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Coimbatore-641043, Tamil Nadu, India

Evaluation of *in vitro* Antidiabetic and Antioxidant Activities of PVA Encapsulated Silver Nanoparticles of Ethanolic Extract of *Basella rubra* and *Celosia argentea*

By

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21PBC003
II M.Sc. BIOCHEMISTRY

Department of Biochemistry, Biotechnology and Bioinformatics
A thesis submitted to Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore - 641 043.

In partial fulfilment of the requirement for the degree of
MASTER OF SCIENCE IN BIOCHEMISTRY

MAY 2023

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Signature of the Supervisor

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Signature of Head of the Department

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INTRODUCTION

1.0 INTRODUCTION

Nanotechnology has received a lot of attention recently due to the unique properties of nanoparticles (Chandhru *et al.* 2022). The continuous use of nanotechnology in the manufacture of nanoscale products in research and development is increasing. Nanoparticles are small fragments with nanoscale ranging from 1 to 100 nm that have excellent thermal conductivity, catalytic reactivity, nonlinear optical efficiency and chemical stability due to their high surface-to-volume ratio (Mustapha *et al.*, 2022). Nanostructured materials come in different forms, such as nanoparticles and nanotubes. There are two main categories of nanoparticles which are inorganic and organic nanoparticles (Ijaz *et al.*, 2022). They are usually classified into different groups according to their size, shape and characteristics. Different groups include carbon-based nanoparticles, metal nanoparticles, ceramic nanoparticles and polymer nanoparticles among many others (Chandhru *et al.* 2022).

Nanoparticles can be made of metals or non-metals, depending on their basic shape. Metal nanoparticles (NPs) are mainly composed of gold, silver, copper, magnetic (cobalt, nickel) and semiconductors (Alharbi *et al.*, 2022). They have unique advantages due to their excellent physicochemical properties such as strong optoelectronic, thermal, catalytic properties and high surface-to-volume ratio and their ease of synthesis with tunable morphology and clear crystallinity. Among metal NPs, noble metal NPs, i.e., gold, silver and platinum NPs, have attracted considerable attention due to their multiple advantages such as tunable size and colorimetric properties, high biocompatibility, surface plasmon resonance (SPR) effects, effective sensing features and a clear developmental mechanism (Jemilugba *et al.*, 2019). In contrast, non-metallic nanoparticles are mostly composed of carbon-based materials. Metal nanoparticles have been intensively studied for their electrical, optical and catalytic properties (Alharbi *et al.*, 2022).

In recent years, nanotechnology has become a major area of research in health and medicine. Nanomedicine has had a rapid and far-reaching impact on healthcare and has provided many opportunities in various industries and research. Nanoparticles (NPs) are more biocompatible than traditional therapeutic agents. Thus, NPs can be used for drug encapsulation and site-specific delivery, which increases drug efficacy, compared to larger particles and helps reduce unwanted drug toxicity (Saratale *et al.*, 2017). Because these structures are nanosized, they penetrate the tissue system, facilitate the uptake of drugs into cells, enable efficient drug delivery and ensure action at the target site (Bagyalakshmi *et al.*, 2022). This could potentially make many biotech tools more customized, versatile, cheaper, safer and easier to manage (Sudha *et al.*, 2018). These nanoparticles increase the effectiveness of the drug due to their targeted action. Their small size gives them an advantage in that they can avoid immune reactions and also allow them to pass through relatively impermeable membranes (Balan *et al.*, 2016).

Metallic nanoparticles especially platinum, silver and gold have been investigated for a variety of applications, including antibacterial, antifungal, anticancer, antidiabetic, antioxidant, catalytic and electronic devices. Among these nanoparticles, silver nanoparticles have recently attracted the interest of researchers due to their unique chemical and physical properties, especially in electrical, thermal, catalytic, industrial and biomedical fields (Majeed *et al.*, 2022). Silver nanoparticles (AgNPs) are metal nanoparticles in a broad absorption band in the visible region of the electromagnetic solar spectrum. AgNPs are used in several scientific fields due to their excellent optical properties (Nagaraja *et al.*, 2022). Treatment with nano-based engineered drugs may be an effective clinical option for people with significant disorders resulting from such properties compared to essential drugs (Naveed *et al.*, 2022).

Silver nanoparticles (AgNPs) among other metal NPs, have intensive applications in pharmaceutical fields. It has unique thermal, electrical and optical properties, making it very important for applications. AgNPs have previously been reported to retain excellent antibacterial, anticancer, antiviral, antidiabetic, antimicrobial and anti-inflammatory potential, as well as exhibit better biodegradability and lower toxicity (Das *et al.*, 2019). AgNPs are very prominent and interesting nanoformation among a variety of precious metal-based nanoparticles administered through many biological pathways to overcome critical diseases (Naveed *et al.*, 2022). AgNPs have potential applications in medical device coatings, sensors, pharmaceuticals, drug delivery, orthopedics and oncology. It is also possible to modify the physical, biological and chemical properties of metal nanoparticles due to their low surface to volume ratio (Majeed *et al.*, 2022).

Additionally, silver was reported to be the most widely used nanoparticle with a production rate of five hundred tons per year (Chandhru *et al*,. 2022).Nanoparticles can be synthesized by various methods, including chemical, physical and biological methods. However, chemical and physical methods are expensive, quite complex and potentially dangerous for the environment due to the toxic chemical compounds used as reducing agents (Ijaz *et al.*, 2022). However, these processes are energy-intensive and require hazardous reagents (stabilizers and reducing agents), making them neither environmentally friendly nor cost-effective. Therefore, there is a high demand to define less demanding manufacturing technologies that would increase the affordability of the entire nanotechnology industry (Naveed *et al.*, 2022).

Recently, more and more attention has been paid to the biological method, which is called the green synthesis method, because the need for development has increased for environmentally friendly technology in the synthesis of nanoparticles. Due to the simple processes and cost-effectiveness, both scientific and industrial sectors are currently interested in the green production of silver nanoparticles, which are used in a wide range of biomedical, environmental and industrial applications (Mustapha *et al.*, 2022). Biological processes called green synthesis are mainly carried out with the help of medicinal plants which offer advantages over chemical and physical methods because they are cost-effective, environmental friendly and readily available (Alharbi *et al.*, 2022). Interestingly, the biologically prepared AgNPs have high yield, solubility and high stability. Several studies have reported the synthesis of AgNPs by green, cost-effective and biocompatible methods without the use of toxic chemicals in biological methods. The biological synthesis of nanoparticles depends on three factors such as solvent, reducing agent and non-toxic material (Bagyalakshmi *et al.*, 2022).

Green synthesis is achieved by combining metal salts with natural reducing agents (such as plant extracts, fruit extracts and their secondary metabolites), microbial extracts and their by-products (such as vitamins, sugars and biodegradable polymers) to create nanomaterials. The green synthesis of NPs using the principles of green chemistry is gaining a lot of attraction in the development of many future nano-sized material (Jemilugba *et al.*, 2019). Plant synthesis of nanoparticles depends on the presence of bioactive metabolites such as polysaccharides, amino acids, aldehydes, flavones, alkaloids, proteins, phenols, saponins, terpenoids, tannins, ketones and vitamins in plants. These are metabolites that act as reducing and limiting agents in the formation of nanoparticles (Naveed *et al.*, 2022).

The synthesis of plant-based nanoparticle extracts is a non-toxic, environmentally friendly, sustainable and economical method and it can work under aquatic conditions, with low energy requirements and without toxic chemicals. Furthermore, the synthesis of nanoparticles using plant extracts is relatively much faster than the microbial route and easily scalable to produce NPs in huge quantities (Majeed *et al.*, 2022). Green synthesized metal nanoparticles have incredible anti-diabetic applicability and regulate diabetic function through pancreatic α-amylase, colon, α-glucosidase, insulin levels, glycemic absorption and other histochemical properties *in vivo* and *in vitro* studies. Metal-based nanoparticles have been found to have antioxidant properties that scavenge free radicals and reduce the production of reactive oxygen species (ROS) (Naveed *et al.*, 2022).

Diabetes refers to a group of diseases that cause high blood sugar due to insulin secretion or dysfunction. Diabetes develops due to decreased insulin production (type 1) or resistance to its effects (type 2), both of which lead to hyperglycemia. When the renal reabsorption threshold of glucose is exceeded, glucose spills into the urine (glycosuria) and causes osmotic diuresis (polyuria), which in turn causes dehydration, increased thirst and drinking (polydipsia) (Bagyalakshmi *et al.*, 2022). The incidence of type 2 diabetes (T2DM) is more severe than the incidence of type 1 diabetes (T1DM). T2DM has an incidence of approximately 90% and affects approximately 460 million people worldwide, with statistics predicting an increase to more than 700 million within 25 years. T1DM is caused by the autoimmune destruction of insulin-producing beta cells. T2DM is associated with insulin resistance and impaired glucose tolerance (Nagaraja *et al*., 2022).

In hyperglycemic conditions, reactive oxygen species (ROS) are produced, leading to lipid peroxidation, membrane damage and secondary problems in organs such as kidney, eyes, blood vessels and nervous system damage. The antioxidant is mainly involved in preventing the degradation of lipid oxidant in the radical chain reaction, removing free radicals and increasing the reactive process to activate oxygen species (Suchithra *et al.,* 2021). Many medications are used to treat diabetes but herbal medicines are often thought to be less toxic and without side effects. However, a complex drug molecule weakens the absorption of the drug and limits the bioavailability of the drug. By using nanotechnology, the bioavailability of the drug can also be improved (Nagaraja *et al*., 2022). Studies have reported the use of nanotechnology in the treatment of diabetes. Nanoparticles can be used to develop glucose sensor technology that can accurately measure glucose levels in the body. Controlled insulin is possible with nanomedicine because it can detect changes in blood sugar levels and automatically control the release of insulin to keep blood sugar levels normal (Majeed *et al.*, 2022).

Organic stabilizers, proteins, peptides and synthetic polymers were used to control the shape of AgNPs and other structured nanomaterials. It is now recognized that surface functionalization of AgNPs is very important for biomedical applications and targeted drug delivery. The binding ability of water-soluble polymers such as Poly Vinyl Pyrrolidone (PVP) and Poly Vinyl Alcohol (PVA) to the silver surface provides a basis for mediating the nucleation, growth, organization and shape regulation of Ag nanostructures (Kyrychenko *et al.*, 2017). Due to its excellent heat resistance, chemical resistance, high mechanical strength, water solubility and moderate and dopant-dependent electrical conductivity, PVA can be considered as a good metal host material, which is among the best polymers as the main matrix of silver nanoparticles. PVA can effectively protect nanoparticles from aggregation (Ghanipour *et al.*, 2013).

The simple structure and geometric shape of PVA facilitate its chemical modification at the secondary alcohol group. Any compound capable of reacting with a hydroxyl group can be used as a potential PVA crosslinker. It has been reported that PVA is derived by carbonization, esterification, etherification and click chemistry, and the resulting products have unique properties and wide applications (Iqbal *et al.*, 2020). Chemical modification of silver nanoparticles (AgNPs) with a stabilizer such as Poly Vinyl Alcohol (PVA) plays an important role in seed shape-directed growth and colloidal stability (Kyrychenko *et al.*, 2017). One of the functions of PVA as a coating agent in the synthesis of silver nanoparticles is to protect them from self-aggregation in solution. The structural flexibility and conformational dynamics of the stabilizing backbone or polymer chain adsorbed on the nanoparticle surface play an important role in the colloidal stabilization, shape-controlled growth and water protection effect of silver nanoparticles (Nguyen *et al*., 2021).

Traditional medicine is undoubtedly a reliable alternative approach to health care in the metropolis because it is cheap, easily available, effective and offers a natural way to treat many human diseases. Therefore, medicinal plants and herbal preparations should be included as possible sources of new drugs. Despite the availability of chemically synthesized drugs for millions of different diseases; plant natural products remained the most important and valuable source of new medicines. One such medicinal plant is *Basella rubra* (Sharma *et al*., 2022).

Basella rubra (*B. rubra)*, commonly known as Indian or Malabar spinach, belongs to the family Basellacae, is an annual or biennial herb found in tropical and subtropical regions. It is a fleshy, branched, smooth, herbaceous vine several meters long. The stems are purple or green. The leaves are fleshy, oval or heart-shaped, 5-12 cm long, petiolate, tapering towards the tip, with a heart-shaped base. Blades are axillary, single, 5-29 cm long. The fruit is fleshy, sessile, egg-shaped or globose, 5-6 mm long and ripe purple and contains only one seed. The flowers are pink and about 4 millimeters long (Singh *et al.*, 2016).

Celosia argentea L. is a herbaceous plant of the Amaranthacaceae family. *Celosia argentea* L is a 2 metre tall erect annual plant. The stem is ridged and smooth. The leaves are alternating, simple, and without stipules; the petiole is indistinctly delimited; the blade is ovate to lanceolate-oblong or broadly linear, up to 15 cm long, tapering at the base, acute to obtuse and briefly mucronate at the apex, whole, glabrous, and pinnately veined. a thick Inflorescence , many-flowered spike, first conical but growing cylindrical up to 20 cm long, braceate, slivery to pink in decorative types, totally or partially sterile, and in a variety of hues. Flowers are tiny, bisexual, regular five merous, tepal free, narrowly elliptical-oblong, 6-10 mm long and stamen united at base (Kanu *et al.*, 2017).

With this background, the present study "Evaluation of *invitro* Antidiabetic and Antioxidant Activities of PVA Encapsulated Silver Nanoparticle of Ethanolic Extract of *Basella rubra* and *Celosia argentea*" was undertaken with the following objectives

- To synthesis silver nanoparticles of ethanolic extract of *Basella rubra* and *Celosia argentea*
- To encapsulate the synthesized silver nanoparticles with Poly Vinyl Alcohol (PVA)
- To characterize the PVA encapsulated silver nanoparticles of ethanolic extract of leaves of *Basella rubra* and *Celosia argentea*
- To determine the *in vitro* antioxidant activity of PVA encapsulated silver nanoparticles of ethanolic extract of *Basella rubra* and *Celosia argentea*
- To determine the *in vitro* antidiabetic activity of PVA encapsulated silver nanoparticles of ethanolic extract of *Basella rubra* and *Celosia argentea*

REVIEW OF LITERATURE

2.0 REVIEW OF LITERATURE

Nanotechnology is one of the emergent cutting-edge technologies in a variety of different fields of science including biology, chemistry and material science (Mustapha *et al.*, 2022). The area of nantechnology deals with particles between 1 and 100 nm in size (Majeed *et al.*, 2022). The study of the basic characteristics of nanoscale objects is included in the well-known modern scientific field known as nanoscience. As a result, the manufacturing of nanoparticles using biologically inspired technology has become a unique and great sector in the field of nanotechnology and nanostructure (Ijaz *et al.*, 2022). Nanomaterials, which affect the frontiers of nanomedicine from biosensors, microfluidics, drug delivery and tissue engineering, may be simply characterised as materials with diameters ranging from 1 to 100 nm. Since nanostructures are used as delivery agents to encapsulate or attach therapeutic pharmaceuticals and transport them to target tissues more accurately with a controlled release, nanomedicine have gained popularity recently (Bagyalakshmi *et al.*, 2022).

The review of literature pertaining to the research entitled “Evaluation of *in vitro* Antidiabetic and Antioxidant Activities of PVA Encapsulated Silver Nanoparticles of Ethanolic Extract of *Basella rubra* and *Celosia argentea*” is pertinently presented below the subsequent heading

2.1 Nanotechnology
2.2 Nanoparticles
2.3 Synthesis of Silver Nanoparticles
2.4 PVA encapsulation of silver nanoparticles
2.5 Charcterization of silver nanoparticles
2.6 Oxidative stress
2.7 Diabetes mellitus
2.8 Herbal medicine
2.9 Medicinal plants selected for the study

2.1 NANOTECHNOLOGY

Nanotechnology refers to atomic, molecular and macromolecular scale research and development that leads to the controlled manipulation and study of structures and devices with length scales ranging from 1 to 100 nanometers. Nanotechnology also provides the possibility to monitor and detect molecular and cellular changes associated with disease states by creating structures capable of merging many capabilities into a single nanoscale entity (McNeil, 2005). Nanometre-sized structures, materials and particles are critical to nanomedicine advancements. They feature distinct physical, chemical, or biological characteristics, enabling novel building blocks for the development of devices and systems for illness diagnosis and treatment (Sindhwani *et al.*, 2021). It has received a lot of interest in materials science, medicine, and biomedical engineering in recent decades (Yin *et al.*, 2020).

