prepared using *Lemongrass* extract. These peaks are indexed as 31.74° (100), 34.40° (002), 36.23° (101), 47.52° (102), 56.59° (110), 62.82° (103), 66.30° (200), 67.88° (112), 69.00° (201) and 76.93° (202). This pattern also correlates to the Hexagonal wurtzite structure of ZnO.

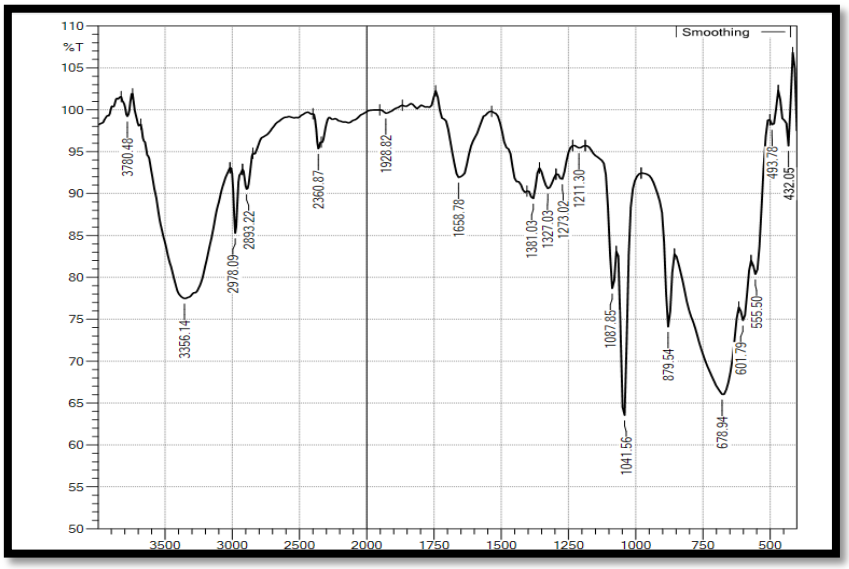
4.2.3FOURIER TRANSFORM INFRARED SPECTROSCOPY (FT-IR) OF SYNTHESIZED SILVER AND ZINC NANOPARTICLES OF FRUIT EXTRACT OF *Helicteres isora*

An infrared spectrum (IR) represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. An IR result is a positive identification (qualitative analysis) of every different kind of material. In addition, the size of the peaks in the spectrum is a direct indication of the amount of material present. With modern software algorithms, IR is an excellent tool for quantitative analysis. Because all of the frequencies are measured simultaneously, most measurements by FT-IR are made in a matter of seconds rather than several minutes (Ali *et al*., 2015 and Vahabi*et al*., 2011).

Fourier Transform Infrared Spectroscopy (FT-IR**)** images of silver nanoparticles of fruit extract of *Helicteres isora* (AgNP*Hi*) is depicted in the following figure 15

FIGURE 15

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FT-IR) OF SILVER NANOPARTICLE OF *Helicteres isora*



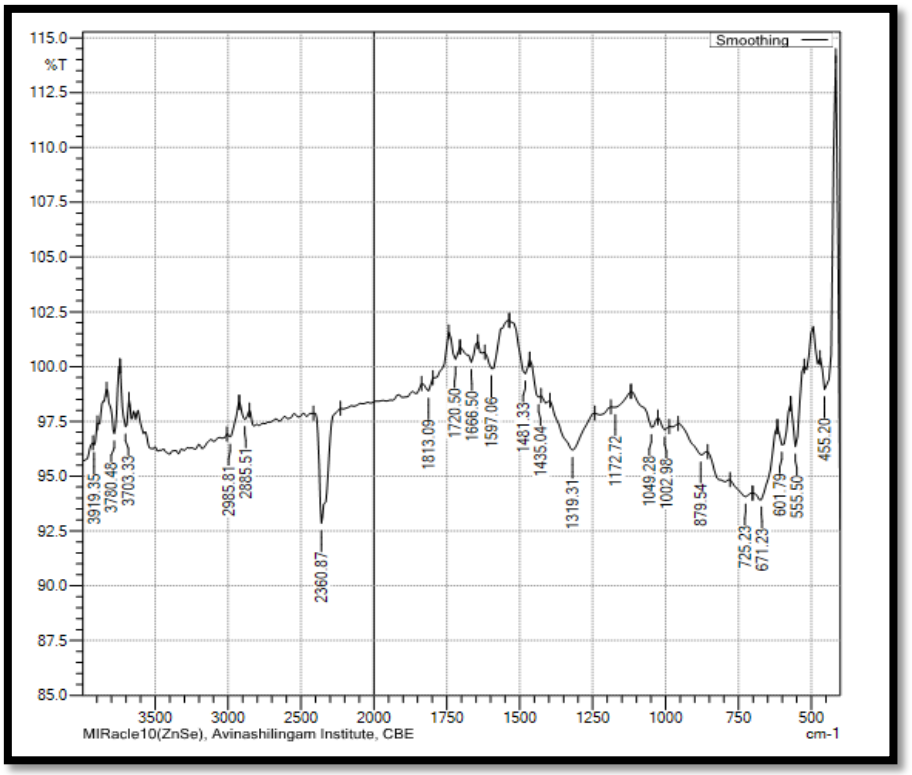
It is clear from the above figure that the peak 3780.49 cm^{-1} can be E-H stretching (E=B, C, N, O), 3356.14 cm^{-1} represents OH stretching vibration, 2978.09 - 2893.22 cm^{-1} represents C-S stretching modes in alkyls, 2360.87 cm^{-1} reveals C=N=O asymmetric stretch vibration, 1928.82 cm-1 represents E-X-double bonds (E=X=C, N, O), 1658.78 cm^{-1} represents C=C stretching of alkenes and aromatic functional groups, peaks between 1381.10cm^{-1}-1211.30 cm^{-1} reveals C-O stretch of alcohol, ethers and carboxylic acids, 1087.85 and 1041.56 cm^{-1} reveal stretch in C-N of amines, 879.546 cm-1 reveals C-H bending vibrations of alkenes, and the peaks between 862.12-476.38 cm^{-1} (678.94 cm^{-1}, 601.94 cm^{-1}, 601.79 cm^{-1}, 555.50 cm^{-1} , 493.78 cm^{-1} , 432.05 cm^{-1}) represents CHOH stretching.

