

***In vitro* ANTIOXIDANT AND ANTICANCER
ACTIVITY OF GREEN SYNTHESIZED SILVER
NANOPARTICLES AND NANOLIPOSOMES OF
Camellia sinensis and *Durio zibenthinus***

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(19MPBCF001)
M.Phil in Biochemistry**

**A Thesis submitted in
Partial Fulfilment of the
Degree of Master of Philosophy (M.Phil)**

**Department of Biochemistry, Biotechnology and Bioinformatics
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore-641043**

January, 2021

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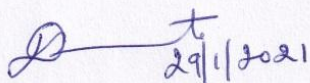
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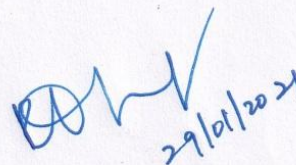
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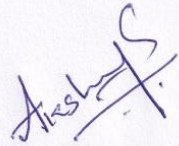
Signature of the Head of the Department


29/01/2021

Signature of the Supervisor

DECLARATION

I declare that the dissertation entitled *In vitro* Antioxidant and Anticancer Activity of Green Synthesized Silver Nanoparticles and Nanoliposomes of *Camellia sinensis* and *Durio zibenthinus* submitted by me for the degree of Master of Philosophy (M.Phil.) is the record of work carried out by me during the period from July, 2019 to January, 2021 under the guidance of Dr.NIRMALADEVI. R and has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship, Titles in this University or any other University or other similar institution of Higher Learning.



Signature of the Candidate

CERTIFICATE FROM THE SUPERVISOR

I certify that the dissertation entitled *In vitro* Antioxidant and Anticancer Activity of Green Synthesized Silver Nanoparticles and Nanoliposomes of *Camellia sinensis* and *Durio zibenthinus* submitted for the degree of Master of Philosophy (M.Phil.) is the record of research work carried out by her during the period from July, 2019 to January, 2021 under my guidance and supervision, and that this work has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other Titles in this University or any other University or other institution of Higher Learning.

Handwritten signature in blue ink, followed by the date 29/01/2021.

Signature of the Supervisor with designation

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1.0 INTRODUCTION

The medicinal plants have been used globally since ancient times and also considered as the origin of modern medicine. Compounds of plants origin are the most important source of compounds for drugs. Half the population of all vascular plants are used as medicinal plants and they are approximately estimated to be between 650,000 and almost half a million species of them. Technological and scientific advances have made prominent role in the identification of their bioactive compounds has allowed *in vitro*, *in vivo* and clinical studies to prove their efficacy and mechanism of action in the treatment of various diseases like cancer. Losses in the life battle against cancer of modern medicine, have led some of the cancer patients to use various medicinal plants to increase life expectancy and to reduce the side effects of chemo treatment (Lyubchyk *et al.*, 2019). Nanotechnology is the emerging technology which offers an immense application in various industries. The rapid growth of this technology provides various invention and innovations in all the fields, especially in pharmaceutical sciences where high level research has been carried out for the development of diagnosis and treatment of various diseases. Over the past 20 years, the exponential development has been observed in the field of nanotechnology based medicine and drug delivery systems. Nowadays the pharmaceutical industries are come up with a strategy to overcome the problems and challenges associated with the applications of nanoparticles and also focused the low cost, safe and highly sensitive techniques for the drug development (Sharma *et al.*, 2018).

Nanoparticles are considered as a special group of materials because of its unique properties in the development of nanoparticle based products for diverse applications. The nanomaterials have attained a great importance of their unique optical properties, catalytic nature and electromagnetic applications. Nanoparticles are small in size ranges from 1 to 100 nm and it has the influence on the discovery of many nano based materials for tissue engineering. Nanoparticles comprises of materials designed at atomic or molecular level so that it can move candidly in the human body compared to other macro molecules. In recent times, the nano sized particles are used as a drug delivery system by encapsulating the compounds or attaching to the therapeutic molecules and deliver the drug molecules to the targeted area with a controllable release (Patra *et al.*, 2018).

Wide range of methods have been deployed for the production and development of nanoparticles such as physical and chemical methods. But they contribute some disadvantages like, use of hazardous chemical for the nanoparticles production, generation of waste products during its synthesis which pollutes the environment, high energy consumption and low efficiency. Despite of this drawback, the researchers and scientists are looking for the alternative strategy for the production of nanoparticles with low side effects and high efficiency. Green biosynthesis of nanoparticles has gained a lot of interest for the development of drug delivery system in pharmaceutical industries. The major advantage of the biosynthesis method for the production of nanoparticles is compatibility with the environment and it gradually decreases the economic burden (Seifipour *et al.*, 2020).