Nanomedicine, a sub-discipline of nanotechnology, is typically defined as science, engineering and technology performed at the nanoscale (Mostafavi *et al.*, 2019). It makes use of various nanotechnology-based techniques to provide faster and more efficient solutions to medical problems or disease management. It not only overcomes the problems that conventional medicine faces, but it also allows for the comprehension of numerous physiological and pathological processes. Utilisation of such processes opens up new opportunities and treatment strategies for current issues (Sayed *et al.*, 2018). Nanomedicine has enormous potential to improve the diagnosis and treatment of human diseases (Sudha *et al.*, 2018).

Scientists are most concerned about the synthesis of safe, effective and above all cheaper and less toxic drugs to fight diseases such as diabetes, cancer and epilepsy (Balan *et al.*, 2016). The promise of nanotechnology has been realized to achieve scientific and technological progress in several fields (Sudha *et al.*, 2018). Nanotechnology offers a platform to improve herbal extracts and plant active ingredients, modify their release and prevent their unwanted side effects (Khodeer *et al.*, 2022). It often known as nanomedicine, has emerged as the dominant and most commercially developed technology aimed at improving the quality of health-care solutions. Despite significant restrictions, numerous pharmaceutical and medical equipment businesses have already used medical nanotechnology (Sahu *et al.*, 2021).

2.2 NANOPARTICLES

Nanoparticles are objects with sizes ranging from 1 to 100 nm that, due to their size, vary from the bulk material. Nanoparticles are employed for a variety of reasons, including medicinal treatments and the creation of solar and oxide fuel batteries for energy storage (Hasan, 2015). According to the American Society for Testing and Materials (ASTM), nanoparticles, the most extensively used nanotechnology platforms in nanomedicine, are particles having two or more dimensions on the nanometer scale. When compared to their bulk counterparts, these nanoparticles exhibit increased physical and chemical characteristics. They have special electrical structures and qualities such as high surface area-to-volume ratio and a distinctive quantum size effect (Mauricio *et al.*, 2018).

The nanoparticles vary in form, size and structure. It can be spherical, cylindrical, tubular, conical, hollow core, spiral and flat or irregular, with sizes ranging from 1 nm to 100 nm. The surface might be smooth or uneven, with surface changes. Some nanoparticles are either crystalline or amorphous, having single or many crystal solids that are either loose or agglomerated (Ealia *et al*., 2017). Nanoparticles have a wide range of applications in research, including medical, micro-wiring, food and agriculture electronics, and energy harvesting. A nanoparticle's potential and applicability are determined by its unique properties (Ijaz *et al*., 2020). Some nanoparticles are antioxidants and they may ameliorate vascular dysfunction caused by hypertension, diabetes or atherosclerosis (Mauricio *et al*., 2018).

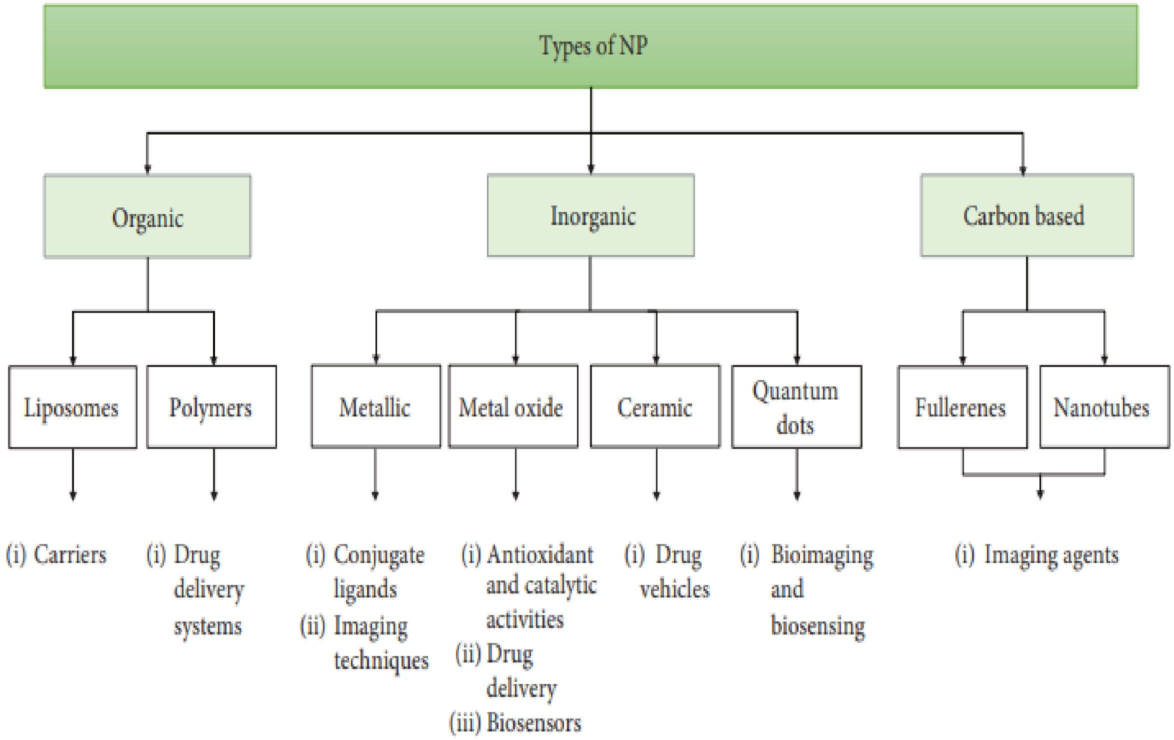
The optical characteristics of the nanoparticle include its colour, light penetration, absorption, and reflection abilities and UV absorption in a solution or when deposited onto a surface. It also comprises mechanical qualities such as elastic, ductile, tensile and flexibility, which are important in their application (Ealia *et al*., 2017). The chemical characteristics of the nanoparticles, such as their reactivity with the target, as well as their stability and susceptibility to variables such as moisture, environment, heat and light, define their uses. The nanoparticles' antibacterial, antifungal, disinfection and toxicological capabilities make them attractive for biomedical and environmental applications (Bruna *et al*., 2021). Because of these characteristics, nanotechnology platforms can be used in biology and medicine. The motivation derives from fundamental biological interactions at the nanoscale regime that preserve cell function and vitality (Sindhwani *et al*., 2021).

2.2.1 TYPES OF NANOPARTICLES

Nanoparticles (NPs) are classified into three types based on their chemical composition: organic nanoparticles (liposomes and polymers), inorganic nanoparticles (metals, metal oxide, ceramics and quantum dots), and carbon-based nanoparticles (Mauricio *et al*., 2018). Figure 1 represents different types of nanoparticles.

FIGURE 1

DIFFERENT TYPES OF NANOPARTICLES



(Mauricio *et al*., 2018)

Organic nanomaterials can be synthesised synthetically or biologically from polymers, proteins, nucleic acids, carbohydrates and lipids. Organic nanoparticles or polymers are often known as dendrimers, micelles, liposomes and ferritin among other things. These nanoparticles are biodegradable, non-toxic and some have a hollow core known as nanocapsules, and are sensitive to thermal and electromagnetic radiation such as heat and light. Because of their distinguishing properties, they are an excellent source for medication administration (Ealia *et al*., 2017). Organic nanoparticles are often biocompatible and functionally diverse. Since they are largely composed of carbon, nitrogen, and oxygen, they are normally harmless and have modest immune reactions (Sindhwani *et al*., 2021).

Inorganic nanoparticles do not include carbon. The inorganic nanoparticles are not toxic. They are biocompatible and hydrophilic in nature. Inorganic nanoparticles are more stable than organic nanoparticles. Metal and metal oxide nanoparticles are the two types of inorganic nanoparticles (Ijaz *et al*., 2020). Inorganic nanoparticles are being developed for medicinal uses as well. The adjustable features of these nanoparticles distinguish them. By modifying the physicochemical design of the inorganic nanoparticle, the researcher may adjust its electrical, optical and magnetic capabilities (Sindhwani *et al*., 2021). Metal-based nanoparticles are those that are synthesised to nanometric sizes from metals using either destructive or constructive processes. Almost any metal can be synthesised into nanoparticles. Aluminium (Al), cadmium (Cd), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), silver (Ag), and zinc (Zn) are the most widely employed metals for nanoparticle production (Ealia *et al.*, 2017).

Metal oxide based nanoparticles are synthesised to modify the properties of their respective metal based nanoparticles. For example, iron (Fe) nanoparticles instantly oxidise to iron oxide (Fe_2O_3) in the presence of oxygen at room temperature, increasing their reactivity compared to iron nanoparticles (Hasan, 2015). Metal oxide nanoparticles are produced primarily for their improved reactivity and efficiency. Aluminium oxide (Al_2O_3), Cerium oxide (CeO_2), Iron oxide (Fe_2O_3), Magnetite (Fe_3O_4), Silicon dioxide (SiO_2), Titanium oxide (TiO_2) and Zinc oxide (ZnO) are the most regularly synthesised. When compared to their metal counterparts, these nanoparticles offer outstanding characteristics (Ealia *et al.*, 2017).

Carbon-based nanoparticles are those formed entirely of carbon. Fullerenes, graphene, carbon nano tubes (CNT), carbon nanofibers and carbon black are occasionally activated carbon in nano size are among them (Ijaz *et al.*, 2020).

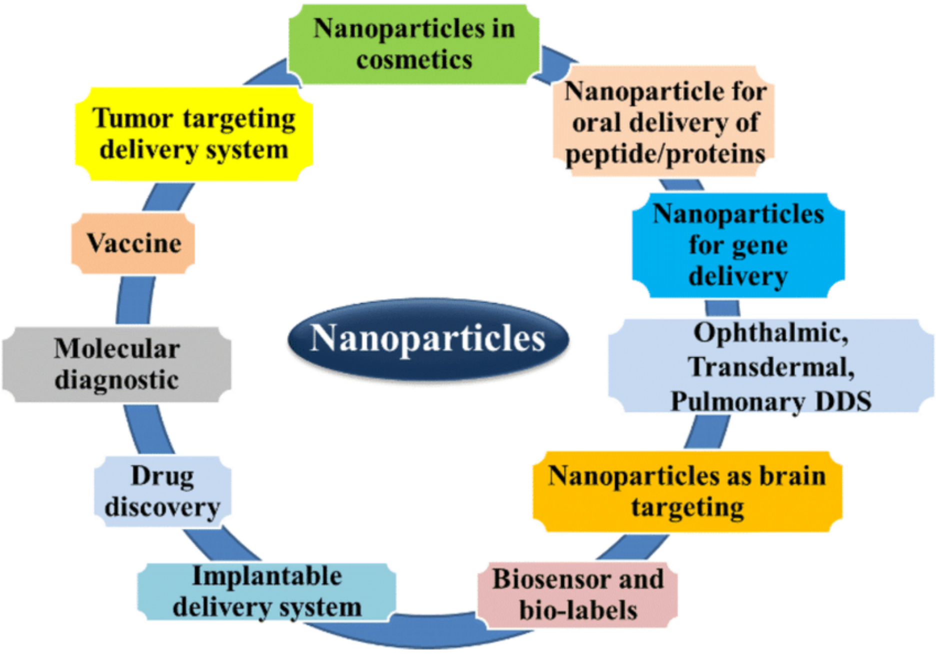
2.2.2 APPLICATIONS OF NANOPARTICLES

Nanoparticles have grown important in numerous industries in recent years due to their remarkable qualities, including energy, health care, the environment and agriculture. Nanoparticle technologies offer enormous potential for converting poorly soluble, poorly absorbed and labile physiologically active compounds into viable deliverable chemicals (Hasan, 2015). Nanomaterials are commonly employed as drug delivery vehicles to carry medications to sick sites. There are potential medications that show excellent effectiveness *in vitro* in numerous disorders. Nanoparticles are also emerging as an important technology for *in vitro* diagnostic devices (Sindhwani *et al.*, 2021).

Nanoparticles have a site-specific effect that requires only a safe and prescribed dose of drug molecules, helping to reduce unwanted toxicity (Balan *et al.*, 2016). According to studies, the nanoparticles can be utilised to create glucose sensor technology that accurately measures the body's blood glucose levels. Nanomedicine's ability to detect changes in blood glucose levels and automatically regulate the release of insulin to maintain normal blood glucose levels makes controlled insulin administration conceivable (Majeed *et al.*, 2022). Nanomaterials are commonly employed as drug delivery vehicles to carry medications to diseased sites. One of the most investigated topics of nanomedicine is the use of nanoparticles in cancer treatment. This area of study is known as cancer nanomedicine (Dykman *et al*., 2012).

Nanomedicine has previously been used for a wide range of ailments, including the treatment of uncommon hepatic disorders, the delivery of nucleic acid-based vaccinations, iron deficient anaemia and the treatment of antifungal infections. In preclinical animal models, researchers are also creating nanomaterials to detect and treat atherosclerosis, Alzheimer's disease, skin illnesses, toxic overdoses and inflammatory bowel disease, as well as to modify gut microbes (Kim *et al*., 2010). Nanoparticles are also being studied as carriers of CRISPR tool for genome editing. Finally, great effort is being made to produce nanoparticle probes for MRI, CT and PET imaging. Vaccines are another area where nanoparticles can be used. Viruses are nucleic acid-carrying nanoparticles that may enter infected cells and reproduce. In the present COVID-19 pandemic era, nanoparticle-based mRNA and DNA vaccines are being developed at a rapid pace (Shin *et al*., 2020). Figure 2 represents biomedical applications of nanoparticles.

FIGURE 2
BIOMEDICAL APPLICATIONS OF NANOPARTICLES



(Khan *et al*., 2020)

2.2.3 SYNTHESIS OF NANOPARTICLES

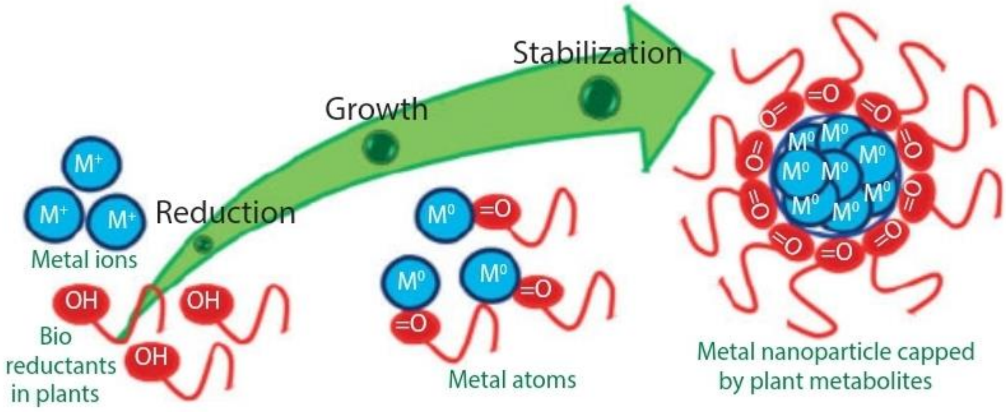
Plant biomasses have shown beneficial in the production of sustainable nanoparticles (NPs), which are widely utilised in biological techniques, in the mission meant for green and eco-friendly means of synthesising nanomaterials for the creation of new goods (Das *et al*., 2019). Nanostructures are significantly more readily absorbed by cells than big particles between 1 and 10 m in size. As a result, they work together more effectively with little or no side effects (Bagyalakshmi *et al.*, 2022). It has been discovered that both physical and chemical procedures are very time-consuming, costly and could be bad for the environment and people's health. The biological approach, which uses fungus, bacteria, plants and algae as nanofactories, offers a wide range of applications in biomedical, industrial and pharmaceutical contexts and has been proved to be nontoxic, cost-effective and ecologically benign (Majeed *et al.*, 2022).

The synthesis of plant-based nanoparticle extracts is a non-toxic, environmental friendly, sustainable and economical method and it can work under aquatic conditions, with low energy requirements and without toxic chemicals. Furthermore, the synthesis of nanoparticles using plant extracts is relatively much faster than the microbial route and easily scalable to produce NPs in huge quantities (Majeed *et al.*, 2022). Green synthesized metal nanoparticles have incredible anti-diabetic applicability and regulate diabetic function through pancreatic α-amylase, colon, α-glucosidase, insulin levels, glycemic absorption and other histochemical properties *in vivo* and *in vitro* studies. Metal-based nanoparticles have been found to have antioxidant properties that scavenge free radicals and reduce the production of reactive oxygen species (ROS) (Naveed *et al.*, 2022).