FT-IR spectra of synthesized silver nanoparticles of fruit extracts of *Terminaliabellirica* indicates the obtained at 3718.76 – 3487.30 cm^{-1} assigned for C-H, O-H, N-H stretching vibrations of alcohol and phenolic compounds, the peaks 2978.09 – 2862.36 cm^{1} represents C-H stretching mode in alkanes. The distinct peaks 1728.22 cm^{-1} which is attributed to C=O stretching vibrations of aldehyde, ketones, carboxylic acids, esters, 1442.75-1334.74 cm^{-1} reveal scissoring and bending in C-H of alkanes, 1211.30 cm^{-1} reveal C-O stretch of alcohol, ethers, carboxylic acids, 1041.56 cm^{-1} reveal stretch in C-N of amines, 964.41–702.09 cm^{-1} reveal C-H bending vibrations of alkenes (Ali *et al*., 2011 and Vahabi*et al*., 2011).

Fourier Transform Infrared Spectroscopy (FT-IR**)** images of zinc nanoparticles of fruit extract of *Helicteres isora* (ZnONP*Hi*) is depicted in the following figure 16

FIGURE 16

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FT-IR) OF ZINC NANOPARTICLE of *Helicteres isora*



It is clear from the above figure, that the peak between 4000-3700cm^{-1} (3919.35,3780.48) represents be E-H-stretching (E=B, C, N, O), 2985.81 cm^{-1} represent C-H stretch in alkanes, 2360.87 cm^{-1} represent *C≡C*, peak between 1813.09 - 1666.50cm^{-1} represents –C- stretching, 1442.75-1334.74 cm^{-1} reveals scissoring and bending in C-H of alkanes; (1481.03,1435.04,13119.31cm^{-1}), 1280-1360cm^{-1} (1172.72,1049.28, 1002.98) reveals reveal stretch in C-N of amines, 964.41–702.09 cm^{-1} (879.54,725.23cm^{-1}) indicates C-H bending vibrations of alkenes and 862.12-476.38cm^{-1} (671.23,601.79,555.50, 455.20cm^{-1}) will represents CHOH bending.

Yedukar*et al*. (2016) shows different peaks of biosynthesized zinc oxide nanoparticles using *Ixoracoccinea* leaf extract. The peaks are at 3398.56 cm^{-1}OH represents stretching vibrations 2912.09 cm^{-1} represents the C-H stretch in alkanes, 2845.74 cm^{-1} indicates O-H stretch in carboxylic acid, 1561.06cm^{-1} gives C=C stretch in aromatic ring and C=O stretch

in polyphenols, 1461.45 cm^{-1} reveals C-N stretch of amide-I in protein, 1018.12cm^{-1} indicates C-O stretching in amino acid and 533.71cm^{-1} indicates hexagonal phase ZnO.

Another observation of Vennila*et al*. (2017) shows that the Zinc Oxide nanoparticles synthesized by gooseberry extracts have peaks at 3764cm^{-1} which is related to the (O-H) stretch, free hydroxyl alcohols, phenols compounds, 3448 cm^{-1} reveals (O-H) alcohols, 2881 cm^{-1} is (OH) stretch of carboxylic acids. The peak 2350 cm^{-1} (C=H) stretch indicates the alkynes group compounds and this wave number shifted to 1611cm^{-1} (2350 -1611 cm-1), 1611 cm^{-1} (C=O) indicates about the amides groups, 1398 cm^{-1} (C-H) indicating bent by an alkynes compounds, 1216 cm^{-1} (C-O) alcohols, ethers, carboxylic acids, 712cm^{-1} indicates alkynes (-C=CH) bent, 516cm^{-1} and 432 cm^{-1} is (C-Br) and stretch (C-I) stretch of very strong bonds and groups contain an alkyl halide. 516 cm-1 and the absorption at 432cm^{-1} band indicates Zn-O stretching vibration.

4.3 IN VITRO ANTIDIABETIC ACTIVITY OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF FRUIT EXTRACTS OF *Helicteres isora*

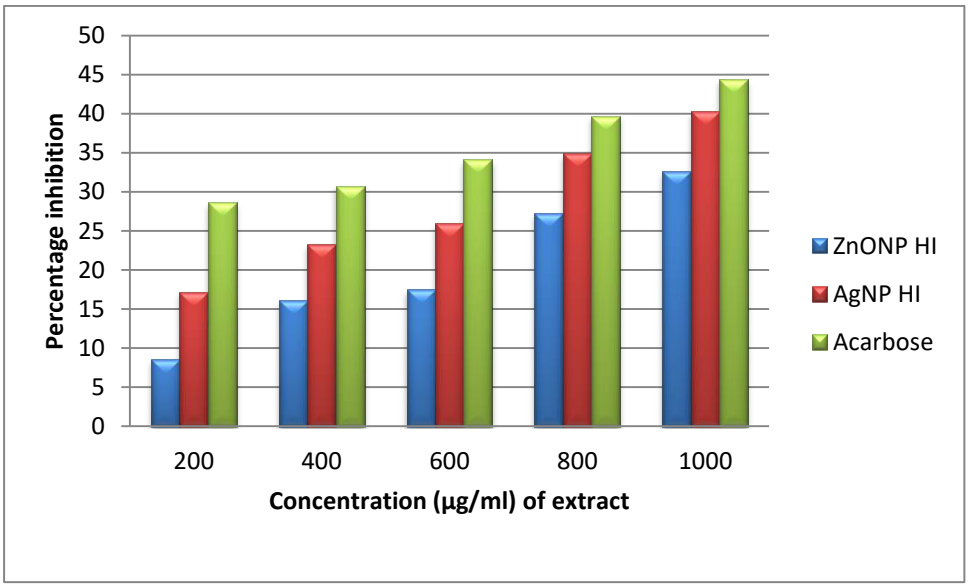
Diabetes mellitus is a chronic disorder in the metabolism of proteins, fats and carbohydrates. This can be described as an increase in blood glucose after any type of meal. Diabetes mellitus can results from either insulin deficiency or malfunction. In the past three decades, even though there is a significant progress made in the treatment of diabetes, the results of treatment in patients is still far from perfect. These treatments have some disadvantages that include drug resistance (reduction of efficiency), side effects and even toxicity. Therefore, today in order to overcome these disadvantages the use of medicinal plants is recommended to treat the disorder. Most plants contain carotenoids, flavonoids, terpenoids, alkaloids, glycosides and can often have antidiabetic effects. The antihyperglycaemic effects which results from treatment with plants are often due to their ability to improve the performance of pancreatic tissue which is done by increasing insulin secretions or reducing the intestinal absorption of glucose (Kooti*et al*., 2016).