Various types and forms of nanoparticles have been reported based on their characteristics such as Core shell NPs (nanoparticles), photo-chromic polymer NPs, polymer coated magnetize NPs, inorganic NPs, Ag NPs, Cu NPs, Au NPs, Pt NPs, Pd NPs, Si NPs and Ni NPs. ZnO NPs, CuO NPs, FeO NPs, MgO NPs, TiO₂ NPs, CeO₂ NPs and ZrO₂ NPs are classified under metal oxide and metal dioxide nanoparticles. Wide range of sources can be used to produce the nanoparticles using biosynthesis method and it includes plants, algae, fungi, yeast and viruses. Especially, the plant sources are the rich sources for the production of nanoparticles due to the presence of compounds such as alkaloids, tannins, steroids, phenols, saponins and flavonoids in the plants which facilitates the biosynthesis of nanoparticles. The compounds present in the plant can help to reduce the silver ions to silver nanoparticles by changing the various functional groups such as hydroxyl, ketone and aldehydes (Ahmad *et al.*, 2020).

The demand for the synthesis of silver nanoparticles (AgNPs) is enhanced gradually in the field of science. The silver nanoparticles have dynamic applications in various fields including, drug delivery, ointments, nanomedicine, chemical sensing, data storage, cell biology, agriculture, cosmetics, textiles, food industry, photocatalytic organic dye-degradation activity, antioxidants and antimicrobial agents. Apart from the pharmaceutical industry, nanoparticles application is continuously growing in textiles industry for its expanding uses in sportswear, underwear, military clothing and medical textiles. Food industry has applied the AgNPs for storage and processing of food, to

increase the shelf life of the product and to maintain the hygienic of the product (Syafiuddin *et al.*, 2017).

The green synthesis of nanoparticles has been emerged as a alternative method to minimize the hazardous, harmful effects and serious consequences and it referred as a user friendly method. Biosynthesis of silver nanoparticles is a cost effective method and it produces minimum defects and it has a homogenous chemical composition. The plant extract has open up many usefulness and economically friendly alternative strategy for the synthesis of silver and other nanoparticles. The silver nanoparticles produced from the different parts of the plant include (leaf, bark, root, fruit, seed, stem and flower) has become attained a great importance due to its quality of being biodegradable, biocompatible, readily available, renewable and exhibit low level of toxicity (He *et al.*, 2019).

Nanotechnology has recently gained increased attention for its capability to effectively diagnose and treat various tumors. Nanocarriers have been used to circumvent the problems associated with conventional antitumor drug delivery systems, including their nonspecificity, severe side effects, burst release and damaging the normal cells. Nanocarriers improve the bioavailability and therapeutic efficiency of antitumor drugs, while providing preferential accumulation at the target site. A number of nanocarriers have been developed; however, only a few of them are clinically approved for the delivery of antitumor drugs for their intended actions at the targeted sites. (Din *et al.*, 2017).

Liposomes attained a great attention during the last few decades in biomedicine, especially as a drug delivery system for antitumor drugs. They showed a number of advantages over conventional systems which are, but not limited to, enhanced delivery of drug, protection of active drug from environmental factors, preventing early degradation of the encapsulated drug, cost-effective formulations of expensive drugs and efficient treatment with reduced systemic toxicity. Moreover, drugs associated with liposomes have markedly altered pharmacokinetic properties compared to free drugs in solution. They can be covered with polymers such as polyethylene glycol (PEG; PEGylated or stealth liposomes) to exhibit prolonged half-life in blood circulation. Liposomes are spherical vesicles having an aqueous core enclosed by lipid bilayers. They

have single or multiple bilayered membrane assembly formed of natural or synthetic lipids. Those containing one bilayer membrane are termed as small unilamellar vesicles or large unilamellar vesicles based on their sizes. If more than one bilayer is present, then they are called as multilamellar vesicles. Liposomes vary with respect to composition, size, surface charge and method of preparation. Liposomes are commonly used as model cells or carriers for various bioactive agents including drugs, vaccines, cosmetics and nutraceuticals (Xu *et al.*, 2020).