For the creation of metal NPs, using natural materials rather than manufactured compounds has various benefits. These include quick and easy synthesis, affordability and the utilisation of biocompatible materials as a non-toxic reducing agent. Additionally, isolated proteins, amino acids and carbohydrates (such as cellulose, starch and glucose) can be employed. The wide and varied plant flora is one of these green materials that has drawn scientists from all around the world, and it has been used to synthesise various metal nanoparticles. Plant extracts can operate as both reducing and stabilising agents for metal nanoparticles (Jemilugba *et al.*, 2019). In addition, there are alternative techniques including ultrasonics, ball milling and supercritical fluid synthesis. Ultimately all these procedures are involved in the oxidation of Ag+ to Ag0 in order to create silver nanoparticles. This can also be accomplished by employing biomolecules found in plants, bacteria and fungus such as flavonoids, tannins, proteins and aldehydes as capping or reducing agents (Ijaz *et al.*, 2022). Figure 3 represents mechanism of green synthesis of nanoparticles.

FIGURE 3

MECHANISM OF GREEN SYNTHESIS OF NANOPARTICLES



(Sajjad *et al.*, 2018)

2.3 SYNTHESIS OF SILVER NANOPARTICLES

Nanoparticles can be made of metals or non-metals, depending on their basic shape. Metal nanoparticles are mainly composed of gold, silver, copper, magnetic (cobalt, nickel) and semiconductors. In contrast, non-metallic nanoparticles are mostly composed of carbon-based materials (Alharbi *et al*., 2022). Among these nanoparticles, silver nanoparticles have recently attracted the interest of researchers due to their unique chemical and physical properties, especially in electrical, thermal, catalytic, industrial and biomedical fields (Majeed *et al*., 2022). Due to its numerous uses in the fields of health, solar energy conversion, waste water treatment and catalysis, research into the production of silver nanoparticles is expanding. There has been several research that concentrate on bio-inspired nanoparticles as innovative delivery strategies for the treatment of eye disorders (Chandhru *et al*., 2022).

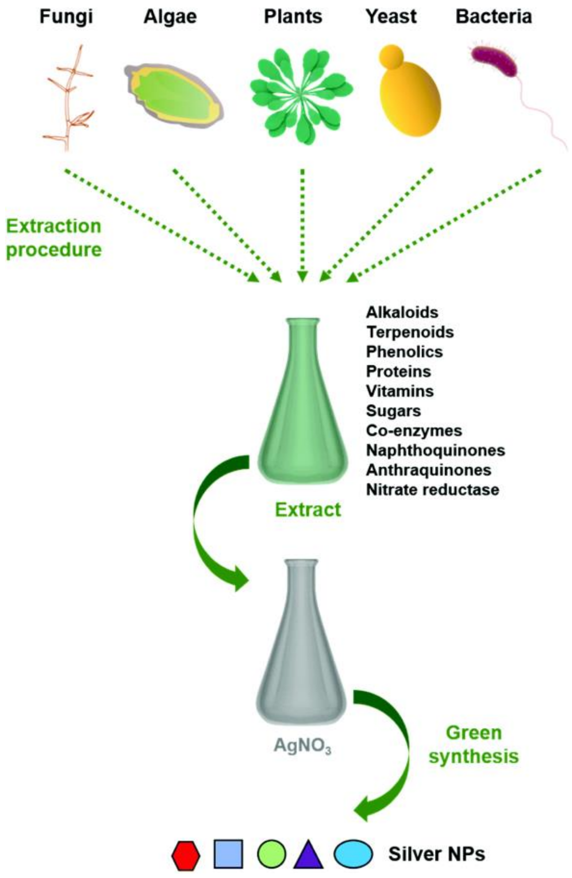
Silver nanoparticles have previously been reported to retain excellent antibacterial, anticancer, antiviral, antidiabetic, antimicrobial and anti-inflammatory potential, as well as exhibit better biodegradability and lower toxicity (Das *et al*., 2019). Silver nanoparticles are a very prominent and interesting nanoformation among a variety of precious metal-based nanoparticles administered through many biological pathways to overcome critical diseases (Naveed *et al*., 2022). Typically, chemical or physical processes have been used to synthesise NPs with regulated shapes and sizes. However, the use of harmful chemicals as well as costly physical methods and instruments has resulted in the development of ecologically friendly techniques. Natural-source NPs are the nontoxic, recyclable and most successful approach, providing a safe and nontoxic alternative (Das *et al*., 2019).

The use of plants as the production assembly of silver nanoparticles has drawn attention, because of its rapid, eco-friendly, non-toxic, economical protocol and affording a single step technique for the biosynthetic processes (Ahmed *et al.*, 2016). In addition, plant extracts comprise bioactive polyphenols, alkaloids, proteins, sugars, phenolic acids and terpenoids which are made up to have a vital role in initially reducing the metallic ions and then stabilizing them. The difference in conformation and concentration of these energetic biomolecules among many plants and their resulting collaboration with aqueous metal ions are thought to be one of the key factors associated with the diverse of nanoparticle sizes and shapes fabricated (Chokkareddy *et al.*, 2018).

The reduction and stabilization of silver ions by amalgamation of biomolecules like proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolics, saponins, terpenoids and vitamins which are already established in the plant extracts having medicinal values and are environmental safe, yet chemically complex structures (Ahmed *et al.*, 2020). These biological molecules extracted from different origin are externally added to metal salts to make nanoparticles. To synthesize silver nanoparticles, biological extract in situ reduces silver salts (Ag+) to metallic silver, Ag0. In the method of nanoparticle synthesis in a green way, biological molecules not only reduce the metal salts but also cover the formed nanoparticles or acts as *in situ* reducing and capping agent. This capping is advantageous over as it acts as multifunctional way; (i) prevents the agglomeration of the nanoparticles, (ii) reduces the toxicity and (iii) improves antimicrobial activity (Roy *et al.*, 2019). Figure 4 illustrate schematic representation of the procedure for green synthesis of silver nanoparticles using various biological entities.

FIGURE 4

SCHEMATIC REPRESENTATION OF GREEN SYNTHESIS OF SILVER NANOPARTICLES USING VARIOUS BIOLOGICAL ENTITIES



(Roy *et al.*, 2019)

2.4 PVA ENCAPSULATION OF SILVER NANOPARTICLES

Poly Vinyl Alcohol (PVA) is a water-soluble polymer that is thermally stable, chemically resistant and mechanically strong. PVA, which functions as an excellent metal host material, can protect nanoparticles from agglomeration (Bulla *at al*., 2021). PVA's simple structure and geometric shape make chemical modification of the secondary alcohol group easier. All chemicals that can react with the hydroxyl group can be employed as possible PVA cross-linking agents. PVA has been reported to be derivatized by carbamation, esterification, etherification and click chemistry, with the resulting compounds having unique characteristics and a wide range of uses (Iqbal *et al*., 2020).

In the preparation of nanoparticles, agglomeration is the natural tendency of nanoparticles to be bigger in size from nanoscopic to microscopic to macroscopic dimension. So, in order to manage the size, morphology, form, stability, colour and physicochemical characteristics of advanced nanomaterials in nanoscopic dimensions, a stabiliser as well as a protective agent is required (Das *et al*., 2019).Selecting a suitable capping agent is frequently required for nanoparticle stabilisation. Capping agents appear to function through a number of processes, including electrostatic stabilisation, steric stabilisation, hydration force stabilisation, depletion force stabilisation and van der waals force stabilisation. The selection of a capping agent for nanoparticles is critical since the capping agent frequently determines the nanoparticle's size, shape and interactions with the surrounding solvent. Therefore, the selection of the capping agent plays a vital role in the nanoparticle synthesis process and capping agents also affect nanoparticle properties (Ajitha *et al*., 2016).

In the production of Ag NPs, a variety of compounds have been utilised as protective agents. Polymers are the most often used stabilisers and protective agents in nanoparticle manufacturing, including gelatin, D-sorbitol, PVP (Poly Vinyl Pyrrolidone) and PVA (polyvinyl alcohol) (Das *et al*., 2019). PVA is a multi-hydroxyl group (O-H) polymer that has been used for both electrical and medicinal uses due to its high tensile strength, flexibility and degradability. PVA, on the other hand, has a high hydrophilic property due to its chemical makeup, which allows it to interface directly with fluids in live cells while also reducing calcification (Kader *et al*., 2021). PVA's role as a coating agent in the manufacture of silver nanoparticles includes protecting them from self-aggregation in solution. The structural flexibility and conformational dynamics of a stabilising agent backbone or polymer chain adsorbed onto a nanoparticle surface play a key role in colloidal stabilisation, shape-controlled growth and the water-protecting effect of silver nanoparticles (Nguyen *et al*., 2021).

The PVA-encapsulated silver nanoparticles produced by different methods are stable for several months and can be kept at room temperature without any special care (Chandran *et al*., 2016). PVA also has a variety of great features, including non-toxicity, strong chemical stability, biocompatibility and excellent film forming capacity and it is frequently blended with natural polymers to increase their mechanical performance (He *et al*., 2017). Because of its strong temperature stability and chemical resistance, PVA might be regarded a promising host material for metal. PVA can efficiently prevent nanoparticle. On the other hand, PVA functions as both stabilizing and reducing agent, from the fact that the vinyl polymers with high density of polar groups enhance the reduction process. The incorporation and production of silver nanoparticles inside PVA will significantly alter the characteristics of the polymer involved (Nimroth *et al*., 2011).

2.5 CHARCTERIZATION OF SILVER NANOPARTICLES

Characterization of nanoparticles is important to understand and regulate nanoparticle synthesis and application. The morphological, structural and chemical composition of Silver NP’s were analyzed by employing Scanning Electron Microscopy, Energy Dispersive X-ray, X-ray diffraction, Fourier Transform Infrared Spectrometry and UV Spectroscopy. Moreover, orientation, intercalation and dispersion of nanoparticles and nanotubes in nanocomposite materials could be determined by these techniques (Nour *et al.*, 2010).

2.5.1 UV-VISIBLE SPECTROMETRY

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

UV-Vis spectrophotometry is perhaps the most ordinarily utilized strategy for the portrayal of blended nanoparticles. Likewise, UV-vis spectrophotometry is basic, simple, quick, delicate and specific for various kinds of nanoparticles (Tomaszewska *et al*., 2013). The assimilation spectrum of AgNPs relies upon the dielectric medium, morphology, shape, size and environmental factors of blended nanoparticles. Many considerations have shown that the AgNPs produces the retention groups at around 200-800 nm in the UV visible spectra frequency and it utilized for the portrayal of nanoparticles with a scope of 2-100 nm (Cabral *et al*., 2013).

2.5.2 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FT-IR)

FTIR spectroscopy is utilized to research the metal nanoparticle surface science and to see if bio-atoms are associated with the nanoparticles combination (Gurunathan *et al*., 2014).Fourier transform infrared (FT-IR) spectroscopy is generally employed to use the expression of characteristic spectral bands to reveal nanomaterial biomolecule conjugation. Proteins bound to nanoparticle surfaces and to illustrate the conformational states of the bound proteins. Moreover, FT-IR has also been expanded to study nano-scaled materials, such as confirmation of functional molecules covalently grafted onto carbon nanotubes (Lin *et al.,* 2014).

The FT-IR spectrometers attain the IR spectrum by Fourier transformation of the signal from an interferometer with a moving mirror to produce an optical transform of the infrared signal. Numerical Fourier assessment gives the relation of intensity and frequency, that is, the IR spectrum. The FT-IR technique can be used to analyze gases, liquids and solids with minute preparation (Barrios *et al.,* 2012). FTIR has additionally been utilized to contemplate the communications happening among catalyst and substrate during the synergist cycle, affirmation of utilitarian atoms covalently joined onto silver (Rohman, 2010).

2.5.3 SCANNING ELECTRON MICROSCOPE (SEM)

Among a few electron microscopy strategies, SEM is a surface imaging strategy, fit for deciding distinctive molecule shapes, surface morphology, sizes and size dispersions of the coordinated nanoparticles at the miniature and nano scale levels (Buhr *et al*., 2009). SEM uses a high energy electron bar which is looked over the outside of the AgNPs test and afterward back-dispersed electrons perception gives trademark highlights (Anandalakshmi *et al*., 2016).

2.5.4. ENERGY DISPERSIVE X-RAY (EDX)

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

EDX investigation is engaged with various biomedical field of study because of its high affectability in recognizing the various components in tissues. EDX procedure is made especially valuable in the investigation of medications conveyance in which the EDX is significant device to identify nanoparticles. Ordinarily, EDX is incomparable strategy permitting a natural investigation of the outside of the samples (Scimeca *et al*., 2018).

2.5.5 X-RAY DIFFRACTION (XRD)

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

2.5.6 ZETA POTENTIAL

The zeta potential is a critical metric for determining the stability of AgNPs in aqueous solutions. Particles with a high negative or positive zeta potential resist each other, resulting in improved suspension stability. If the particles have low zeta potential values, attraction forces between them become dominant, and the particles aggregate and flocculate. In general, a zeta potential of at least 30 mV is required to verify a stable nanosuspension (Ajitha *et al*., 2016).

Dynamic light scattering (DLS) was used to examine the particle size of synthesised silver nanoparticles (Patil *et al*., 2012). The DLS technique does not assess size but rather the diffusivities of AgNPs-PVA in an aqueous solution. Given a monodisperse size distribution, that is, all particles of the same size, a size measurement by SEM should provide a size similar to that recorded by DLS. If a particle size distribution exists in a solution, DLS weights the distribution differently than SEM, by size to the power of 6, resulting in a z-average distribution (Nguyen *et al*., 2021).

2.6 OXIDATIVE STRESS

Oxidative stress is a state in which the equilibrium between the antioxidative defence of the cell and oxidants is disrupted by the effect of excess oxidants, such as reactive oxygen or nitrogen species (ROS or RNS, respectively) and organic compounds containing sulphur that produce alkyl sulfanyl radicals (RS•) (Bedlovicova *et al*., 2020). Many normal biological activities in our bodies, such as breathing, digestion, metabolising alcohol and drugs and converting lipids to energy, generate harmful substances known as free radicals. Natural antioxidant system of human normally destroys free radicals. If this system fails to function properly, free radicals can cause a negative chain reaction in the body, destroying the cell membrane, blocking the action of major enzymes, preventing cellular processes required for proper body functioning, preventing normal cell division, destroying deoxyribonucleic acid (DNA), and blocking energy generation (Kurutas, 2015).

Although the idea of oxygen free radicals (ROS) has been known for over 50 years, it was only in the last two decades that their function in disease development was recognised, and therefore the positive benefits of antioxidants have been thoroughly explored (Liu, 2019). High ROS levels damage lipids, proteins, and DNA. ROS, in particular, can disrupt the lipid membrane, increasing membrane fluidity and permeability. Protein damage includes amino acid modification at particular sites, peptide chain breakage, aggregation of cross-linked reaction products, electric charge changes, enzyme deactivation and proteolysis susceptibility (Ayala *et al.*, 2014). ROS have the ability to damage DNA by oxidising deoxyribose, breaking strands, deleting nucleotides, altering bases and crosslinking DNA-protein (Liang *et al.*, 2020).

Chronic disorders such as cardiovascular disease, diabetes, neurological diseases and cancer are all influenced by oxidative stress. Long-term exposure to pro-oxidant substances can produce structural errors in mitochondrial DNA as well as functional changes in many enzymes and cellular structures, resulting in gene expression aberrations (Rad *et al.*, 2020). Superoxide and hydroxyl radical are the primary oxygen free radicals. They are created by chemical reduction of molecular oxygen. Excessive levels of these free radicals can cause cell damage and apoptosis, which can contribute to a variety of disorders including cancer, stroke, myocardial infarction, diabetes, and other serious problems (Padureanu *et al.*, 2019).

A range of endogenous and exogenous antioxidants protect against oxidative damage and persistent illnesses (Cadet *et al.*, 2012). Many metabolic instances can result in oxidative stress. Type 2 diabetes is a disease in which oxidation plays a significant pathogenetic role. Insulin resistance is the fundamental component of this condition, which is connected to compensatory insulin hypersecretion. Reactive oxygen species can cause insulin resistance by inactivating signalling processes between insulin receptors and the glucose transport system (Chen *et al.*, 2018).

2.7 DIABETES MELLITUS

Diabetes mellitus is a serious metabolic illness characterised by increased blood glucose levels, which can be acute or chronic. The most typical symptoms are increased hunger, frequent urination and increased thirst. Diabetes mellitus, if ignored, can result in major health issues such as chronic kidney disease, stroke, cancer, neuropathy, eyesight loss,and cardiovascular disease. Diabetes affects people in both industrialised and underdeveloped nations, with estimates around 25% of the world's population is afflicted (Majeed *et al.*, 2022). It is a chronic condition caused by a combination of inherited and environmental factors that result in an abnormally high blood sugar level. Despite the fact that several medicines are available for the treatment of diabetes, they do not entirely cure the condition and have severe adverse effects (Jini *et al*., 2020).