In the present study, antidiabetic activity of synthesized silver and zinc oxide nanoparticles of different concentrations (200 – 1000 µg/ml) was compared by performing different *in vitro* assays such as alpha-amylase inhibition, non-enzymic glycosylation, glucose diffusion and glucose uptake by yeast cells.

4.3.1 INHIBITION OF ALPHA-AMYLASE ACTIVITY OF SYNTHESIZED SILVER AND ZINC NANOPARTICLES OF ETHANOLIC EXTRACTS OF FRUITS OF *Helicteres isora*

α-amylase is a key enzyme in carbohydrate metabolism. Inhibition of α-amylase is one of the strategies for treating diabetes. Amylase inhibitors are also known as starch blockers because they contain substances that prevent dietary starches from being absorbed by the body. Amylase inhibitor with starchy meal will reduce the usual rise in blood sugar levels (Bhagyalakshmi and Haritha, 2017). The inhibition of different concentrations of synthesized silver and zinc oxide nanoparticles of fruit extracts of *Helicteres isora* against α-amylase was evaluated and the results are depicted in figure 17.

FIGURE 17
ALPHA AMYLASE ACTIVITY OF SILVER AND ZINC OXIDE NANOPARTICLES OF *Helicteres isora*



From the above figure, it is clear that the α- amylase activity of the silver nanoparticles synthesized from the fruits of *Helicteres isora* was determined to be increased with increase in concentration from 200 –1000 µg/ml. The inhibitory effect of silver nanoparticle was found to be better compared to that of zinc oxide nanoparticle. The inhibition varied from 17.9 to 40.3percent for silver and 8.5 to 32.6percent for zinc oxide in the concentration range of 200 to 1000µg/ml whereas standard showed an inhibition ranging from 28.62 to 44.44percent. Thus according to results of our study, silver and zinc nanoparticle of plant extract inhibited the enzyme as that of Acarbose and also inhibited the hydrolysis of complex polysaccharides.

Vijayalakshmi*et al*. (2015) found that methanol extract of *S. brevistigma* showed significant inhibitory activity when compared with other plant extract. The inhibition varied from 49 to 68percent in the concentration range of 250 to 1000µg/ml. The results obtained

clearly suggested that the methanol extract of *S. brevistigma* is capable of effectively inhibiting the α-amylase activity.

Similar findings of antidiabetic activity of Ashwagandha root aqueous extract (ARAE) and Ashwagandha root aqueous extract nano particles (ARAENP) were given by Uddin*et al*. (2018). At 10mg/L concentration of each of acarbose, ARAE and ARAENP percentage inhibition was found as 58.16percent, 29.08percent and 81.67percent respectively. Percentage inhibition produced by acarbose is more than ARAE at all concentrations whereas percentage inhibition produced by ARAENP is more than both Acarbose and ARAE at all concentrations indicating its effectiveness.

The *in vitro* α-amylase inhibitory studies of Rehana*et al*. (2017) showed that the nanoparticles synthesized using *Tamarindusindica* exhibited higher α-amylase inhibition activity when compared with ZnO nanoparticles synthesized using other plant extracts. The percentage of inhibition showed a concentration-dependent reduction, which means that percentage of inhibition increased with increase in concentration. The percentage of inhibition varied from 89 to 3.2 percentresulting an increase in percentage inhibition with increase in concentration.

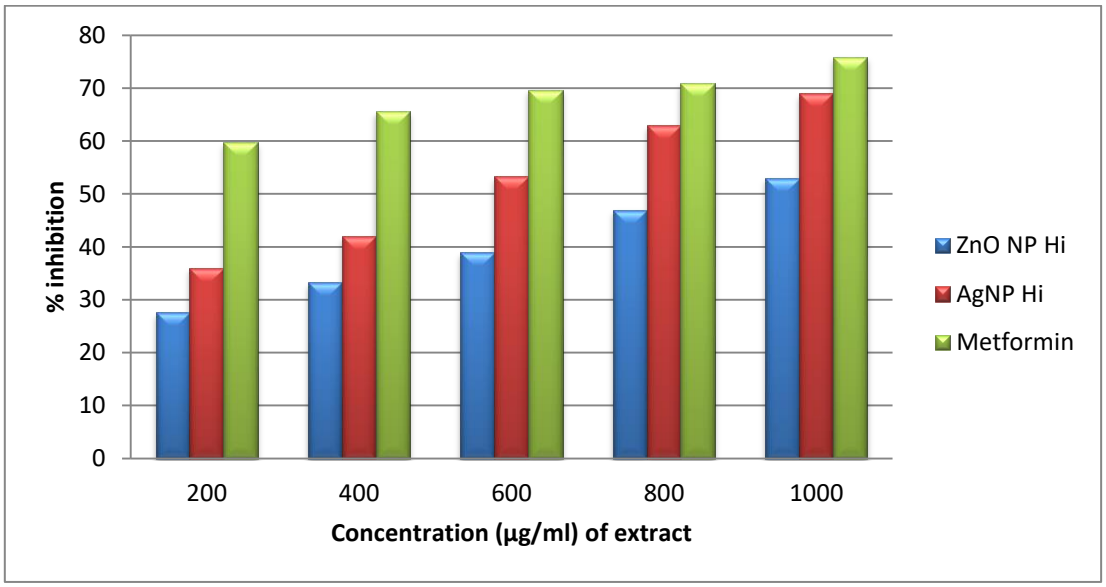
4.3.2 INHIBITION OF NON-ENZYMATIC GLYCOSYLATION OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT FRUITS OF *Helicteres isora*

Glucose reacts non-enzymatically with proteins *in vivo*, chemically forming covalently attached glucose-addition products and crosslinks between proteins. The excessive accumulation of rearranged late-glucose-addition products, or advanced glycosylation end products (AGEs), is believed to contribute to the chronic complications of diabetes mellitus.

The inhibition of different concentrations (200-1000µg/ mL) of synthesized silver (AgNP) and zinc oxide nanoparticles (ZnONP) of fruit extracts of *Helicteres isora* against non-enzymatic glycosylation was evaluated and the values are depicted in Figure 18.