Camellia sinensis is a species of evergreen shrubs or small trees in the flowering plant family Theaceae whose leaves and leaf buds are used to produce tea. This plant is cultivated in many countries such as China, India and Japan. It has become universally consumed beverage and second most consumed drink after water. Inhabitants of Europe mainly Britain are the largest consumer of tea (approximately 540ml per day). In average, world population consumes approximately 120ml of tea per day. Tea is available in three major forms Green tea, Oolong tea and Black tea based on the degree of fermentation. Approximately 3 billion kilograms of tea is produced and consumed worldwide per year. Tea mainly consists of polyphenols, alkaloids, flavanoids, trace amounts of vitamins, amino acids and carbohydrates. Polyphenols are the major component in green tea leaves. The principal polyphenol present in green tea is catechin. They include two non-ester catechins, epicatechin and epigallocatechin and two ester catechins epigallocatechin gallate and epicatechin gallate. These catechins had shown potential health benefits such as anticancer, antiobesity, antibacterial, antioxidant and antiviral effects (Luo *et al.*, 2020).

According to the European Food Safety Authority (EFSA), 126 mg of catechins are present per 100 mL of green tea. However, according to the Food and Drug Administration (FDA), 71 mg of epigallocatechin gallate are present per 100 mL of green tea. In the case of black tea, 200 mg of flavonoids are present per 100 mL. Tea is considered as a functional food since it can impart many physiological benefits apart from its nutritional contents. Its antioxidant property makes it a predominant regulator in mediating free radicals, which is of significant use in healthcare. Downstream carcinogenesis signalling pathway by green tea polyphenols has a role in chemoprevention and also in treatment of cancers. Epigallocatechin gallate improves insulin sensitivity or liver insulin signalling pathway. Epigallocatechin gallate

significantly decrease brain amyloid β production by increasing the levels of α -secretase. Low dose of epigallocatechin gallate effectively reduced the cell viability and increased the apoptosis rate of myeloid derived suppressor cells *in vitro* (Mhatre *et al.*, 2020)

Durian fruit is only recently familiar in India, being exported as exotic produce, is actually a very popular fruit native to Southeast Asia, where it is acknowledged as the 'King of all Fruits'. Durians grow particularly in Southeast Asian countries, which include Malaysia, Thailand, Philippines, and Indonesia. The name Durian originally comes from the Malay word "Duri", which mean 'thorn' of the fruits. It is native to the Indonesian island of Borneo and Sumatra, where more than 30 varieties of the durian plants sprout are available, but only 9 types are used. This large fruit has a hard outer shell, with spiky outer parts either light green or brownish in colour. The fruit generally weighs 1-3 kilograms, with a soft inner pulp that tastes rich, delicious and intensely sweet, in addition to comprising vast nutritional elements. Of these, only one durian fruit variant is available commercially in many countries all over the world, bearing the scientific name *Durio zibethinus* (Durian pulp) is an excellent source of various nutrients important in the human diet. It is rich in vitamins such as vitamin A, vitamin C, a natural antioxidant and health benefiting B-complex vitamins like folic acid, thiamine, riboflavin, niacin and vitamin B6 (Aziz *et al.*, 2019).

Durian plant also contains efficient amount of minerals such as potassium, iron, calcium, magnesium, zinc, and phosphorus. Durian fruit is known to be natural multivitamin and also a multi-mineral supplement. It is also a rich source of dietary fat fiber. It has high antidiabetic and antidepressant property. Pulp extract of Durian fruit as high antiproliferative activity, which acts on human leukemia cancer cell lines HL60. Antioxidants present in durian fruit have the great potential to scavenge free radicals. Durian extracts were reported to have inhibitory effect on nitric oxide induced cell proliferative activity in the breast cancer cell line (Saminathan and Doraiswamy, 2020).

The present study aims at investigating the efficacy of liposomes loaded with Green synthesized nanoparticles using plants that are used as traditional medicines, namely *Camellia sinensis* and *Durio zibethinus* with the following objectives.

- To synthesize silver nanoparticles of aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*.
- To characterize the synthesized silver nanoparticles of aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*.
- To determine the *in vitro* antioxidant activity of synthesized silver nanoparticles.
- To prepare synthesized silver nanoparticles loaded liposomes as nanocarriers for drug delivery under *in vitro*.
- To evaluate the cytotoxicity of synthesized silver nanoparticles loaded liposomes against Molt-3 leukemic cell lines.