Diabetes is divided into four types: type 1 diabetes, type 2 diabetes, gestational diabetes and diabetes caused by other factors. Diabetes classification is critical for determining treatment. Type I diabetes, also known as insulin-dependent diabetes or juvenile diabetes, is caused by a lack of insulin in the body as a result of cell mass loss in the pancreatic islets of Langerhans. Type 2 diabetes, also known as insulin-independent diabetes, occurs when the body fails to respond proportionally to insulin released by the body, and this kind of diabetes is expected to eventually develop to type 1 diabetes (Devadasu *et al*., 2017). Type 2 diabetes mellitus (T2DM) has a higher prevalence than type 1 diabetes mellitus (T1DM) and gestational diabetes. T2DM affects around 460 million individuals globally, with projections indicating a growth to over 700 million in the next 25 years. T1DM is caused by the autoimmune destruction of beta cells, which generate insulin. With an annual incidence rate of 2% to 5%, it is one of the most common paediatric diseases (Nagaraja *et al*., 2022).

2.7.1 ALPHA AMYLASE

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

2.7.2 NON ENZYMIC GLYCOSYLATION OF HEMOGLOBIN

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

2.7.3 PROTEIN GLYCATION

Non enzymatic protein glycation in the body results vascular and renal complications of hyperglycemia. Diabetic patients tend to accumulate glycated proteins in their body tissues as a result of their blood glucose concentration are more than that in healthy people. The initial chemical modification step is that the reaction between the free amino group of proteins and carbonyl group of glucose, that results in formation of fructosamines via Schiff bases, followed by the Amadori rearrangement. The fructosamines are successively oxidized, dehydrated and condensed to make cross-linked proteins and eventually advanced glycation end products (AGEs). Various attempts are created to spot effective glycation inhibitors. Aminoguanidine has the capability to prevent the diabetes-induced formation of AGEs, together the inhibition of protein cross-linking. Aspirin as well as vitamin B6, taurine, quercetin and other natural inhibitors have also been reported (Matsuura *et al.,* 2014).

2.7.4 GLUCOSE UPTAKE

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

2.7.5. GLUCOSE DIFFUSION

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

2.8 HERBAL MEDICINE

India has the most extravagant assorted social custom related with the home grown therapeutic plants for restoring various sicknesses (Tiwari, 2008). India is the biggest maker of therapeutic spices and is called as Professional flower bed of the World (Kumar *et al.*, 2009). It has an incredible biodiversity because of its topographical and climatic conditions. Nearly, 80% of the total populace is depending on home grown meds for their medical services benefits since plant-based therapies are protected, monetary and profoundly effective. Currently, natural prescriptions are getting more significance in the therapy of diabetes (Newmaster, 2011).

The bioactive mixtures of restorative plants are utilized as hostile to diabetic, chemotherapeutic, mitigating against joint specialists where no palatable fix is available in present day medicines. Medicinal plants have been utilized as dietary aid and in the therapy of various sicknesses without appropriate information on their function. Plants have been a praiseworthy wellspring of drugs that have been got explicit route from them. Around 800 plants are hostible to diabetic potential (Ramprasad *et al.*, 2016).

Organic bioactive compounds have challenges with solubility, stability and oral bioavailability in the application, which nanotechnologies might address. Green synthesis of nanoparticles (NPs) using plant extract, on the other hand, is a promising method for NP manufacture due to its safety advantage, but the bioactive plant components that might be more than an aid of the green synthesis of NPs (Mynit *et al.*, 2021). Nanoparticles act as antioxidants which may offer novel ways to combat the pathogenicity of these microbes and their biofilm development, both of which are linked to oxygen levels and ROS generation (Merouane *et al.*, 2019). The antioxidant capabilities of numerous biological samples, pure chemicals, and separated molecules are widely documented. Aside from the use of AgNPs in a variety of applications, a considerable number of studies dealing with the antioxidant capabilities of silver nanoparticles have been published in the last decade (Bedlovicova *et al.*, 2020).

Plant and microbe based nanoparticles, which can protect tissues from free radical damage, have currently received study prominence due to their low cost and safety (Baskaran *et al.*, 2019). The use of nanotechnology in the diagnosis and treatment of diabetes will allow for the use of minuscule amounts of analytes to detect glucose levels and to access clinically important interior cellular areas to effectively treat the condition. Furthermore, nanotechnology delivers very small materials with exceptional durability and electrical conductivity for glucose sensing. Nanotechnology is also assisting in closed loop insulin delivery, in which insulin is administered automatically based on glucose levels. The following sections go into further depth about this occurrence (Devadasu *et al.*, 2017).

2.9 MEDICINAL PLANTS SELECTED FOR THE STUDY

2.9.1 Basella rubra

Basella rubra L. (Basellaceae), often as Malabar spinach, is a herbaceous annual or biennial climbing herb found in tropical and subtropical climates. It is a succulent, branching, smooth, twining herbaceous vine that can grow to be several metres long. The stems are either purple or green. The fleshy leaves are oblong or heart-shaped, 5 to 12 cm long, stalked, and taper to a pointy tip with a cordate base. Spikes are 5-29 cm long, axillary, and solitary. When mature, the fruit is fleshy, stalkless, ovoid or spherical, 5-6 mm long, purple and contains only one seed. The pink blossoms are around 4 millimetres long. Flavonoids are abundant in the plant's leaves. The flower comprise phenolic compounds such as Rutin, Quercetin, Scopoletin, Coumarin, β-xanthin and β-cyanin pigments and Caffeic, Homo-protocatechuic, Chlorogenic, trans and cis-p-coumaric, p-hydroxy-benzoic, phloretic, trans and cis-sinapic, cinnamic acids; and the fruit is composed of β-cyanin, gomphrenin I, gomphrenin II and gomphrenin III. *Basella rubra* is also high in nutrients and minerals (Singh *et al*., 2016).

It is commonly grown as a pot herb in India. Various studies have shown that the plant is high in vitamin A, vitamin C, flavonoids, saponins, carotenoids, a variety of amino acids, organic acids, calcium and iron. As recorded in many Samhitas and Nighantus, it was utilised to heal a wide range of human illnesses. *Basella rubra*'s major biological activities include androgenic, antiulcer, antioxidant, cytotoxic, antibacterial activity, anti-inflammatory, CNS depressant activity, nephroprotective, antidiabetic, antimicrobial, antiviral, hepatoprotective, sleep inducing and wound healing properties. Aside from these actions, the plant has important ethnomedicinal value. It nourishes, strengthens the body, cleanses the blood, rejuvenates and functions as an aphrodisiac. Its inclusion in a regular diet would aid in the prevention of bone weakness, anaemia, cardiovascular disease, colon cancer and other disorders (Sharma *et al*., 2022).

Kingdom	**Plantae**
Division	**Tracheophyta**
Subdivision	**Spermatophytina**
Class	**Magnoliopsida**
Superorder	**Caryophyllanae**
Order	**Caryophyllales**
Family	**Basellaceae**
Genus	***Basella* Linn.**
Species	***Basella rubra* Linn**

2.9.2 *Celosia argentea*

Celosia argentea L. is a 0.4 - 2 m tall, upright, coarse, simple or branching, and smooth annual herb with many ascending branches and brilliantly coloured bedding plants. Stem and herb branches are ridged and frequently parallel, but otherwise glabrous. Leaves are alternate, lanceolate-oblong to narrow linear, entire, 4 to 14 cm long, acute to obtuse, shortly mucronate with the excurrent midrib, glabrous, bitter taste and odour, light green, lamina of the leaves from the centre of the main stem 2-15 x 0.1-3.2 cm, tapering below into an indistinctly demarcated, slender petiole; upper and branch leaves smaller, markedly reducing; leaf axils often with small leaved sterile shoots (Thorat *et al.*, 2018).

Celosia species is used in traditional medicine to treat a variety of ailments such as fever, diarrhoea, wounds, mouth sores, gonorrhoea, itching, jaundice and inflammation. *C.argentea* is a kind of leafy vegetable in the Amaranthaceae family. It's also known as plumed cockscomb. *C. argentea* is a tropical herbaceous plant that is recognised for its bright colour and traditional applications. *C. argentea* has been associated with a number of phytoconstituents, including alkaloids, tannins, flavonoids, saponins, carbohydrates, proteins, steroids and amino acids. All of the previously stated phytoconstituents were found in the leaf and stem, whereas the flower included alkaloids, carbohydrates, proteins and steroids (Fayaz *et al.*, 2019).

Kingdom	**Plantae**
Division	**Magnoliophyta**
Subdivision	**Spermatophyte**
Class	**Magnoliopsida**
Order	**Caryophyllales**
Family	**Amaranthaceae**
Genus	***Celosia***
Species	***C. argentea***

PROCEDURE

EXPERIMENTAL PROCEDURE

3.0 EXPERIMENTAL PROCEDURE

3.1 COLLECTION OF PLANT SAMPLE

3.1.1 *Basella rubra*

The leaves and stem of *Basella rubra* (*B. rubra*) were collected from Omalur, Salem, Tamil Nadu, India. The sample was identified and authenticated by Botanical Survey of India, TNAU, Coimbatore. The authentication number is BSI/SRC/5/23/2023-24/Tech-414. Plate 1 represents *Basella rubra*.

Basella rubra is commonly known as Indian spinach or Malabar spinach. It belongs to the family Basellacae. It is an annual or biennial herb found in tropical and subtropical regions. The plant has important ethnomedicinal value. It nourishes, strengthens the body, cleanses the blood, rejuvenates and functions as an aphrodisiac (Sharma *et al*., 2022). Plate 1 represents *Basella rubra*.

PLATE 1

Basella rubra

3.1.2 *Celosia argentea*

The leaves of *Celosia argentea* (*C. argentea*) were collected from Coimbatore, Tamil Nadu, India. The sample was identified and authenticated by Botanical Survey of India, TNAU, Coimbatore. The authentication number is BSI/SRC/5/23/2023-24/Tech-413.

Celosia argentea L. is a herbaceous plant of the Amaranthacaceae family. It is a 2 metre tall erect annual plant. The stem is ridged and smooth. *Celosia* species is used in traditional medicine to treat a variety of ailments such as fever, diarrhoea, wounds, mouth sores, gonorrhoea, itching, jaundice and inflammation (Fayaz *et al.*, 2019). Plate 2 represents *Celosia argentea.*

PLATE 2

Celosia argentea

3.2 PREPARATION OF ETHANOLIC EXTRACT

Collected *Basella rubra* and *Celosia argentea* plants were thoroughly washed several times in water to remove dust and the samples are air-dried in the shade at room temperature to achieve complete drying. The dried sample was powdered. 10 g of the powdered plant sample was weighed and dissolved in 100 ml of ethanol and kept in continuous shaking for 7 days. The mixture was then filtered through Whatman No. 1 filter paper. The filtrate was then evaporated using rotary evaporator. The final extract was collected and stored at 4°C for further experiments.

3.3 SYNTHESIS OF SILVER NANOPARTICLES

500 mg of *Basella rubra* and *Celosia argentea* ethanolic extract was dissolved in 100ml of deionized water. 1mM silver nitrate (Ag) was prepared using deionized water and used to synthesize silver nanoparticles according to the method explained by Harbone (1998). 90 ml of 1mM aqueous silver nitrate solution was added to 10 ml of ethanolic extract and exposed to bright sunlight, a color change occurs from green to reddish brown within minutes (Sulaiman *et al.*, 2013).

3.4 ENCAPSULATION OF SILVER NANOPARTICLES IN PVA MATRIX

Poly Vinyl Alcohol (PVA) of 0.14g was mixed with 100 ml of double distilled water and stirred for 2 h. The solution was then slowly added with 120 ml of plant extract with silver nitrate (Chandran *et al*., 2016).

3.5 CHARACTERIZATION OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

The PVA encapsulated silver nanoparticles of ethanolic extract of *Basella rubra* and *Celosia argentea* were characterized as per the methods explained below.

3.5.1 FOURIER TRANSFORM INFRARED (FT-IR) ANALYSIS OF *Basella rubra* AND *Celosia argentea*

FT-IR analysis is performed to obtain infrared spectra of absorption, emission and to confirm the formation of silver nanoparticles. This helps to identify possible interactions between silver, PVA and bioactive molecules that can lead to the formation and stabilization of PVA encapsulated silver nanoparticles. The advantage of using FT-IR is that it simultaneously collects spectral data from a wide spectral range (Zaheer *et al*., 2012). FT-IR is mainly used to detect functional groups on PVA encapsulated silver nanoparticles. Fourier transform infrared (FTIR) analysis was performed in the wavenumber range from 500 to 3500 cm^{-1} to identify the functional groups of the colloidal form of PVA-encapsulated silver nanoparticles (Chandran *et al*., 2016).

3.5.2 UV VISIBLE SPECTRUM OF *Basella rubra* AND *Celosia argentea*

The primary method used to monitor the generation of AgNPs was the colour change of extract upon treatment with AgNO3 (1 mM). Using UV-visible spectra, the bio-reduction of Ag ions in an aqueous extract was observed. At room temperature, spectrophotometer readings of UV-visible spectra from 300 to 700 nm were taken. As a reaction blank, double distilled water was used (Premasudha *et al*., 2015). In addition, a greater peak on the absorbance axis indicates that there are more silver nanoparticles in the aqueous solution and more of this specific wavelength of light is absorbed. As a result, this feature aids in determining the synthesis of PVA encapsulated silver nanoparticles enables preliminary prediction of the concentration of silver nanoparticles created (Nguyen *et al*., 2021).

3.5.3 SCANNING ELECTRON MICROSCOPY (SEM) OF OF *Basella rubra* AND *Celosia argentea*

Morphology of the synthesized silver nanoparticles in the PVA matrix was observed using the SEM micrographs (Chandran *et al.*, 2016). The interaction between the beam's electrons and the sample results in a variety of signals that may be utilised to learn more about the surface composition and topography (Nguyen *et al.*, 2021). The samples were created by simply dropping a very little quantity of the sample onto a copper grid covered with carbon, followed by blotting out any excess solution. Each suspension of PVA AgNPs was coated individually. The film on the grid for scanning electron microscopy was then given five minutes to dry under a mercury lamp. After that, it underwent SEM examination (Srirangam *et al.*, 2017).

3.5.4 ZETA POTENTIAL OF *Basella rubra* AND *Celosia argentea*

Zeta potential is a crucial factor in determining how stable AgNPs are in aqueous solutions. Particles that have a large negative or positive zeta potential are likely to repel each other, and thereby have enhanced suspension stability. However, if the particles have low zeta potential values, then attractive forces between the particles become dominant and particles undergo aggregation and flocculation (Ajitha *et al.*, 2016).

3.5.5 DYNAMIC LIGHT SCATTERING (DLS) OF *Basella rubra* AND *Celosia argentea*

Dynamic light scattering (DLS) is used to determine the size distribution of particles by measuring dynamic fluctuations of light scattering intensity caused by the Brownian motion of the particles. The measurement gave the average hydrodynamic diameter of the particles. If a particle size distribution exists in a solution, DLS weights the distribution differently than SEM, by size to the power of 6, resulting in a z-average distribution (Elamawi *et al.*, 2018).

3.6 *IN VITRO* ANTIOXIDANT ACTIVITY OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* and *Celosia argentea*

3.6.1 DPPH Radical Scavenging Assay of *Basella rubra* and *Celosia argentea*

The radical scavenging activity of PVA encapsulated silver nanoparticles of *Basella* *rubra* and *Celosia argentea* was determined by DPPH assay explained by Chang *et al.* (2017) and the detailed procedure is given in Appendix I.

3.6.2 Hydroxyl radical scavenging assay of *Basella rubra* and *Celosia argentea*

Hydroxyl radical scavenging activity of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argentea* was determined by the method of Halliwell *et al*. (1981) and the detailed procedure is given in Appendix II.

3.6.3 Hydrogen peroxide radical scavenging assay of *Basella rubra* and *Celosia argentea*

Hydrogen peroxide radical scavenging activity of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argentea* was determined by the method explained by Patnaik *et al*. (2017) and the detailed procedure is given in Appendix III.

3.6.4 Ferric reducing antioxidant power (FRAP) assay of *Basella rubra* and *Celosia argentea*

In-vitro ferric reducing activity of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argentea* was determined by the method of Oyaizu *et al*. (1986) and the detailed procedure is given in Appendix IV.

3.7 *IN VITRO* ANTIDIABETIC ACTIVITY OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* and *Celosia argentea*

3.7.1 Alpha amylase inhibitory activity of *Basella rubra* and *Celosia argentea*

In- vitro alpha amylase inhibitory activity of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argentea* was done by the method explained by Subramanian *et al*. (2008) and it is given in Appendix V.