Figure 18

NON-ENZYMATIC GLYCOSYLATION OF SILVER AND ZINC OXIDE NANOPARTICLES OF *Helicteres isora*



The above results showed an increase in the concentration of haemoglobin, resulting in an increased inhibition of glycosylation which is concentration dependent. The inhibition of glycosylation was greater with silver nanoparticles when compared with zinc oxide nanoparticles and both of them showed a good inhibitory activity. Metformin which is used as a standard expresses better inhibition of glycosylation when compared with the samples. At the highest concentration of silver and zinc oxide nanoparticle tested (1000µg/mL), maximum inhibition of glycosylation was observed (69.00percent and 64 percent). At the lowest concentration tested (200µg/mL) minimum inhibition of glycosylation was observed which is found to be 36 percent in silver nanoparticles and 27percent in zinc oxide respectively. The standard metformin drug showed maximum and minimum inhibition of 59.7 and 79.7 at the concentration 1000µg/mL 200µg/mL.

Our results were similar with that of the work done by Wilson *et al*. (2015) who have reported that silver nanoparticle synthesized from *Centellaasiatica* showed a good result when compared to that of standard drug α-tocopherol. The highest concentration 1000 µg/ml of silver nanoparticles and α-tocopherol showed a maximum inhibition 52.91.± 0.421 and 75.13 ± 0.314 while the lowest concentration 200µg/ml showed a minimum inhibition of 19.87 ± 0.633 and 24.19 ± 0.215 respectively.

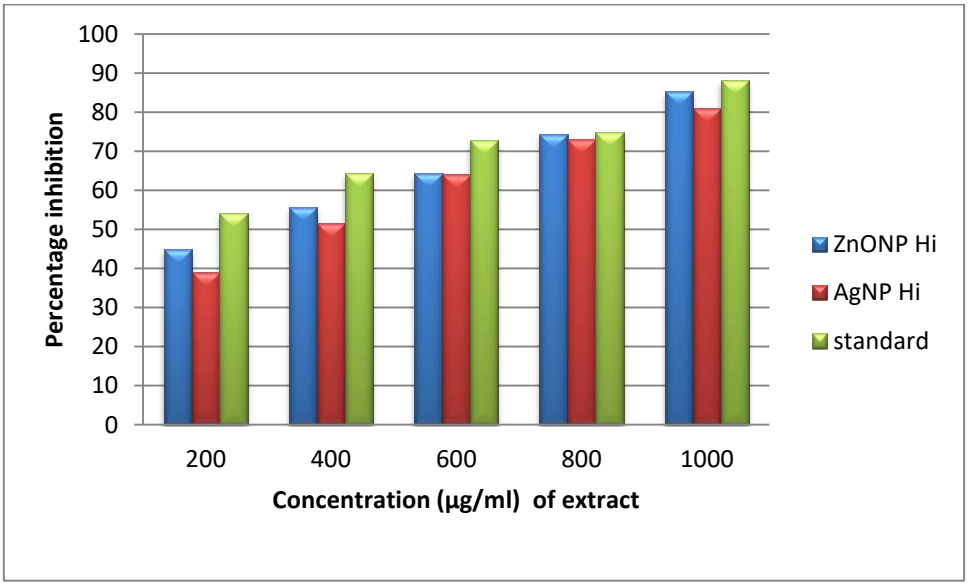
4.3.3 PROTEIN GLYCATION INHIBITION OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT FRUITS OF *Helicteres isora*

Glycation is a regular process that occurs in our body at a very low rate and is also a major cause of aging. The process mainly involves a non-enzymatic reaction between reducing sugars and protein residues. In the presence of elevated sugar levels, the formation of the glycation end products accelerates and hence becomes responsible for the secondary complications in patients with diabetes mellitus (Sachindra*et al*., 2018).

The results of inhibitory effect of silver (AgNP) and zinc oxide (ZnONP) nanoparticles towards AGE formation is depicted in the following figure 19

FIGURE 19

INHIBITION OF PROTEIN GLYCATION OF SILVER NANOPARTICLE AND ZINC OXIDE NANOPARTICLE OF *Helicteres isora*



From the above figure it is clearly indicated that the zinc oxide of *Helicteres isora* nanoparticle showed a better inhibition activity than silver nanoparticles when compared to the standard. Percentage inhibition of advanced glycation end products (AGE) by silver nanoparticles at 200µg/mL and 1000 µg/mL was 39 percent and 81 percent, and for zinc nanoparticles is 45 percent and 85 percent respectively. Also meteronidazol (200µg/mL and 1000µg/mL) also exhibited a significant reduction in AGEs formation when introduced to BSA-fructose medium.

Elosta*et al*. (2017) shows the increase in percentage inhibition of glucose uptake by yeast cells with extract concentration ranging from 1mg/ml to 5mg/ml. The amount of glucose remaining in the medium after a specific time serves as an indicator of the glucose uptake by the yeast cells. At lower concentration (i.e.) 1mg/ml the increase in percentage inhibition was somewhat linear but as concentration increases higher till 5mg/ml it tends to be more exponential.

4.3.4 GLUCOSE UPTAKE ASSAY BY YEAST CELLS OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACT FRUITS OF *Helicteres isora*

The characteristics of sugar transport system in yeast are similar to that of human. All sugars can be utilised by yeast. The inhibition of glucose uptake by yeast cell is important for the evaluation of antidiabetic activity.

Table 1 show results of glucose uptake assay by yeast cells of synthesized silver (AgNP Hi) and zinc oxide nanoparticles (ZnONP*Hi*) of ethanolic extract fruits of *Helicteres isora*

TABLE 1

GLUCOSE UPTAKE ASSAY BY YEAST CELLS OF SILVER AND ZINC NANOPARTICLE OF *Helicteres isora*

Glucose uptake at 5mM glucose concentration			
Concentration (µg/ml)	**Standard percentage**	**AgNP*Hi* Percentage**	**ZnONP*Hi*percentage**
200	6.89±1.15	8.30 ± 1.08	4.96 ± 0.52
400	9.50±2.39	13.33 ±0.47	9.13 ± 0.93
600	15.70±0.66	25.90 ± 0.65	16.40 ±0.56
800	23.74±1.75	32.13 ± 0.93	21.70 ± 0.93
1000	27.19±0.66	37.90 ±0.50	30.20 ±1.03
Glucose uptake at 10mM glucose concentration			
Concentration (µg/ml)	**Standard percentage**	**AgNP*Hi* Percentage**	**ZnONP*Hi*percentage**
200	13.81±0.51	11.90 ± 0.14	7.50 ± 0.42
400	14.41±0.90	18.60 ± 0.84	9.90 ± 0.47
600	23.72±1.37	32.10 ± 0.42	24.00 ± 042
800	25.52±0.51	42.30 ± 0.73	34.50 ± 0.42
1000	29.42±0.51	46.50 ±0.42	39.90 ±0.42
Glucose uptake at 25 mM glucose concentration			
Concentration (µg/ml)	**Standard percentage**	**AgNP*Hi* Percentage**	**ZnONP*Hi*percentage**
200	14.64±0.36	20.30 ± 0.47	13.70 ± 0.16
400	16.77±0.36	25.00 ± 0.51	20.60 ± 0.50
600	26.53±0.36	39.20 ± 0.28	25.37 ± 0.64
800	28.44±0.97	45.90 ± 0.65	36.60 ± 0.40
1000	31.43±0.39	62.10 ± 0.32	44.00 ± 0.70

From the above table, we can observe that the glucose uptake rate was increased with the increasing concentration of the nanoparticles and decreased with the increasing extracellular glucose concentration. It is stated that the transport of glucose across yeast cell membrane occurs by facilitated diffusion down the concentration gradient. Hence glucose transport occurs only if the intracellular glucose is effectively reduced (utilized). In our studies Silver nanoparticle showed maximum results than zinc oxide while comparing with the standard drug metronidazole.