2.0 REVIEW OF LITERATURE

Medicinal plants have long been used for the treatment of many diseases. Recently several experimental studies have contributed scientific evidence for the pharmacological effects of medicinal plants observed in folk medicine. The World Health Organization estimated that 80% of the people living in the developing countries use a traditional medicine (Krishna *et al.*, 2018)

Herbal medicines are still the mainstay of primary health care because of the general belief that herbal drugs are without any side effect, being cheap and locally available. Ayurvedic medicines are essentially promotive and preventive in therapeutic approach (Singh, 2015). The use of plants for healing purpose predates human history and forms the origin of much modern medicine. Thus search for crude drugs of plant origin with antioxidant activity has become a central focus of protecting body from various diseases including cancer. Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments (Aslam and Ahmad, 2016).

In spite of the overwhelming influences and our dependence on modern medicine and tremendous advances in synthetic drugs, a large segment of the world population still likes drugs from plants. For a long time, plants are being used in the treatment of cancer. The search for anticancer agents from plant sources started in 1950s and resulted in the discovery and development of the vinca alkaloids, vincristine and the isolation of the cytotoxic podophyllotoxins. More than 60 % of currently used anticancer agents are derived in one way or another from natural sources (Oladeji, 2016).

Nanoparticles can be synthesised from a wide variety of biological entities. These entities differ from the degree of the biochemical processing abilities that can be effectively used to synthesize particular metallic or metallic oxide nanoparticles. The use of plants or plant extracts are the most adopted method of green, eco-friendly production

of nanoparticles because the plants are widely distributed, easily available, much safer to handle and act as a source of several metabolites. These nanoparticles loaded into the nano-carriers for the targeted action of the synthesized nanoparticles. Thus, it can be used as a valuable resource for the treatment and management of diseases in human, including cancer (Khan *et al.*, 2019).

To the possible extent sincere efforts have been made to collect the relevant literature of the study. After thorough reviewing of all possible sources, it was observed that no studies on the antioxidant and anticancer role of synthesized silver nanoparticles of *Camellia sinensis* and *Durio zibenthinus* leaf extracts, also preparation and anticancer activity of synthesized silver nanoparticles loaded nanoliposomes for drug delivery activity have been conducted. Hence the review of literature pertaining to the present research entitled “*In vitro* Antioxidant and Anticancer activity of green synthesized silver nanoparticles and nanoliposomes of *Camellia sinensis* and *Durio zibenthinus*” is appropriately presented under following headings.

2.1 CONCEPT OF NANOTECHNOLOGY AND BIONANOTECHNOLOGY

2.2 NANOPARTICLES

2.2.1 TYPES OF NANOPARTICLES

2.2.1.1 ORGANIC NANOPARTICLE

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2.9 HERBAL MEDICINE - *Durio zibenthinus*

2.1 CONCEPT OF NANOTECHNOLOGY AND BIONANOTECHNOLOGY

Nanotechnology is a level of research and innovation concerned with building things, generally materials and devices on the scale of atom and molecule. A nanometre is one billionth of a metre, ten times the diameter of hydrogen atom. The diameter of a human hair is average 80000 nanometre. Nanotechnology is accredited as having the potential to increase the efficiency of energy consumption, help clean the environment and solve major health problems. In the developing world, Brazil, Chile, China, India, Philippines, South Korea, South Africa and Thailand have shown their commitment to nanotechnology by establishing government funded programmes and research institutes. In Jan 2005, researchers at US used “optical tweezers” - pairs of tiny glass needles to study the elasticity of red blood cells that are infected with malarial parasite (Zhu *et al.*, 2020).

Nanotechnology is the emerging area of research and it is one of the rapid growing fields in science and technology. It has been applied to other fields of science such as electronics, engineering, physical sciences, material sciences, biomedical sciences and many others. Nanotechnology has given a greatest impact towards society and simultaneously applied in the health risks of nano objects. Recently, many types of nanoparticles have been identified and utilised in several areas. Scientific areas exhibits advantages in the development of nanotechnology mainly in the areas of forensic science, health sciences and various areas of automotive engineering as well. The growing demand of nanotechnology today have been enabled most of the scientist and analyst to go in the efficient strategic objectives by combining the most advanced chemical and

physical technologies with the needs of modern applications of research (Chauhan *et al.*, 2017).