3.7.2 Non enzymatic glycosylation of haemoglobin inhibitory activity of *Basella rubra* and *Celosia argentea*

Nonenzymatic glycosylation of haemoglobin inhibitory activity of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argentea* was measured by the method of

Daksha *et al.* (2012) and detailed procedure is given in Appendix VI.

3.7.3 Glucose uptake capacity by yeast cells

Glucose uptake capacity of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argentea* was studied by the method of Vijayalakshimi *et al.* (2015) and detailed procedure is given in Appendix VII.

3.7.4 Glucose diffusion inhibitory activity of *Basella rubra* and *Celosia argentea*

Glucose diffusion inhibitory activity of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argentea* was studied by the method of Gallager *et al.* (2003) and detailed procedure is given in Appendix VIII.

3.8 STATISTICAL ANALYSIS

All experiments were carried out in triplicates and the results are expressed as percentage inhibition and as mean ± standard deviation.

RESULTS AND DISCUSSION

4.0 RESULTS AND DISCUSSION

The medicinal plants are a rich source of medicines used as herbal remedies and are involved in the development of the modern pharmaceuticals. It has gained increased attention in solving the health care problems worldwide. The research on plants having medicinal importance is growing tremendously and has revealed that many plants synthesize and accumulate in them the natural constituents that have active physiological and psychological effects on the human body. India is a country with a vast reverse of the natural resources (Mamedov *et al*., 2015).

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

The present study was formulated to produce PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argentea* and to compare their efficacy on antidiabetic and antioxidant activities. The results obtained were furnished and discussed below.

4.1 SYNTHESIS OF SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

The green synthesis of silver nanoparticles begins with the reduction of silver ions by phytochemicals as the first step in the generation of nanoparticles. Phytochemicals also play a role in the next phases by stabilising and controlling the form and size of nanoparticles (Ahmed *et al*., 2016).

Synthesis of silver nanoparticles from natural sources has gained importance in recent days. The synthesis exhibited a notable change in color from yellow to brown and the intensity of brown colour is directly proportional to the increase in incubation period and temperature, which indicated the reduction of silver nitrate by the extract (Parveen, 2015). Plate 3 represents synthesis of silver nanoparticles of ethanolic extract of *Basella rubra* and *Celosia argentea.*

PLATE 3

SYNTHESIS OF SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

Plant extract +
1mM silver nitrate solution

Synthesized silver nanoparticles
of *Basella rubra*

Plant extract +
1mM silver nitrate solution

Synthesized silver nanoparticles
of *Celosia argentea*

The silver nanoparticles of ethanolic extract of *Basella rubra* and *Celosia argentea* are synthesized on exposure of sunlight for 20 minutes. Both *Basella rubra* and *Celosia argentea* was found to act as a stabilizing agent for the reduction of Ag+ to Ag 0. As the silver nanoparticles are formed, the color of the solution changed from green to dark brown which is an indication of the presence of silver nanoparticles. The variation of the color was due to the change in surface plasmon resonance of silver nanoparticles during the formation.

According to Srikar *et al*. (2016), plant extract of *Prunus amygdalus* when added to $AgNO_3$ the color of the solution change from pale yellow to dark brown which indicates the formation of AgNPs. The colour formation occurs due to excitation of the surface plasmon resonance effect and the reduction of $AgNO3$.

Similar results were observed by Sumitha *et al.* (2018), that aqueous extract of durian seeds was utilized to reduce $AgNO_3$ to Ag0 where the observed color change from colorless to yellowish brown confirms the reduction. The maximum absorbance exhibited by the reaction solution at 420 nm is the characteristic surface plasmon resonance (SPR) peak of *Durio zibethinus* silver nanoparticles.

4.2 PVA ENCAPSULATION OF SYNTHESIZED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

Green synthesis of metal nanoparticles is an essential technology in the manufacture of environmental friendly nanoparticles. Despite the fact that silver nanoparticles reduced by medicinal plants have a significant therapeutic potential, employing silver alone is difficult for applications in the food sector and pharmaceutical technology. As a consequence, research has been focused on polymer metal composites to enhance the preservation of biological active chemicals from degradation, regulate medication release and increase therapeutic agent absorption (Ahmed *et al*., 2016). The manufacture of nanoparticles from plant extract is inexpensive. The polyvinyl alcohol (PVA) encapsulated silver nanoparticles synthesized from such technique are stable for several months and can be stored at room temperature without any special attention (Chandran *et al*., 2016). Plate 4 represents PVA encapsulation of synthesized silver nanoparticles of ethanolic extract of *Basella rubra* and *Celosia argentea.*

PLATE 4

PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

B.rubra ***C.argentea***

The silver nanoparticles synthesized from the ethanolic extract of *Basella rubra* and *Celosia argentea* are encapsulated with Poly Vinyl Alcohol (PVA). It improves the stability of synthesized silver nanoparticles and prevent them from agglomeration.

The incorporation of metal nanoparticles into composite polymers has created a new class of materials that may be used in a variety of applications. Polymer is often employed as a stabiliser in order to sustain particle formation, stabilise metal dispersion and reduce particle oxidation. Polymers are an excellent host material with excellent optical and electrical properties for metal and semiconductor nanoparticles (Bulla *et al*., 2021). The synthesized silver nanoparticles are encapsulated with a polymer called poly vinyl alcohol (PVA), since it acts as a capping agent. It is also used as a stabilizing agent that prevents aggregation or coagulation in colloidal synthesis (Chandran *et al*., 2016). PVA is a water-soluble polymer that is thermally stable, chemically resistant and mechanically strong. It is a suitable metal host material that can protect nanoparticles from agglomeration (Bulla *et al*., 2021).

4.3 CHARACTERIZATION OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

The characterization of PVA encapsulated silver nanoparticles synthesized from *Basella rubra* and *Celosia argentea* was done by UV-Visible spectroscopy, Fourier-Transform infrared spectroscopy (FT-IR), Scanning Electron Microscopy (SEM), Zeta potential and Particle size analyser.

4.3.1 FOURIER TRANSFORM INFRARED ANALYSIS (FT-IR) OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

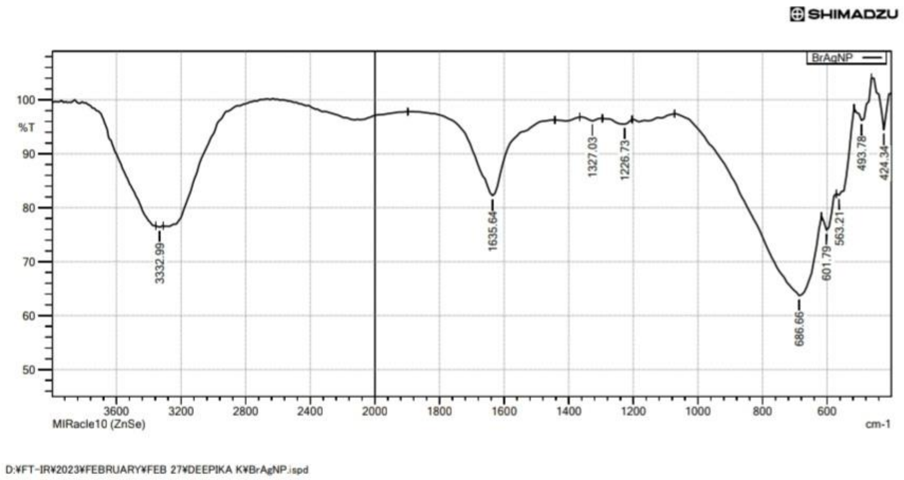
Fourier Transform Infrared Spectrophotometer (FT-IR) is perhaps the most powerful tool for identifying the types of functional groups present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption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

FT-IR spectra of the silver nanoparticles were carried out to identify the functional groups and the possible interaction between the compounds and the silver nanoparticles.

The FT-IR spectrum of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argentea* are presented in the Figure 5.

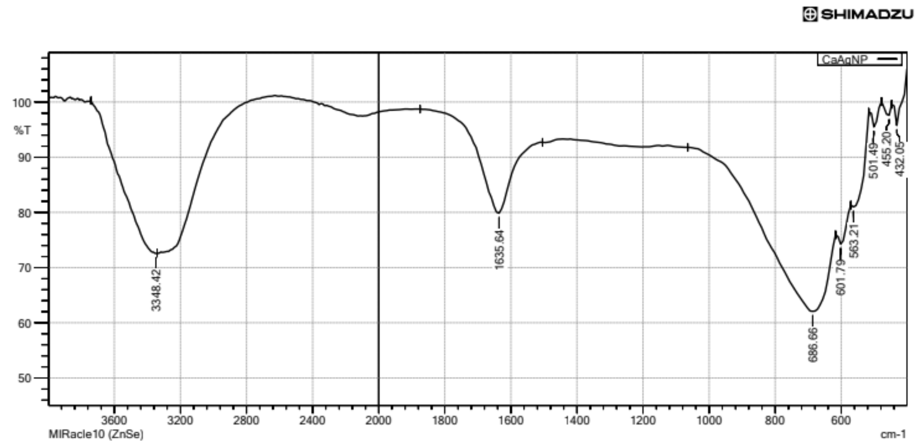
FIGURE 5

FOURIER TRANSFORM INFRARED ANALYSIS OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra*



D:¥FT-IR¥2023¥FEBRUARY¥FEB 27¥DEEPIKA K¥BrAgNP.ispd
MIRacle10 (ZnSe)

FOURIER TRANSFORM INFRARED ANALYSIS OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Celosia argentea*



D:¥FT-IR¥2023¥APRIL¥APR 20¥DEEPIKA K¥CaAgNP.ispd
MIRacle10 (ZnSe)

	Item	Value
2	Sample name	
3	Sample ID	
4	Option	
5	Intensity Mode	%Transmittance
6	Apodization	Happ-Genzel
9	No. of Scans	45

The FT-IR spectrum of PVA encapsulated silver nanoparticles of *Basella rubra* indicates the functional groups. The peak 3332.99 cm^{-1} reveals O-H stretching of alcohols. The peak 1653.64 cm^{-1} reveals C-H bending of aromatic compounds. 1327.03 cm^{-1} represents O-H bending of phenol. The peak 1327.03 - 1226.73 cm^{-1} shows C-O stretching of alkyl aryl ether. The peak 1087 cm^{-1} reveals C-O stretching of primary alcohol. 686.66 cm^{-1} represents C=C bending of alkenes. 601.79 - 424.34 cm^{-1} represents C-Br stretching of halo compounds.

Different peaks observed in the FTIR spectrum indicate the functional groups on PVA encapsulated silver nanoparticles. The peak 3348.42 cm^{-1} reveals O-H stretching of alcohols. The peak 1635.64 cm^{-1} reveals C-H bending of aromatic compounds. The peak 1327.03 - 1226.73 cm^{-1} shows C-O stretching of alkyl aryl ether. 686.66 cm^{-1} represents C=C bending of alkenes. 601.79 - 432.05 cm^{-1} represents C-Br stretching of halo compounds.

Nilavukkarasi *et al.* (2020) report that the peak of *Capparis zeylanica* leaves ranges between 400 and 4000 cm^{-1}. The NH group produced a wide band at 3577.28 cm^{-1}. The bands at 3455.17 and 1637.5 cm^{-1} correspond to vibrations of the hydroxyl (OH) and carbonyl (C-O) groups, respectively. The C-H stretching and C-N stretching variants of amine are responsible for the band at 2426.90 cm^{-1}. The band at 1120.99cm^{-1} is caused by the C-O group of aliphatic esters, whereas the bond at 1014.60 cm^{-1} might be caused by the C-O stretch. The measured peaks of 886.22 and 838.75 cm^{-1} correspond to the C-CH2 group and the PH bent phosphines group, respectively. The remaining linkages resembled the acetylenic C-H bend with alkynes (630-770 cm^{-1}).

Chandhran *et al.* (2016) reported that peaks of *Ocimum sanctum* were observed at 3340$cm^{−1}$, 1637$cm^{−1}$, 659$cm^{−1}$ to 553$cm^{−1}$. The peak observed at 3340$cm^{−1}$ indicates the presence of a hydrogen bond between the PVA polymer and leaf causing OH/NH2 stretching. The peak at 1637$cm^{−1}$ may be attributed to CC, stretching mode. The peaks from 659$cm^{−1}$ to 553$cm^{−1}$ might be caused by wagging mode of OH groups. The FTIR results confirm that the PVA, as a capping agent, plays an important role in the formation of silver nanoparticles.

4.3.2 UV-VISIBLE SPECTROSCOPY OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

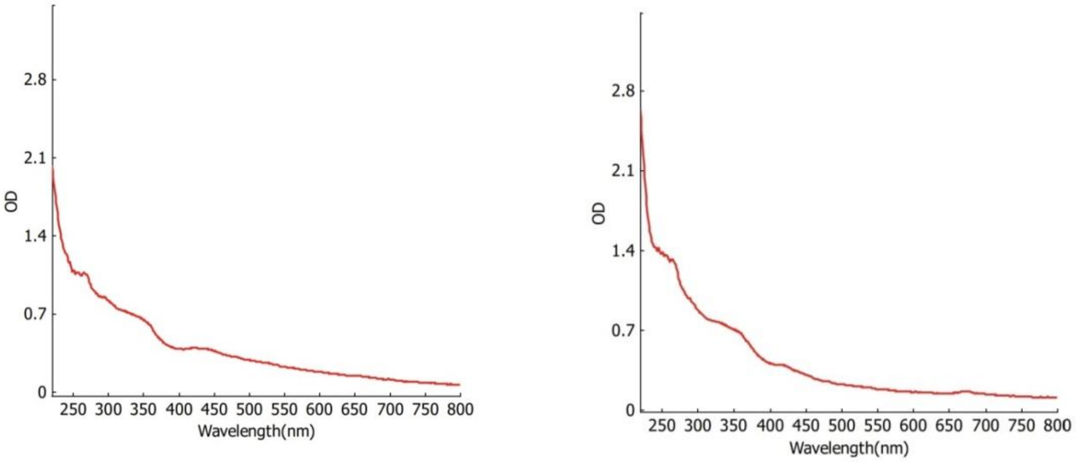
The reduction of silver ions to silver nanoparticle was reflected in spectral data obtained using a UV-Visible spectrometer. The synthesis of silver nanoparticles was analyzed using UV-Visible (UV-Vis) spectrometry. The absorbance of nanoparticles was measured in the range 300-700nm.

The silver ions reduced to silver nanoparticles by the plants *Basella rubra* and *Celosia argentea* was monitored by UV-Visible spectroscopy.

The UV-Visible absorption spectrum of *Basella rubra* and *Celosia argentea* is shown in the Figure 6.

FIGURE 6

UV-VISIBLE SPECTROSCOPY OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra and Celosia argentea*



B.rubra ***C.argentea***

The UV visible spectrum of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argentea* clearly indicates that the synthesized silver nanoparticles has absorption spectra of maximum at 420nm and the remarkable broadening of peak indicated that the particles polydispered. The broad peak of 400 to 450nm depicts the stability of PVA encapsulation. This clearly indicates that there is an interaction between silver nanoparticles and bimolecules present in the leaves of *Basella rubra* and *Celosia argentea.*

Johnson *et al*. (2020) reported that *O. chinensis* extract showed that the absorption peak (SPR) was observed in the visible range at 260 nm. Thus it clearly indicates that the synthesis of silver nanoparticles.

Chandhran *et al*. (2016) reported that a broad surface plasmon resonance peak was observed for the PVA encapsulated silver nanoparticles of *Ocimum sanctum* and the absorption spectra was observed from 450nm to 480nm. The broad nature of the band might be due to the presence of polymer in the solution.

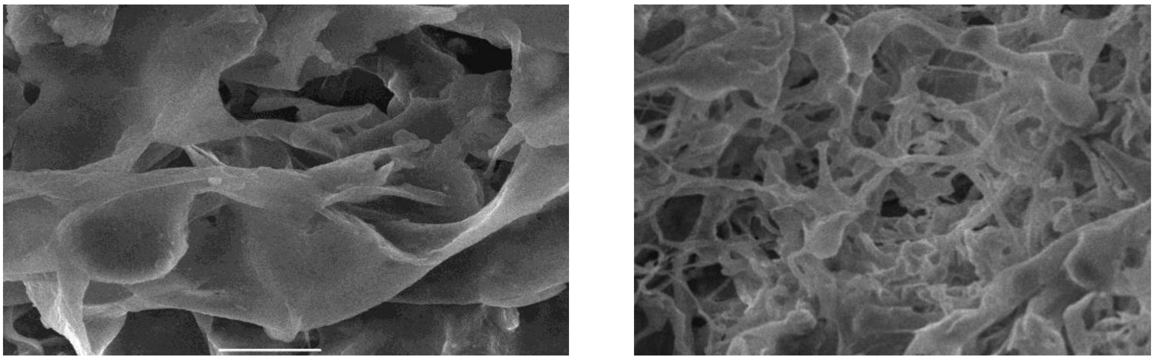
4.3.3 SCANNING ELECTRON MICROSCOPY (SEM) OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

The shape and size of the PVA encapsulated silver nanoparticles synthesized from *Basella rubra* and *Celosia argentea* was identified using SEM.