Sinha*et al*. (2013) showed the increase in percentage inhibition of glucose uptake by yeast cells with extract concentration ranging from 1mg/ml to 5mg/ml. The amount of glucose remaining in the medium after a specific time serves as an indicator of the glucose uptake by the yeast cells. At lower concentration (i.e.) 1mg/ml the increase in percentage inhibition was found to be linear but as concentration increases higher till 5mg/ml it tends to become more exponential.

4.3.5 INHIBITION OF GLUCOSE DIFFUSION OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF FRUIT EXTRACT OF *Helicteres isora*

The potential of *Helicteres isora* synthesized silver and zinc nanoparticle to inhibit glucose diffusion into the external solution was investigated at certain time intervals. The obtained glucose diffusion inhibition results for silver and zinc oxide nanoparticles of *Helicteres isora* and acarbose standard are depicted in the following Table 2.

TABLE 2

INHIBITION OF GLUCOSE DIFFUSION OF SILVER AND ZINC OXIDE NANOPARTICLES OF *Helicteres isora*

Glucose diffusion at 1st hour			
Concentration (µg/ml)	**Standard**	**AgNP*Hi* Percentage**	**ZnONP*Hi*percentage**
200	24.68±2.13	22.20 ± 2.98	22.30 ± 5.83
400	32.09±4.27	28.60 ± 3.33	26.90 ± 6.00
600	35.79±5.66	33.10 ± 5.17	29.20 ± 4.99
800	39.50±2.14	37.90 ± 5.16	30.10 ± 2.06
1000	44.44±3.70	39.70 ± 4.32	33.90 ± 4.41
Glucose diffusion at 2nd hour			
Concentration (µg/ml)	**Standard percentage**	**AgNP*Hi* Percentage**	**ZnONP*Hi*percentage**
200	26.68±3.36	25.90 ± 2.32	22.20 ± 2.98
400	34.44±1.92	29.70 ± 2.74	30.33 ± 5.79
600	39.99±3.33	34.13 ± 2.97	32.60 ± 4.78
800	43.33±3.33	47.60 ± 4.90	36.10 ± 4.60
1000	49.99±3.33	43.10 ± 3.02	39.50 ± 4.42
Glucose diffusion at 3rd hour			
Concentration (µg/ml)	**Standard percentage**	**AgNP*Hi* Percentage**	**ZnONP*Hi*percentage**
200	27.95±1.86	27.10 ± 2.68	25.80 ±3.22
400	39.77±1.86	30.30 ± 5.73	32.60 ± 4.78
600	44.08±4.92	44.20 ±4.10	36.10 ± 4.62
800	50.53±1.86	50.66 ±2.49	39.50 ± 4.42
1000	55.90±1.86	53.10 ± 4.08	42.90 ± 4.19

From the above table it is clear that the silver nanoparticle showed the most effective inhibitory effect on the glucose movement than zinc oxide nanoparticles. Silver nanoparticle showed an effect that is almost equal to the effect of standard and also zinc oxide nanoparticles showed comparative good results. In the presence of 1000g/ml of silver and zinc oxide nanoparticles, fructose diffusion was significantly decreased and external fructose concentration was39.7 percentage (silver nanoparticles) and 32.2 percentage (zinc oxide nanoparticle) in the first 60 minutes, whereas for standard it is 44.4 percentage. The external fructose concentration after 60min for silver nanoparticle is 43.1 percentage and for zinc oxide it is 39.5 percentage which can be compared with the result of standard (47 percentage). The inhibitory percentage is again increasing after 60 minute which were found to be 42.9 percentage for zinc oxide nanoparticles 53.1 percentage for silver nanoparticles and 55.9 percentage for metformin.

Our results for glucose diffusion inhibition were similar to Roy and Mahalingam, (2017). Maximum inhibition of glucose was shown by ethanolic extract of *Phoenix roebelenii*for both 400 µg/ml (69.77 ± 1.00 percentage) and 200 µg/ml (58.72 ± 0.43 percentage) after 180 min. Methanolic extract of the same plant also showed a significant amount of inhibition for 400 µg/ml (64.87 ± 0.9 percentage). For 200 µg/ml, the inhibition of the ethanolic (57.11 ± 0.56 percentage) and methanolic extracts (57.05 ± 0.93 percentage) after 180 min was nearly similar.

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 (Vijayalekshmi*et al*., 2015).

4.4 ANTIBACTERIAL ACTIVITY OF SILVER AND ZINC OXIDE NANOPARTICLES OF FRUIT EXTRACTS OF *Helicteres isora* AGAINST BACTERIA

Silver nanoparticles and zinc oxide nanoparticles can be considered as antimicrobial agents and they are potential agents to help or manage and prevent infections. They can be used in several applications against bacteria resistant to common antibiotics or even multi-resistant bacteria. The antibacterial activity of synthesized silver and zinc nanoparticles was investigated against various pathogenic bacteria of gram positive (*Bacillus subtilis, Staphylococcus aureus*) and gram negative strains (*Klebsiellapneumoniae, Pseudomonas aeruginosa*) using well diffusion technique.