Nanotechnology lead to cheaper and more reliable system for drug delivery. For example. Materials build on nanoscale provide encapsulation system that protect and secrete the enclosed drugs in a slow and controlled manner. Nanoscale size ranges below 0.1 μm or 100 nm. Nanoscale science or nanoscience studies the phenomena, properties and responses of materials at atomic, molecular and macromolecular scales and normally at sizes between 1-100 nm. In this scale and particularly below 5 nm, the properties of matter differ significantly (that is quantum scale effects play a vital role) from that at a larger particulate scale. Nanotechnology is then the layout, the manipulation, the building, the production and application, by regulating the shape and size, the properties responses and functionality of structures, devices and systems of the order or less than 100 nm (Logothetidis, 2012).

Nanobiotechnology has the most powerful impact of research on plants and different plant products and are widely used in the synthesis of nanoparticles (NPs). In recent years, synthesis of metal nanoparticles with the help of plants has become an interesting subject of nanoscience and nanobiotechnology. However, there is a growing attention for biosynthesis of nanoparticles using metal-, accumulating organisms. Among these biological entities, plant extract is supremely suitable for large-scale biosynthesis of nanoparticles. Nanoparticles produced by plant extracts were stable and the rate of synthesis is much faster compared to microbe mediated synthesis (Meenakshi *et al.*, 2017).

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

2.2 NANOPARTICLES

Nanotechnology has gained huge attention over time. The fundamental, basic component of nanotechnology is said to be nanoparticles. Nanoparticles are between 1 and 100 nanometres in size and are made up of carbon, metal, or organic matter. Nanoparticles exhibit a distinctive physical, chemical and biological properties. These nanoparticles consists of macromolecular material in which the active principle is dissolved, entrapped or even encapsulated, to which the active principle is absorbed or attached. Nanoparticles represent very promising carrier system for the targeting of anti-cancer agent to tumours. They exhibit significant tendency to accumulate in tumors after iv injection. They are also used in brain drug targeting. Poly nanoparticles are successfully used for *in vivo* delivery of drugs to brain. They are rapidly biodegradable, hexapeptide dalargin is the first drug to deliver into brain, followed by loperamide, tubocurarine and doxorubicin (Din *et al.*, 2017).

2.2.1 Types of nanoparticles

There are three different kinds of nanoparticles, organic nanoparticles, inorganic nanoparticles and carbon based nanoparticless. Organic nanoparticles like poly-lysine, quaternary ammonia compounds, cationic quaternary polyelectrolytes, N-halamine compounds and chitosan. Organic nanoparticles are typically less stable at high temperatures. The inorganic nanoparticles are metal and metal oxides. Metal. oxide nanoparticles like silver, iron oxide, titanium oxide, copper oxide and zinc oxide are certain examples of inorganic nanoparticles. (Oscar *etal.*, 2016).

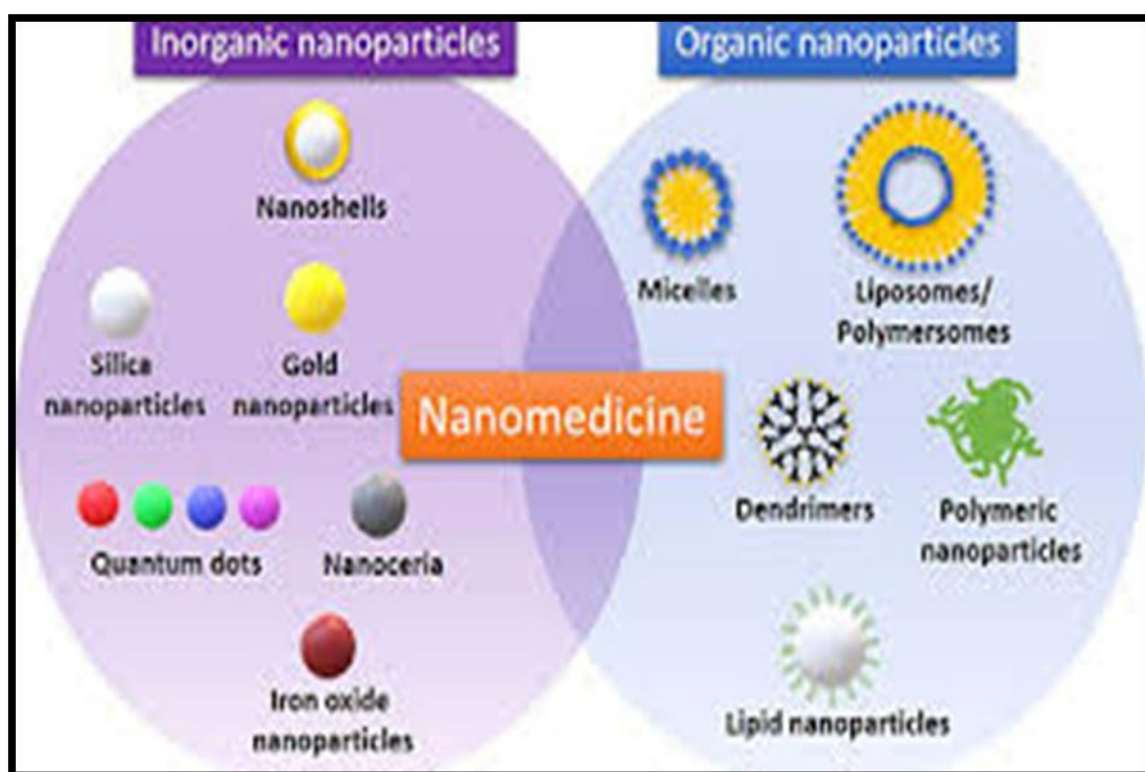
Carbon-based nanomaterials (CBNs) are becoming attractive nanomaterials Due to the existence of diverse allotropes of carbon, from renowned allotropic phases such as amorphous carbon, graphite and diamonds to newly discovered auspicious carbon nanotubes (CNTs), graphene oxide (GO), graphene quantum dots (GQDs) and fullerene. Each member of the carbon family exhibits inimitable features and has been widely exploited in diverse biological applications including biosensing, drug delivery, tissue engineering, imaging, diagnosis and cancer therapy (Bhattacharya *et al.*,2016).