The SEM images of PVA encapsulated silver nanoaparticles of *Basella rubra* and *Celosia argentea* are shown in the figure 7.

FIGURE 7

SEM IMAGES OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra and Celosia argentea*



B.rubra ***C.argentea***

The SEM images of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argentea* revealed that the synthesized silver nanoparticle were in the range of 58.3nm and 52.7nm. It shows a clear image of highly dense silver nanoparticles of plant extracts of *Basella rubra* and *Celosia argentea*. From the image, it is found that the spherical shaped silver nanoparticles were embedded in a sponge-like PVA matrix.

Oves *et al.* (2018) reported that the plant extract of *Salvia spinosa* shows the morphology of the AgNPs was analyzed through SEM images. The majority of particles were spherical in shape and there were a few oval as well. Biosynthesized Ag NPs had been spread thoroughly in the solution. The size of some selected biosynthesized nanoparticles was 19–125 nm according to SEM images.

Bayrami *et al.* (2018) reported that the plant extract of *Vaccinium arctostaphylus* shows the surface morphology of AgNPs which was analysed through SEM images. The majority of particles were almost spherical in shape, but with different sizes and aggregations.

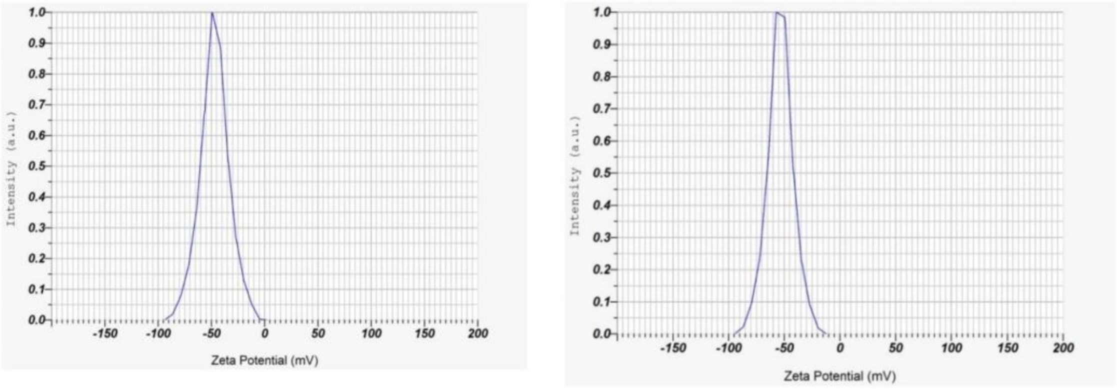
4.3.4 ZETA POTENTIAL OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

The zeta potential is an essential parameter for determining the stability of AgNPs in aqueous solutions. Particles with a high negative or positive zeta potential resist each other, resulting in improved suspension stability. In general, a minimum zeta potential of -30 mV is required to verify a stable nano suspension (Ajitha *et al*., 2016).

The zeta potential peak of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argentea* is shown in the figure 8.

FIGURE 8

ZETA POTENTIAL OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* and *Celosia argentea*



B.rubra ***C.argentea***

The zeta potential results showed that the synthesised PVA encapsulated AgNPs were more stable. Furthermore, the negative zeta potential value -47.0 mV of *Basella rubra* and -53.3 mV of *Celosia argentea* suggests that the PVA encapsulated AgNPs acts as a capping agent which plays an active role in enhancing the stability of the AgNPs for a longer period.

Saxena *et al.* (2021) reported that the zeta potential analysis of silver nanoparticles synthesized from *Ocimum tenuilforum* and *Catharanthus roseus* were -8.35mV and -16.12 mV.

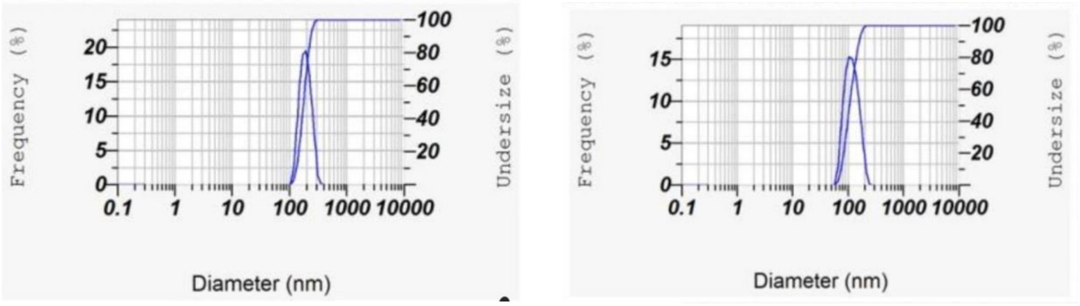
4.3.5 DYANAMIC LIGHT SCATTERING (DLS) OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

The particle size (z-average) and polydispersity index of PVA-stabilized silver nanoparticles of Basella rubra and Celosia argentea were recorded by particle size analyzer.

The DLS plot of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argentea* is shown in the figure 9.

FIGURE 9

DYANAMIC LIGHT SCATTERING OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*



B.rubra ***C.argentea***

Figure 9 presents the plot of number intensity and volume intensity of nanoparticles with the size of PVA AgNPs. The average particle size of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argentea* was 184.8nm and 68.8nm.

Basak *et al.* (2018) reported that the particle size analysis of gelatin PVA silver nanocomposites revealed a particle size of 86.55nm. This denotes to monodispersity of nanoparticles which provides very high stability of nanoparticles for a long time.

Elamawi *et al.* (2018) reported that the particle size analysis of silver nanoparticles of *Trichoderma longibrachiatum* showed an average particle size of 53.7nm. The measurement gave the average hydrodynamic diameter of the particles.

4.4 *IN VITRO* ANTIOXIDANT ACTIVITY OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

It is recognised that free radicals cause oxidative stress, which can lead to the destruction of DNA molecules, lipids, and proteins in biological systems, resulting in a variety of illnesses. Antioxidants are highly able to retard or prevent oxidation of main substances through free radical scavenging. Several phytochemicals in plants namely polyphenols such as phenolic acids, flavonoids, tannins, and anthocyanins, are known to be responsible for free radical scavenging and antioxidant properties (Farag *et al*., 2020).

In the present study, the inhibitory activity of different concentations (250 – 1000 µg/ml) of PVA encapsulated silver nanoparticles synthesized from ethanolic extract of *Basella rubra* and *Celosia argenta* was investigated for its DPPH radical activity, hydroxyl radical scavenging activity, hydrogen peroxide scavenging activity and ferric reducing activities and the results are given below.

4.4.1 DPPH RADICAL SCAVENGING ASSAY OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

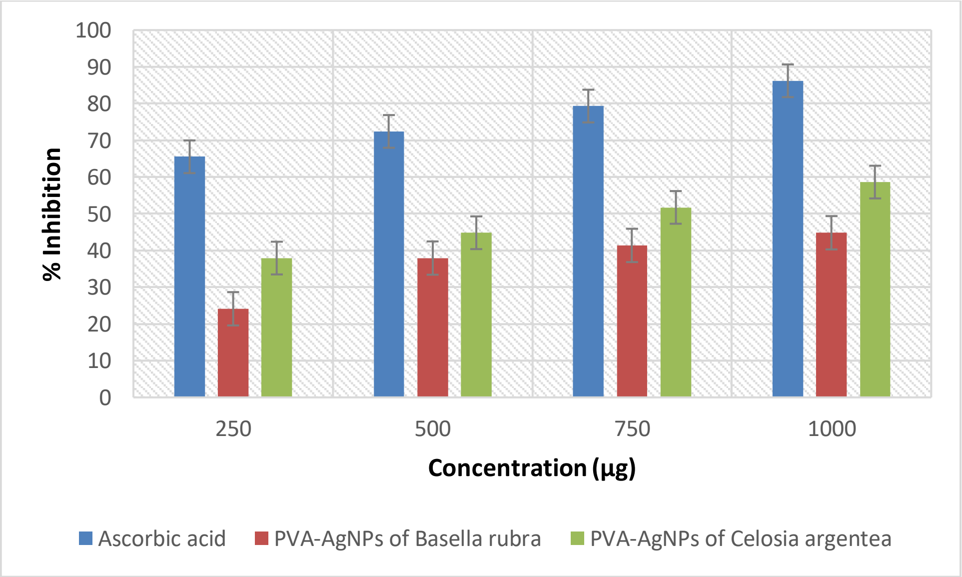
DPPH (2,2-diphenyl-2 picryl hydrazyl hydrate) is a stable free radical with deep violet colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The degree of discolouration indicates the scavenging potential of the antioxidant compounds (Reddy *et al*., 2021).

DPPH is a stable nitrogen-centered free radical commonly used for testing radical scavenging activity of the compound or plant extracts. When the stable DPPH radical accepts an electron from the antioxidant compound, the violet color of the DPPH radical was reduced to yellow colored or colorless diphenyl picrylhydrazine radical which was measured colorimetrically. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Kalaisezhiyen *et al*., 2018).

Figure 10 represents DPPH radical scavenging assay of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argenta.*

FIGURE 10

DPPH RADICAL SCAVENGING ASSAY OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*



The above figure clearly indicates that the DPPH radical scavenging activity of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argenta* was found to be increased with increase in concentration from 250-1000 µg/ml. At a concentration of 1000 µg/ml the PVA encapsulated silver nanoparticles of *Basella rubra* showed 44.8 percentage inhibition and *Celosia argenta* showed 58.6 percentage inhibition. Thus the PVA encapsulated silver nanoparticles of *Celosia argenta* showed potent inhibitory activity against DPPH when compared with *Basella rubra*. The positive control, ascorbic acid has exerted the highest potent inhibitory action against DPPH (86.2 per cent).

Ojemaye *et al.* (2021) showed that the silver nanoparticle synthesized from the extract of *Crataegus ambigua* depicts significant radical scavenging effect, acting as good electron donors in the *in vitro* experiment. The silver nanoparticles showed a maximum scavenging capacity of 68.02% against DPPH radicals with an IC_{50} concentration of 54.5 µg/mL.

Csakvari *et al.* (2021) showed that the silver nanoparticle synthesized from the plant extract of *Cannabis sativa* exhibited 18.4 ± 3.9% inhibition.

4.4.2 HYDROXYL RADICAL SCAVENGING ASSAY OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

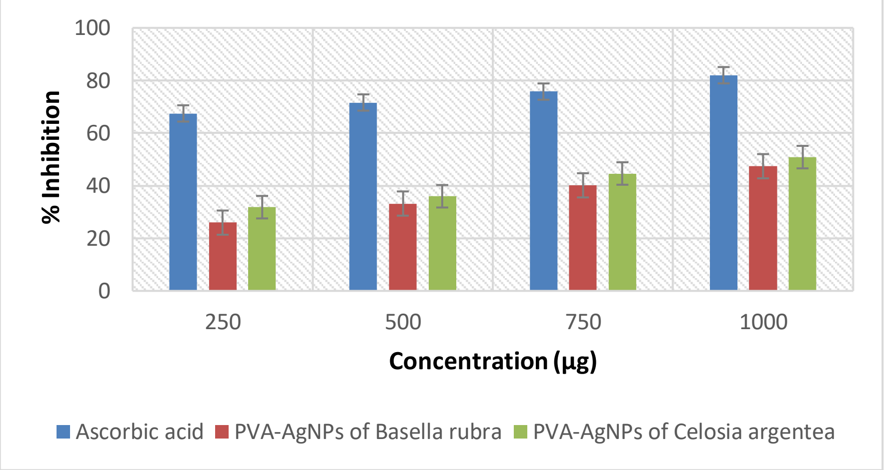
Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acids of cell membranes phospholipids and causes damage to cells. This assay is used to find the scavenging activity of free radicals in the presence of different concentrations of plant sample. The model used is ascorbic acid-iron-EDTA model of hydroxyl radical generating system. This is totally aqueous system in which ascorbic acid, iron and EDTA conspire with each other to generate hydroxyl radical (Thomas *et al*., 2023).

Hydroxyl radical is the most reactive oxygen centered species and causes severe damage to adjacent biomolecule. The hydroxyl radicals were formed by the oxidation reaction with the Dimethyl Sulphoxide (DMSO) to yield formaldehyde, which provides a convenient method to detect hydroxyl radicals by treatment with Nash reagent. Substances which are able to scavenge hydroxyl radicals can be considered as antioxidants and therefore radical scavengers (Kalaisezhiyen *et al*., 2018).

Figure 11 represents hydroxyl radical scavenging assay of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argenta.*

FIGURE 11

HYDROXYL RADICAL SCAVENGING ASSAY OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*



The above figure implies that the hydroxyl radical scavenging activity of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argenta* was found to be increased with increase in concentration from 250-1000 µg/ml. At a concentration 1000 µg/ml the PVA encapsulated silver nanoparticles of *Basella rubra* showed 47.4 percentage inhibition and *Celosia argenta* showed 50.9 percentage inhibition. Thus the PVA encapsulated silver nanoparticles of *Celosia argenta* showed potent inhibitory activity against hydroxyl radical when compared with *Basella rubra*. The positive control, ascorbic acid has exerted the highest potent inhibitory action against hydroxyl radical (82 per cent).

Keshari *et al*. (2018) reported that the hydroxyl radicals scavenging activity of *C.roseus* extract, CrAgNPs and Vitamin C results confirmed that *C. roseus* extract, CrAgNPs and Vitamin C have 67.5, 73.2 and 70.3 per cent hydroxyl radicals scavenging activity.

4.4.3 HYDROGEN PEROXIDE RADICAL SCAVENGING ASSAY OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

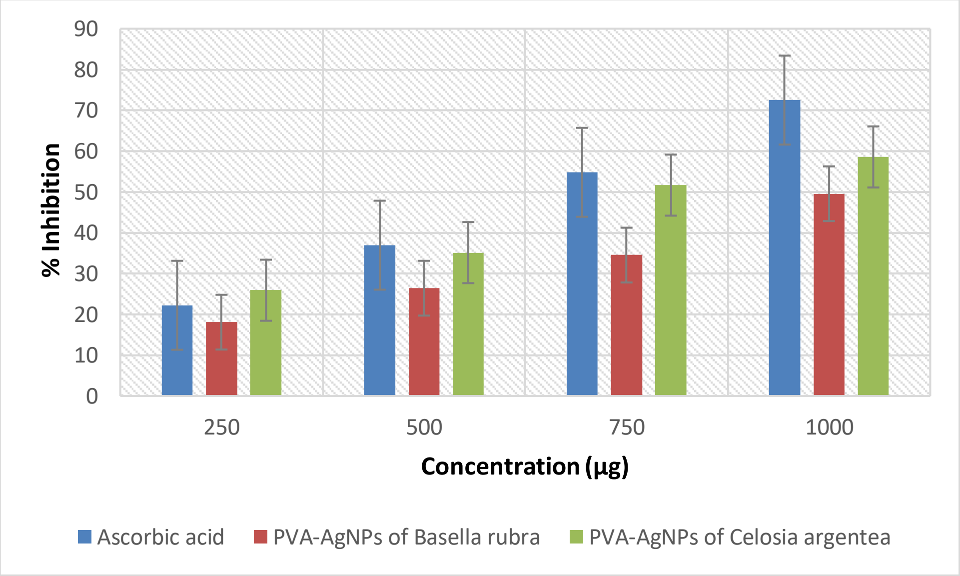
Scavenging of hydrogen peroxide by extracts may be attributed to their phenolics, which can donate electrons to hydrogen peroxide, thus neutralizing it to water. The results show that the PVA encapsulated synthesized silver nanoparticles of ethanolic extract of

Boerhavia diffusa had potent hydrogen peroxide scavenging activity which may be due to the antioxidant compounds. As the antioxidant components present in the extracts are good electron donors, they may accelerate the conversion of hydrogen peroxide to water (Kalaisezhiyen *et al*., 2018).

Figure 12 represents hydrogen peroxide radical scavenging assay of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argenta*.

FIGURE 12

HYDROGEN PEROXIDE RADICAL SCAVENGING ASSAY OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*



From the above figure, it is clear that the hydrogen peroxide radical scavenging activity of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argenta* was found to be increased with increase in concentration from 250-1000 µg/ml. At a concentration 1000 µg/ml the PVA encapsulated silver nanoparticles of *Basella rubra* showed 49.6 percentage inhibition and *Celosia argenta* showed 58.6 percentage inhibition. Thus the PVA encapsulated silver nanoparticles of *Celosia argenta* showed potent inhibitory activity against hydrogen peroxide when compared with *Basella rubra*. The positive control, ascorbic acid has exerted the highest potent inhibitory action against hydrogen peroxide (72.51 per cent).