The diameter of zone of inhibition are presented in Table 3 and images are given in Plate 4 and 5

Table 3

ZONE OF INHIBITION (mm) OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF FRUIT EXTRACTS OF *Helicteres isora* AGAINST MICRO-ORGANISM

Micro-organisms	**ZONE OF INHIBITION (mm) OF SYNTHESIZED SILVER NANOPARTICLESOF FRUIT EXTRACTS OF *Helicteres isora* AGAINST MICROORGANISMS**				
	10µl	20 µl	30µl	40 µl	50 µl
Bacillus subtilis	12	14	15	17	18
Staphylococcus aureus	16	17	19	19	20
Klebsiella pneumonia	11	15	16	18	18
Pseudomonas aeruginosa	16	15	17	18	19

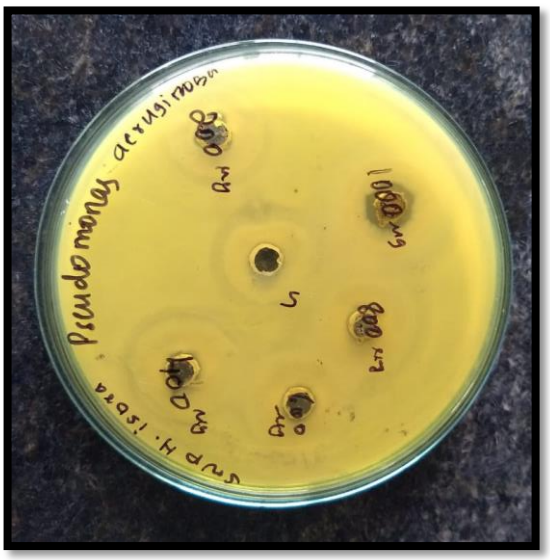
Micro-organisms	**ZONE OF INHIBITION (mm) OF SYNTHESIZED ZINC NANOPARTICLESOF FRUIT EXTRACTS OF *Helicteres isora* AGAINST MICRO-ORGANISMS**				
	10µl	20 µl	30µl	40 µl	50 µl
Bacillus subtilis	12	13	16	19	18
Staphylococcus aureus	15	12	14	14	15
Klebsiellapneumoniae	10	13	14	18	17
Pseudomonas aeruginosa	12	14	16	17	19

PLATE 4

ZONE OF INHIBITION (mm) OF SYNTHESIZED SILVER NANOPARTICLES OF FRUIT EXTRACTS OF *Helicteres isora* AGAINST MICRO-ORGANISMS

Staphylococcus aureus

Bacillus subtilus



PseudomonasaeruginosaKlebsiella pneumonia

PLATE 5

ZONE OF INHIBITION (mm) OF SYNTHESIZED ZINC NANOPARTICLES OF FRUIT EXTRACTS OF *Helicteres isora* AGAINSTMICRO-ORGANISMS

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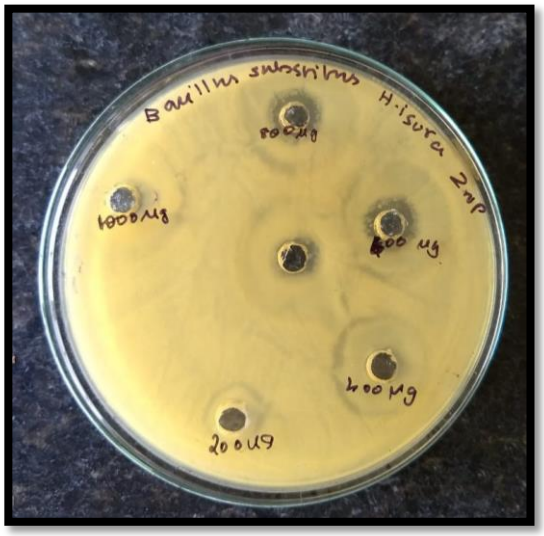
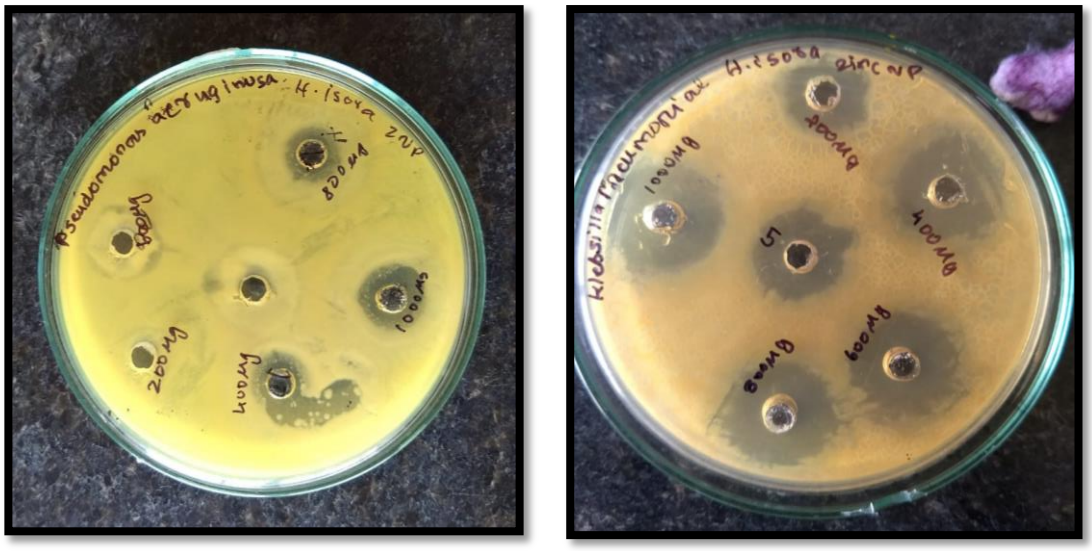


Staphylococcus aureus ***Bacillus subtilus***



Pseudomonas aeruginosaKlebsiella pneumonia

The above Table 6 and Plate 4 proves that the different concentrations of silver nanoparticles synthesized from *Helicteres isora* fruit 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 nanoparticles increased. However the highest zone of inhibition was found for *S.aureus* (20mm) while compared with other bacterial strains at 1000µg/ml concentration of silver nanoparticles followed by, *K.pneumoniae, B.subtilis*(19mm) and *P.aeruginosa*(18mm).

Table 6 and plate 5 shows the zone of inhibition of zinc nanoparticles. Highest zone of inhibition was found for*P.aureus* (19mm), which is followed by *Bacillus subtilis* (18mm), *S.aureus* (17mm) and *K.pneumoniae (*15mm) at 1000µg/ml concentration of zinc nanoparticles.

Karsha and Lakshmi *et al.* (2010) reported the antibacterial activity of silver nanoparticles of black pepper and its mode of action on both Gram-negative and Gram-positive bacteria and experimental outcomes undeniably suggest an effective growth inhibitory activity of the nanoparticles upon the microorganisms. Here also the zone of inhibition increased with increase in concentration.