Keshari *et al*. (2018) reported that hydrogen peroxide scavenging activity of C.roseus extract, CrAgNPs and Vitamin C results showed 55.5, 72.3 and 66.3 per cent hydrogen peroxide scavenging activity.

Govindappa *et al*. (2022) reported that silver nanoparticles from *Alternaria alternate* reported that 100 µg/ml, concentrations of *Alternaria alternata* AgNPs have significantly inhibited the H_2O_2 scavenging activity and were found to be 86.5 per cent.

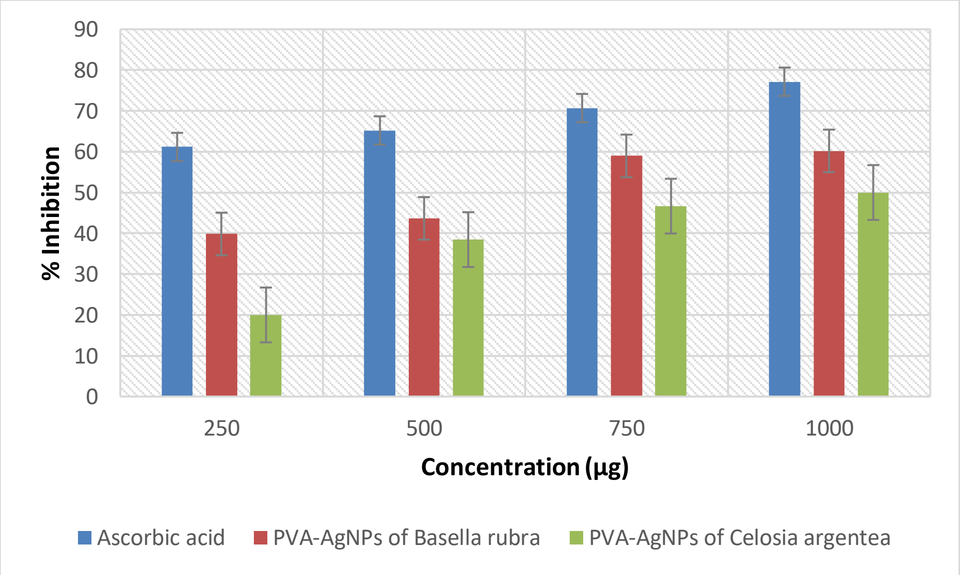
4.4.4 FERRIC REDUCING ABILITY OF PLASMA (FRAP) ASSAY OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

As a measure of antioxidant capacity, the ferric reducing ability of plasma (FRAP) was evaluated. Under acidic conditions (pH 3.6), the FRAP assay measures the reduction of ferric iron and 2,3,5-triphenyl-1,3,4-triaza-azoniacyclopenta-1,4-diene chloride to blue ferrous complex by antioxidants. The FRAP unit is one mole of Fe (ll) reduced to Fe (ll) (Haida *et al*., 2019).

Figure 13 represents ferric reducing ability of plasma (FRAP) assay of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argenta.*

FIGURE 13

FERRIC REDUCING ABILITY OF PLASMA (FRAP) ASSAY OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*



The above figure indicates that the ferric reducing ability of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argenta* was found to be increased with increase in concentration from 250-1000 µg/ml. At a concentration 1000 µg/ml the PVA encapsulated silver nanoparticles of *Basella rubra* showed 60.2 percentage inhibition and *Celosia argenta* showed 50 percentage inhibition. Thus the PVA encapsulated silver nanoparticles of *Basella* *rubra* showed potent ferric reducing power when compared with *Celosia argenta*. The positive control, ascorbic acid has exerted the highest ferric reducing power (77.2 per cent).

Bharathi *et al*. (2019) reported that photo-synthesized silver nanoparticles of *Cassia angustifolia* AgNPs activity improved as the concentration of nanoparticles increased. FRAP IC50 value for AgNPs was estimated to be 63.21 ± 0.75 μg/mL. Ferric ion-reducing activity of the AgNPs was estimated from their ability to reduce oxidized Fe3+ (color less) to Fe2+ (blue color).

4.5 *IN VITRO* ANTIDIABETIC ACTIVITY OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

Diabetes mellitus is a life-threatening metabolic condition characterised by increased blood glucose levels, which can be transient or chronic. The most typical symptoms are increased hunger, frequent urination and increased thirst. Diabetes mellitus, if ignored, can result in major health issues such as chronic kidney disease, stroke, cancer, neuropathy, eyesight loss and cardiovascular disease. Diabetes affects 25 per cent of the world's population (Majeed *et al*., 2022).

In the present study, the inhibitory activity of different concentations (250 – 1000 μg/ml) of PVA encapsulated silver nanoparticles synthesized from ethanolic extract of *Basella rubra* and *Celosia argenta* was investigated for its alpha amylase activity, nonenzymatic glycosylation, glucose uptake capacity and glucose diffusion inhibitory activity activities and the results are given below.

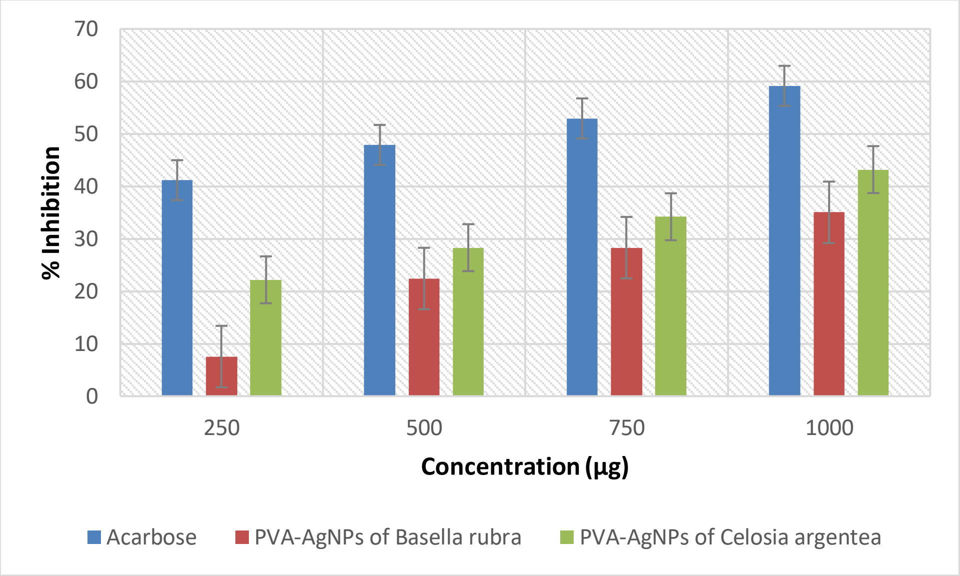
4.5.1 INHIBITION OF ALPHA AMYLASE ACTIVITY OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

The inhibition of alpha-amylase which involved in the digestion of carbohydrates can significantly reduce the post-prandial blood glucose level and therefore can be an important strategy in the management of blood glucose level in type 2 diabetic and borderline patients (Tundis *et al.*, 2010).

Figure 14 represents alpha amylase activity of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argentea.*

FIGURE 14

INHIBITION OF ALPHA AMYLASE ACTIVITY OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*



From the above figure, it is clear that the alpha amylase activity of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argentea* was found to be increased with increase in concentration from 250-1000µg/ml. At a concentration of 1000 µg/ml the PVA encapsulated silver nanoparticles from *Basella rubra* showed 35 percentage inhibition and *Celosia argentea* showed 43.2 percentage inhibition. Thus the PVA encapsulated silver nanoparticles synthesized from *Celosia argentea* showed potent inhibitory activity against alpha amylase when compared *Basella rubra*. The positive control, acarbose has exerted the highest potent inhibitory action against alpha amylase (59.2 per cent).

Agarwal *et al.* (2018) reported that the extract of *Cymbopogon citratus* represents the per cent inhibition effect of silver nanoparticle on the enzyme. Silver nanoparticle exhibited an inhibitory effect on the enzyme in a dose-dependent manner till the dose was increased to 100µg/ml, which resembled the per cent inhibition exhibited by standard Acarbose.

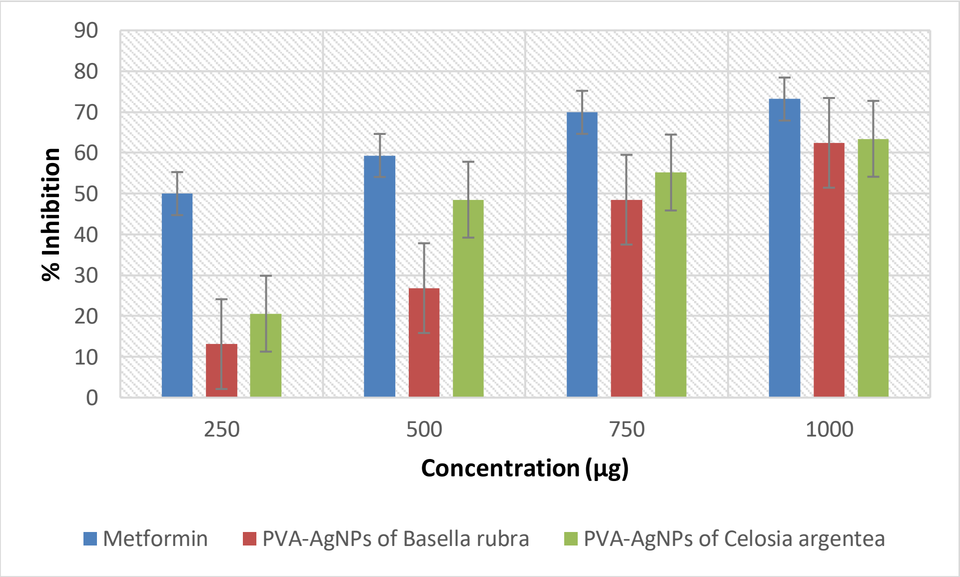
Chouhan *et al*. (2020) reported that the extract of *Cannabis sativa* showed alpha amylase inhibitory effect. The maximum inhibition of aqueous extract of *Cannabis sativa* was showed at 250µg/ml.

4.5.2 INHIBITION OF NON-ENZYMATIC GLYCOSYLATION OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

Nonenzymatic glycation describes a common post-translational process by which D-glucose interacts slowly with intracellular and extracellular proteins, resulting in glucose being covalently bound to the protein. Glycated human hemoglobin (HbA1C) is the first example of an *in vivo* glycated protein. HbA1C is proportionately increased with persistent hyperglycemia and the measurement of HbA1C has been a cornerstone in the monitoring and management of diabetes mellitus (Clark *et al.*, 2013). Figure 15 represents non-enzymatic glycosylation of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argentea*.

FIGURE 15

INHIBITION OF NON-ENZYMATIC GLYCOSYLATION OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*



The above figure clearly indicates that the non enzymic glycosylation activity of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argenta* was found to be increased with increase in concentration from 250-1000 µg/ml. At a concentration 1000 µg/ml, the PVA encapsulated silver nanoparticles from *Basella rubra* showed 62.5 percentage inhibition and *Celosia argenta* showed 63.5 percentage inhibition. Thus the PVA encapsulated silver nanoparticles synthesized from *Celosia argenta* showed potent inhibitory activity against non enzymic glycosylation when compared *Basella rubra*. The positive control, metformin has exerted the highest potent inhibitory action against non enzymic glycosylation (73.2 per cent).

Prabhu *et al.* (2018) reported that silver nanoparticle extract of *Pouteria sapota* obtained results for the enzymatic haemoglobin assay showing the percentage inhibition at the concentrations of 20, 40, 60, 80 and 100 µg/mL in a dose-dependent reduction. The highest concentration 100 µg/mL of 1mM, 2mM, 3mM and metformin showed a maximum inhibition of 26.12, 27.96, 32.68 and 36.89 per cent respectively while the lowest concentration 20 µg/mL of 1mM, 2mM, 3mM and metformin showed a maximum inhibition of 2.78, 3.68, 10.81 and 15.40 per cent respectively.

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

4.5.3 GLUCOSE UPTAKE ASSAY BY YEAST CELLS OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

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

TABLE 1

GLUCOSE UPTAKE ASSAY BY YEAST CELLS OF PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

<table>
<tr><th colspan="4">Glucose concentration at 5mM</th></tr>
<tr><th>CONCENTRATION (µg/ml)</th><th>STANDARD (%)</th><th>PVA-AgNPs OF <i>Basella rubra</i></th><th>PVA-AgNPs OF <i>Celosia argentea</i></th></tr>
<tr><td>250</td><td>31.6±0.02</td><td>5.6±0.02</td><td>2.7±0.01</td></tr>
<tr><td>500</td><td>34.6±0.03</td><td>9.7±0.01</td><td>8.4±0.01</td></tr>
<tr><td>750</td><td>37.1±0.01</td><td>13.5±0.01</td><td>14.7±0.01</td></tr>
<tr><td>1000</td><td>40.7±0.02</td><td>20.1±0.02</td><td>21.1±0.01</td></tr>
<tr><th colspan="4">Glucose concentration at 10mM</th></tr>
<tr><th>CONCENTRATION (µg/ml)</th><th>STANDARD (%)</th><th>PVA-AgNPs OF <i>Basella rubra</i></th><th>PVA-AgNPs OF <i>Celosia argentea</i></th></tr>
<tr><td>250</td><td>36.4±0.01</td><td>11.7±0.02</td><td>23.4±0.02</td></tr>
<tr><td>500</td><td>38.6±0.01</td><td>17.7±0.02</td><td>26.9±0.01</td></tr>
<tr><td>750</td><td>40.6±0.01</td><td>21.5±0.01</td><td>30.9±0.01</td></tr>
<tr><td>1000</td><td>44.7±0.02</td><td>25.7±0.03</td><td>31.7±0.02</td></tr>
<tr><th colspan="4">Glucose concentration at 25mM</th></tr>
<tr><th>CONCENTRATION (µg/ml)</th><th>STANDARD (%)</th><th>PVA-AgNPs OF <i>Basella rubra</i></th><th>PVA-AgNPs OF <i>Celosia argentea</i></th></tr>
<tr><td>250</td><td>39.51±0.02</td><td>12.3±0.01</td><td>25.5±0.02</td></tr>
<tr><td>500</td><td>42.31±0.01</td><td>23.1±0.05</td><td>31.2±0.03</td></tr>
<tr><td>750</td><td>43.67±0.01</td><td>30.8±0.01</td><td>38.7±0.02</td></tr>
<tr><td>1000</td><td>45.49±0.02</td><td>34.4±0.02</td><td>42.3±0.02</td></tr>
</table>

The PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argenta* promoted the uptake of glucose across the plasma membrane of yeast cells (Table 1). The glucose uptake at an initial concentration of 5 mM, 10 mM and 25mM was comparable to that of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argenta.* However, the effect of PVA encapsulated silver nanoparticles of *Celosia argenta* on glucose uptake by the yeast cells at 5mM, 10mM and 25 mM glucose concentration was a bit higher as compared to that of *Basella rubra*. The effect of standard metronidazole on glucose uptake by the yeast cells at 5mM, 10mM and 25 mM glucose concentration was higher as compared to that of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argenta.*

Firoj *et al.* (2021) reported the rate of glucose transport across cell membrane in yeast cells system with the extract of *Barleria gibsoni*. Regulation of glucose level in the blood of the diabetic patient can prevent the various complications associated with the disease and the percent increase in glucose uptake by the yeast cell at different glucose concentrations i.e. 25mM, 10mM and 5mM respectively. At 10mM the glucose concentrations was found to be maximum.

Vani *et al*. (2018) reported that the percentage of glucose uptake by yeast cells increases with increase in the concentration of extract of *H. beccarii*. The amount of residual glucose in the sample after a specific time serves as an indicator of the glucose uptake by the yeast cells. At lower concentration, i.e., 1 mg/ml, the percentage of glucose uptake was 30±0.20 but as concentration increases the percentage of uptake reached maximum 75±0.32.

4.5.4 INHIBITION OF GLUCOSE DIFFUSION ON PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

The blood sugar level in hypergycemic patients tends to rise enormously due to the inability of cell membrane to retain the glucose molecules. Certain viscous components present in plant extracts have shown to decrease glucose diffusion across the membrane. The plant extracts showed a great potential in inhibiting the glucose diffusion across the dialysis membrane. Therefore, they will act as a possible barrier in lowering the blood glucose level by inhibiting the movement of glucose molecule across the plasma membrane into the blood vessel (Akhtar *et al.,* 2016). The PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argenta* were subjected to find out their glucose diffusion across the dialysis membrane and the results obtained are shown in Table 2.