The antibacterial activity of silver nanoparticles synthesized by using whole plant extracts of *Clitoriaternatea* showed positive results. Silver nanoparticles showed antibacterial activity against gram-positive bacteria as well as gram-negative bacteria. The zone of inhibition is for *Bacillus subtilis is* 8mm, *Staphylococcus aureus* is 7mm, *Escherichia coli* is 6mm and for *Klebsiella pneumonia* is 8mm (Malabadi*et al*., 2012).

Pal *et al*. (2018) reported that the antibacterial activity of zinc oxide nanoparticles produced by leaves of the *Moringaoliefera* indicating zone of inhibition against *E.coli* was found to be to be 3mm and *Bacillus subtilis* to be 3.3 mm.

4.4.2 MINIMUM INHIBITORY CONCENTRATION (MIC) OF FRUIT EXTRACTS OF *Helicteres isora*

The MIC was determined as the lowest concentration that inhibited the visible growth of the used bacterium. The MIC of silver and zinc oxide nanoparticles synthesized from ethanolic fruit extract of *Helicteres isora* was done.

The minimum inhibitory concentration (MIC) of silver and zinc nanoparticles is depicted in Table 4

TABLE 4

MINIMUM INHIBITORY CONCENTRATION OF SYNTHESIZED SILVER AND ZINC OXIDE NANOPARTICLES OF ETHANOLIC EXTRACTS OF FRUIT OF *Helicters isora* AGAINST BACTERIA

MICROORGANISM	MINIMUM INHIBITORY CONCENTRATION OF SYNTHESIZED SILVER NANOPARTICLES OF *Helicteres isora* (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 OF *Helicteres isora* (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 fruits of *Helicteres isora* was inoculated against *K.pneumoniae, P.aeruginosa, S.aureus*and*B.subtilis* in the concentration of 180-2.81µg /100µl. The growth of *S.aureus* and *P.aeruginosa* were inhibited by synthesized silver nanoparticles of *Helicteres isora* of 180-2.81µg / 100µl concentration. *K.pneumoniae* was found to have significant killing effect at 180-5.62 µg / 100µl, *B.subtilis* was found to have significant killing effect at 180-5.62 µg / 100µl whereas, *P.aeruginosa* have the killing effect at 180- 22.5 µg / 100µl concentration.

The synthesized zinc nanoparticles of fruits of *Helicteres isora* was inoculated against *K.pneumoniae, P.aeruginosa, S.aureus*and *B.subtilis* in the concentration of 180-2.81µg /100µl. The growth of *S.aureus* was inhibited by synthesized silver nanoparticles of *Helicteres isora* of 180-45µg / 100µl concentration. *P.aeruginosa* was inhibited by synthesized silver nanoparticles of *Helicteres isora* of 180-11.25µg/100µl concentration*K.pneumoniae* and *B.subtilis* was found to have significant killing effect at 180-2.81µg/100µl and *P.aeruginosa* have the killing effect at 180- 11.25µg/100µl concentration.

Netala*et al*. (2015) have reported that the silver nanoparticles produced by leaves of the *C. asiatica* were most effective against the gram-positive bacteria *S. aureus* with a MIC of 5µl, whereas MIC of 10µl was reported against *E. coli*.

Manyasree*et al*. (2017) got significant MIC values of zinc between 2 mg/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

Hence from the results of antimicrobial screening test, it was confirmed that the biologically synthesized silver nanoparticles of fruit extracts of *Helicteres isora* possess effective antibacterial property. Therefore, applications of it can cover a large domain of medical, leather and food technologies.

SUMMARY AND CONCLUSION

5.0 SUMMARY AND CONCLUSION

Nanotechnology is a developing interdisciplinary field of research interspersing material science, bionanoscience and technology. Therapeutics has taken the quickest advantage of advances in nanotechnology. Many methods have been developed for invivo drug delivery via nanoparticles such as nanocrystals, nanospheres, nanocapsules and can also include dendrimer technology. By the nature of their size, these nano delivery systems can cross membrane boundaries and can reach into blood stream (Vijayaraghavan*et al*., 2017).

Diabetes mellitus is a group of metabolic disorders which has been considered as a fast growing epidemic worldwide. Genetic condition and environmental factors are the two main factors that contribute to the development of diabetes mellitus. Large number of synthetic drugs are available in market for the treatment of diabetes but most of them are having various side effects therefore researchers has been carrying out on traditional medicinal plants for the discovery of anti-diabetic drugs as an alternative for synthetically derived drugs. Ulceration of the foot in diabetes is common and disabling and frequently leads to amputation of the leg. Mortality is high and healed ulcers often recur. Foot infections are caused by microorganisms namely *Klebsiellapnueumoniae, Staphylococcus aureus, Pseudomonas aeruginosa*and *Bacillus subtilis*.

Biological route of nanoparticle synthesis has been portrayed as an efficient, low-cost and environmental friendly technique. Hence, the present study was framed to synthesize the silver nanoparticles and zinc oxide nanoparticles from the ethanolic extracts of fruits of *Helicteres isora* and to evaluate its antidiabetic and antibacterial potential.

The silver nanoparticles and zinc oxide nanoparticles are synthesised from the ethanolic extracts of fruits of *Helicteres isora*. The synthesis of nanoparticles was noticed by a change in colour and the increase in absorbance. These synthesized nanoparticles are used for the further studies.

UV- Visible (UV-Vis) spectroscopy was done to characterize the presence of nanoparticles in the synthesised silver and zinc nanoparticles of *Helicteres isora*.

The nature of the functional groups of plant secondary metabolites and their involvement during bioreduction was approximately evaluated using the FTIR spectroscopy. The FT-IR spectrum of synthesized silver nanoparticles of *Helicteres isora* showed the peaks assigned for C=C stretching of alkenes and aromatic functional, stretch of alcohol, ethers and carboxylic acids, C-N of amines, CHOH stretching. The FT-IR spectrum of synthesized zinc oxide nanoparticles of *Helicteres isora* showed the peaks assigned for C-H stretch in

alkanes, C≡C, –C- stretching, bending in C-H of alkanes, C-N of amines, C-H bending vibrations of alkenes and CHOH bending.

Scanning electron microscopy (SEM) provides information about the topography and morphology of the nanoparticle. The elemental composition of metal nanoparticles can be established using energy dispersive X-ray spectroscopy (EDX). The SEM image revealed the presence of highly dense silver nanoparticle and synthesized silver nanoparticle was in the range of 1-10 µm with clearly observed spherical shape. EDX results showed that the silver nanoparticles synthesized from the fruits of *Helicters isora* contains about 81.54 weight percentage Ag, 6.09 weight percentage carbon, 8.95 weight percentage Cl and about 3.42 weight percentage of oxygen. The SEM images of zinc oxide nanoparticles shows the structure of synthesized zinc oxide nanoparticles are flakes of size 50- 100 µm and The zinc nanoparticles synthesized from the fruits of *Helicters isora* contains about 37 weight percentage Zn, 50 weight percentage oxygen and 14 weight percentage carbon.

The X-ray diffraction (XRD) technique is used to study structural information about crystalline metallic nanoparticle. The diffraction peaks observed at 2θ shows the main peaks at 38.1627, 32.5259, 46.5682 corresponding to the (57.35), (100.00) and (33.57) planes. XRD pattern recorded to know about crystalline nature of zinc nanoparticles. XRD pattern of zinc oxide nanoparticles showed the peaks which were obtained at 11.1662, 13.2061, 13.7745, 16.6317, 19.2419, 20.643, 22.091, 24.2526, 25.295, 26.0451, 28.941, 30.507, 3.0081, 32.8822, 33.9253, 34.6375, 36.3089, 37.8952, 44.0210, 44.8346, 46.4898, 47.7989, 49.1110, 54.3611, 56.7813, 59.3068, 63.1097, 68.1208, 69.2636, and 76.9978 at in the 2θ range, the peaks can be indexed to the 30.68, 78.80, 38.23, 19.59, 19.55, 10.59, 7.14, 10.40, 35.03, 45.91, 18.18, 17.48, 49.86, 47.08, 19.30, 60.50, 100.00, 21.97, 48.70, 15.03, 12.48, 29.68, 9.57, 11.02, 37.86, 14.54, 33.75, 28.37, 28.37, 14.09, 7.18 crystal planes.

In the present study, antidiabetic activity of synthesized silver and zinc oxide nanoparticles of different concentrations (200 – 1000 µg/ml) was compared by performing different *in vitro* assays such as alpha-amylase inhibition, non-enzymic glycosylation, glucose diffusion and glucose uptake by yeast cells.

The inhibitory activity of different concentrations (200 – 1000 µg/ml) of synthesized silver nanoparticles and zinc oxide nanoparticles was investigated for its alpha amylase. The activity was found to be increase with increase in concentration from 200 – 1000 µg/ml. The synthesized silver and zinc oxide nanoparticles showed good inhibitory activity against α-amylase while compared with that of standard drug acarbose. At the same time silver nanoparticle showed a better inhibitory than zinc oxide nanoparticles.

The haemoglobin present in the red blood corpuscles has a tendency to get bound to glucose. The inhibition of glycosylation was greater with silver nanoparticles when compared with zinc oxide nanoparticles and both of them showed a good inhibitory activity when compared with metformin which was used as a standard.

In the case of protein glycation inhibition of synthesized silver and zinc oxide nanoparticles of ethanolic extract fruits of *Helicteres isora*, zinc oxide nanoparticle showed a better inhibition activity than silver nanoparticles when compared to the standard Metronidazole.

Glucose uptake rate by yeast cells was increased with the increasing concentration of the nanoparticles and decreased with the increasing extracellular glucose concentration. In our studies Silver nanoparticle showed maximum results than zinc oxide while comparing with the standard drug metronidazole.

From the results of inhibition of glucose diffusion, we can conclude that the silver nanoparticle showed the most effective inhibitory effect on the glucose movement than zinc oxide nanoparticles. Silver nanoparticle showed an effect that is almost equal to the effect of standard and also zinc oxide nanoparticles showed comparative good results.

The different concentrations of silver and zinc oxide nanoparticles synthesized from fruit extracts of *Helicteres isora* fruit extract have potent antibacterial activity against gram positive and gram negative bacteria. It was observed that the zone of inhibition was increased when the concentration of silver nanoparticles increased. For silver nanoparticles, the highest zone of inhibition was found for *S.aureus* while compared with other bacterial strains at 1000µg/ml concentration of silver nanoparticles followed by, *K.pneumoniae, B.subtilis* and *P.aeruginosa*. While for zinc nanoparticles highest zone of inhibition was found for *P.aureus*, which is followed by *Bacillus*, *Staphylococcus* and *Klebsiella*at 1000µg/ml concentration.

The synthesized silver and zinc oxide nanoparticles from fruit extracts of *Helicteres isora* were inoculated against *Klebsiellapnueumoniae, Pseudomonas aeruginosa, Staphylococcus aureus*and *Bacillus subtilis*in the concentration of 180-2.81 µg / 100µl. The growth of *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiellapnueumoniae* and *Bacillus subtilis* were inhibited by synthesized silver and zinc oxide nanoparticles of 8-1.0 mg / 100µl concentration. The growth of *S.aureus* and *P.aeruginosa* were inhibited by synthesized silver nanoparticles of *Helicteres isora* of 180-2.81µg / 100µl concentration. *K.pneumoniae* was found to have significant killing effect at 180-5.62 µg / 100µl, *B.subtilis* was found to have significant killing effect at 180-5.62 µg / 100µl whereas, *P.aeruginosa* have the killing effect at 180- 22.5 µg / 100µl concentration. The growth of *S.aureus* was

inhibited by synthesized zinc nanoparticles of *Helicteres isora* of 180-45µg / 100µl concentration. *P.aeruginosa* were inhibited by synthesized zinc nanoparticles of *Helicteres isora* of 180-11.25 µg / 100µl concentration *K.pneumoniae* and *B.subtilis* was found to have significant killing effect at 180-2.81 µg / 100µl and *P.aeruginosa* have the killing effect at 180- 11.25 µg / 100µl concentration.

Altogether, the present study demonstrated that the silver and zinc nanoparticles synthesized from fruit extracts of *Helicteres isora* via green synthesis possess effective antidiabetic and antibacterial activity. At the same time silver nanoparticles showed much more antidiabetic activity whereas both of them equally have antimicrobial property. Hence, these extract 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.

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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,

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

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

ESTIMATION OF NON-ENZYMATIC GLYCOSYLATION OF HAEMOGLOBIN METHOD

(Chandrasekar*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,

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

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.*, 2015)

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:

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

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.*, 2002)

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.*, 1988)

PROCEDURE

Fructose (1000mM, in 200mM, phosphate buffer pH 7.4) 4.0 mL was incubated with 5.0 mL of BSA (20mg/mL, in 200mM phosphate buffer, pH 7.4), 1.0 mL of FLAE (final concentration: 15.6–500µg/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,

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

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.