TABLE 2

INHIBITION OF GLUCOSE DIFFUSION ON PVA ENCAPSULATED SILVER NANOPARTICLES OF *Basella rubra* AND *Celosia argentea*

<table>
<tr><th colspan="4">Glucose diffusion at 1st hour</th></tr>
<tr><th>CONCENTRATION (µg/ml)</th><th>STANDARD (%)</th><th>PVA-AgNPs OF <i>Basella rubra</i></th><th>PVA-AgNPs OF <i>Celosia argentea</i></th></tr>
<tr><td>250</td><td>17.5±0.12</td><td>12.4±0.03</td><td>8.6±0.19</td></tr>
<tr><td>500</td><td>31.7±0.17</td><td>19.9±0.15</td><td>18.9±0.17</td></tr>
<tr><td>750</td><td>40.7±0.34</td><td>27.2±0.20</td><td>21.9±0.26</td></tr>
<tr><td>1000</td><td>50.9±0.26</td><td>36.3±0.17</td><td>33.8±0.13</td></tr>
<tr><th colspan="4">Glucose diffusion at 2nd hour</th></tr>
<tr><th>CONCENTRATION (µg/ml)</th><th>STANDARD (%)</th><th>PVA-AgNPs OF <i>Basella rubra</i></th><th>PVA-AgNPs OF <i>Celosia argentea</i></th></tr>
<tr><td>250</td><td>18.1±0.24</td><td>16.5±0.14</td><td>10.5±0.23</td></tr>
<tr><td>500</td><td>33.8±0.56</td><td>23.5±0.22</td><td>21.4±0.18</td></tr>
<tr><td>750</td><td>46.5±0.13</td><td>29.5±0.34</td><td>26.1±0.45</td></tr>
<tr><td>1000</td><td>51.7±0.23</td><td>38.2±0.54</td><td>34.3±0.54</td></tr>
<tr><th colspan="4">Glucose diffusion at 3rd hour</th></tr>
<tr><th>CONCENTRATION (µg/ml)</th><th>STANDARD (%)</th><th>PVA-AgNPs OF <i>Basella rubra</i></th><th>PVA-AgNPs OF <i>Celosia argentea</i></th></tr>
<tr><td>250</td><td>18.8±0.32</td><td>19.1±0.45</td><td>14.9±0.09</td></tr>
<tr><td>500</td><td>33.9±0.43</td><td>25.5±0.36</td><td>24.3±0.18</td></tr>
<tr><td>750</td><td>49.7±0.18</td><td>36.5±0.43</td><td>32.1±0.15</td></tr>
<tr><td>1000</td><td>53.3±0.18</td><td>39.4±0.29</td><td>36.5±0.29</td></tr>
</table>

The PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argenta* inhibit glucose diffusion across the dialysis membrane (Table 2). The glucose diffusion inhibition was measured at different time interval like first, second and third hour and glucose diffusion inhibition was comparable to that of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argenta*. However, the effect of PVA encapsulated silver nanoparticles of *Basella rubra* on glucose diffusion measurement at different time interval was a bit higher as compared to that of *Celosia argenta*. The effect of standard acarbose on glucose diffusion measurement at different time interval exhibits higher inhibition.

Elgorban *et al*. (2017) showed that the silver nanoparticle extract of *Curvularia pallescens* exhibited a retardation of glucose into an external solution through dialysis membrane. Biosynthesized silver nanoparticle did not allow the escape of glucose into the external solution which may be due to binding or absorbing it and thus can act as a potential drug candidate for the treatment of non-insulin dependent diabetes.

SUMMARY AND CONCLUSION

5.0 SUMMARY AND CONCLUSION

Nanobiotechnology is an upcoming branch of nanotechnology which plays an important role in the field of medicinal science. Biomedical nanotechnology is an evolving field having enormous potential to positively impact the health care systems. Researchers are increasingly turning their attention to natural products and looking for new leads to develop better drugs against the metabolic disorders. Nanotechnology is already moving from being used in passive structures to active structures, through more targeted drug therapies or "smart drugs". These new drugs therapies have shown to cause fewer side effects and proved to be more effective than traditional therapies. One area of nanotechnology application that holds the promise of providing great benefits for society in the future is in the realm of medicine (Moghimi *et al.*, 2005).

Diabetes mellitus is an endocrine metabolic disorder indicated by chronic hyperglycemia that produces biochemical changes and tissue destruction. Metabolic abnormalities in carbohydrates, lipids and proteins result from the importance of insulin as an anabolic hormone. The severity of symptoms is due to the type and duration of diabetes. Some of the diabetic patients are asymptomatic especially those with type 2 diabetes during the early years of the disease, others with marked hyperglycemia and especially in children with absolute insulin deficiency may suffer from polyuria, polydipsia, polyphagia, weight loss and blurred vision (Ahmed *et al.*, 2016).

Literature for synthetic drugs for diabetes mellitus depicts that most of the drugs have many side effects. Hence, scientists are in search of safe, natural antidiabetic agents that can cure the diabetes without causing harm. The world health organisation has also recommended the development of herbal medicine in this concern. The new era in diabetes treatment facilitates the rapid green synthesis of multifunctional, biocompatible and eco-friendly metal colloidal nanoparticles, as effective nanomedicine against the emerging threat of diabetes mellitus and its associated complications (Kharroubi and Darwish, 2015).

Since this method is safer, stable and inexpensive, it is widely used to treat various diseases especially diabetes. Plant based nanoparticle synthesis have attracted more attention due to growing interest in environmentally conscious products. Hence the present study framed to synthesize the silver nanoparticles from the ethanolic extracts of *Basella rubra* and *Celosia argentea*. The synthesized silver nanoparticles are encapsulated with Poly Vinyl Alcohol (PVA) to improve their stability and to evaluate its antidiabetic and antioxidant property.

The silver nanoparticles are synthesised from ethanolic extract of *Basella rubra* and *Celosia argentea*. The synthesis of nanoparticles was noticed by change in colour and absorbance. The color of the solution changes from green to dark brown which is an indication of the presence of silver nanoparticles. The variation of the colour was due to the change in surface plasmon resonance of silver nanoparticles.

UV-Visible spectroscopy is used to determine the particle formation and properties. The absorption spectrum of PVA encapsulated silver nanopartilces of ethanolic extract of *Basella rubra* and *Celosia argentea* shows intense peak between 400nm and 450nm respectively. This clearly indicates that there is an interaction between nanoparticles and bimolecules present in the plants and the broad peak indicates the stability of PVA encapsulated silver nanoparticles.

Functional groups of the plant secondary metabolites involved in the reduction and capping of nanoparticles were identified by FT-IR technique. The FT-IR spectrum of PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argentea* showed the peaks assigned for O-H stretching of alcohols, O=C=O stretching vibrations of carbon dioxide, C-H bending of aromatic compounds, O-H bending of phenol, C-O stretching of alkyl aryl ether, C-O stretching of primary alcohol, C=C bending of alkenes and C-Br stretching of halo compounds.

The size and morphology of the PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argentea* was determined by Scanning Electron Microscope (SEM). The SEM images revealed that the synthesized silver nanoparticle were in the range of 58.3nm and 52.7nm. It shows a clear image of highly dense PVA encapsulated silver nanoparticles of *Basella rubra* and *Celosia argentea*. A spherical shape was observed.

The surface charges of the nanoparticles are identified by zeta potential. The zeta potential average of PVA encapasulated silver nanoparticles of *Basella rubra* and *Celosia argentea* was determined as -47mV and –53.3mV. The negative zeta potential value indicates the enhanced stability of PVA encapsulated silver nanoparticles.

The average particle size of PVA encapsulated silver nanoparticles was identified by dynamic light scattering (DLS). The measurement gave the average hydrodynamic diameter of the particles. The average particle size of PVA encapsulated silver nanoparticles was detrmined as 184.8nm and 68.8 nm.

In the present study, the inhibitory activity of different concentations (250 – 1000 µg/ml) of PVA encapsulated silver nanoparticles synthesized from ethanolic extract of *Basella rubra* and *Celosia argentea* was investigated for its alpha amylase activity, Nonenzymatic glycosylation, Glucose uptake capacity and Glucose diffusion inhibitory activities. The PVA encapsulated silver nanoparticles synthesized showed potent inhibitory activity against alpha amylase. Thus the PVA encapsulated silver nanoparticles synthesized from *Celosia argentea* showed potent inhibitory activity against alpha amylase when compared *Basella rubr*a. The positive control, acarbose has exerted the highest potent inhibitory action against alpha amylase (59.2per cent).

The inhibition of Nonenzymatic glycosylation activity of PVA encapsulated silver nanoparticles synthesized from *Celosia argentea* showed potent inhibitory activity when compared *Basella rubr*a. The positive control, metformin has exerted the highest potent inhibitory action against non enzymic glycosylation (73.2 per cent).

The PVA encapsulated silver nanoparticles of ethanolic extract of *Basella rubr*a and *Celosia argentea* promoted the uptake of glucose across the plasma membrane of yeast cells. The effect of PVA encapsulated silver nanoparticles of ethanolic extract of *Celosia argentea* on glucose uptake by the yeast cells at 5mM, 10mM and 25 mM glucose concentration was higher as compared to that of *Basella rubr*a. The effect of silver standard metronidazole on glucose uptake by the yeast cells at 5Mm, 10mM and 25 mM glucose concentration was higher as compared to that of PVA encapsulated silver nanoparticles of ethanolic extract of *Basella rubr*a and *Celosia argentea.*

The PVA encapsulated silver nanoparticles of ethanolic extract of *Basella rubr*a and *Celosia argentea* inhibit glucose diffusion across the dialysis membrane. However, the effect of PVA encapsulated silver nanoparticles of ethanolic extract of *Basella rubr*a on glucose diffusion measurement at different time interval was higher as compared to that of *Celosia argentea*. The effect of standard acarbose on glucose diffusion measurement at different time interval was higher as compared to that of PVA encapsulated silver nanoparticles of ethanolic extract of *Basella rubr*a and *Celosia argentea.*

The *in vitro* antioxidant acitivity of PVA encapsulated silver nanoparticles of ethanolic extract of *Basella rubr*a and *Celosia argentea* was investigated by DPPH radical scavenging assay, hydroxyl radical scavenging assay, hydrogen peroxide radical scavenging assay and ferric reducing antioxidant power assay.

The inhibitory activity of PVA encapsulated silver nanoparticles of ethanolic extract of *Basella rubr*a and *Celosia argentea* against DPPH radical was investigated. The PVA encapsulated silver nanoparticles synthesized from *Celosia argentea* showed potent inhibitory activity against DPPH radical when compared to *Basella rubr*a. The positive control, ascorbic acid has exerted the highest potent inhibitory action against DPPH (86.2 per cent).

The inhibitory activity of PVA encapsulated silver nanoparticles of ethanolic extract of *Basella rubr*a and *Celosia argentea* against hydroxyl radical was investigated. Thus the PVA encapsulated silver nanoparticles synthesized from *Celosia argentea* showed potent inhibitory activity against hydroxyl radical when compared to *Basella rubr*a. The positive control, ascorbic acid has exerted the highest potent inhibitory action against hydroxyl radical (82 per cent).

The PVA encapsulated silver nanoparticles of ethanolic extract of *Basella rubr*a and *Celosia argentea* against hydrogen peroxide radical shows potent inhibitory activity. However, the effect of PVA encapsulated silver nanoparticles of ethanolic extract of *Celosia argentea* showed potent inhibitory activity against hydrogen peroxide radical when compared to *Basella rubr*a. The positive control, ascorbic acid has exerted the highest potent inhibitory action against hydrogen peroxide (72.5 per cent).

The PVA encapsulated silver nanoparticles of ethanolic extract of *Basella rubr*a and *Celosia argentea* shows better ferric reducing ability. However, the effect of PVA encapsulated silver nanoparticles of ethanolic extract of *Basella rubr*a showed potent reducing ability when compared to *Celosia argentea*. The positive control, ascorbic acid has exerted the highest ferric reducing power (77.14 per cent).

Hence, the present study clearly proves that the PVA encapsulated silver nanoparticles of ethanolic extract of *Basella rubr*a and *Celosia argentea* possess effective antidiabetic and antioxidant activity. When compared the PVA encapsulated silver nanoparticles of *Celosia argentea* possess better antidiabetic and antioxidant activity than that of *Basella rubr*a. Hence, these plants 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

DPPH RADICAL SCAVENGING ASSAY

(Chang *et al.*, 2017)

PROCEDURE:

0.5 ml of 0.1mM DPPH solution in methanol was mixed with sample solution of varying concentrations. L-Ascorbic acid was used as reference standard. The volume was then made upto 3.0ml with methanol. Mixture of 3.0ml methanol and 0.5ml DPPH solution was used as control. The reaction was carried out in duplicate and the decrease in absorbance was measured at 520nm after 30 minutes in dark. The inhibition % was calculated using the following formula,

$$\textbf{Per cent inhibition} = \frac{\textbf{Abs Control} - \textbf{Abs Sample} \times 100}{\textbf{Abs Control}}$$

Where Abs control is the absorbance of control and Abs sample is the absorbance of the extract.

APPENDIX II

HYDROXYL RADICAL SCAVENGING ASSAY

(Halliwell *et al.*, 1981)

PROCEDURE:

Various concentrations of sample (250, 500, 750, 1000µg) were taken and 1ml of iron EDTA solution, 0.5 ml of EDTA solution, 1ml of DMSO and 0.5ml of ascorbic acid were added to it. L-Ascorbic acid was used as reference standard. The mixture was incubated in a boiling water bath at 80 to 90°C for 15 min. After incubation, 1 ml of ice cold TCA and 3 ml of Nash reagent were added and the reaction mixture was incubated at room temperature for 15 min. The absorbance was read at 412 nm. The % hydroxyl radical scavenging activity is calculated by the following formula,

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

Where Abs control is the absorbance of control and Abs sample is the absorbance of the extract.

APPENDIX III

HYDROGEN PEROXIDE RADICAL SCAVENGING ASSAY

(Patnaik *et al*., 2017)

PROCEDURE:

A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (1 M pH 7.4). Different concentration of sample was added to a hydrogen peroxide solution (0.6 ml, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as standard. The free radical scavenging activity was determined by evaluating percent inhibition.

$$\text{Percent inhibition} = \frac{\text{Abs control-Abs sample} \times 100}{\text{Abs control}}$$

Where Abs control is the absorbance of the control Abs sample is the absorbance of the sample.

APPENDIX IV

FERRIC REDUCING ANTIOXIDANT POWER ASSAY

(Oyaizu *et al*., 1986)

PROCEDURE:

1.0 ml of extract containing different concentrations (250-100µg) of samples was mixed with 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. Reaction mixture was kept in a water bath at 50ºC for 20 minutes. After incubation, 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 6500 rpm for 10 minutes. From the supernatant, 2.5 ml solution was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride. Absorbance of the solution was measured at 700nm. The % ferric reducing ability was calculated by the following formula,

$$\textbf{Per cent inhibition} = \frac{\textbf{Abs Sample} - \textbf{Abs Control} \times 100}{\textbf{Abs Sample}}$$

Where Abs control is the absorbance of control and Abs sample is the absorbance of the extract.

APPENDIX V

INVITRO 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 2 ml 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,

$$\textbf{Per cent inhibition} = \frac{\textbf{Abs Control} - \textbf{Abs Sample} \times 100}{\textbf{Abs control}}$$

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 VI

NON ENZYMATIC GLYCOSYLATION OF HAEMOGLOBIN METHOD

(Daksha *et al.*, 2012)

PROCEDURE:

Glucose (2 per cent), haemoglobin (0.06 per cent) and gentamycin (0.02 per cent) 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,

$$\textbf{Per cent inhibition} = \frac{\textbf{Abs Sample} - \textbf{Abs Control} \times 100}{\textbf{Abs Sample}}$$

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 VII

GLUCOSE UPTAKE BY YEAST CELL

(Vijayalakshimi *et al.*, 2014)

PROCEDURE:

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

$$\textbf{Per cent inhibition} = \frac{\textbf{Abs Sample} - \textbf{Abs Control} \times 100}{\textbf{Abs Sample}}$$

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 VIII

GLUCOSE DIFFUSION ASSAY

(Gallagher *et al*., 2003)

PROCEDURE:

2ml of 0.15 M NaCl containing 0.22mM D-glucose was loaded into a dialysis tube containing plant extract (50g/L) and the dialysis tube was sealed. The sealed tube was then placed in a centrifuge tube containing 45 ml of 0.15 M NaCl and kept in an orbital shaker at a room temperature. The diffusion of glucose into the external solution was monitored by measuring the glucose concentration in the external solution every 60min.