2.2.1.1 Organic Nanoparticles

Organic nanoparticles are biodegradable, non toxic and has a hollow core which are known as nanocapsules and are sensitive to thermal and magnetic radiation. These organic nanoparticles are used in the biomedical field like drug delivery system for

targeted drug delivery. Examples of organic nanoparticles are dendrimers, micelles, liposomes and ferritin. These are normally organic based NPs and in the literature a special term polymer nanoparticle (PNP). They are mostly nanospheres or nanocapsular shaped molecules (Mansha *et al.*,2017). The former are matrix particles whose overall mass is generally solid and the other molecules are adsorbed at the outer boundary of the spherical surface. In the latter case the solid mass is encapsulated within the particle completely (Rao and Geckeler, 2011).

Figure 1: Inorganic and Organic Nanoparticles



(Oscar *et al.*, 2016)

2.2.1.2 Inorganic Nanoparticles

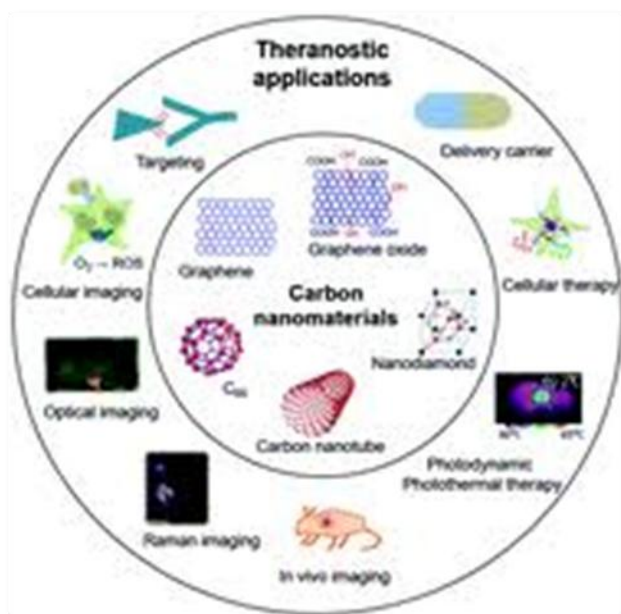
Inorganic nanoparticles are particles that are not made up of carbon. Metal and metal oxide based nanoparticles are categorized under inorganic nanoparticles. Examples of metal based nanoparticles are aluminium, cadmium, cobalt, copper, gold, iron, lead, silver and zinc. Metal oxide based nanoparticles are synthesized to modify the properties of their metal based nanoparticles. For eg. Nanoparticles of Fe (Iron) instantly oxidises to iron oxide in the presence of oxygen at room temperature. Metal NPs are purely made of the metals precursors. Due to well-known localized surface plasmon resonance (LSPR)

characteristics, these NPs possess unique optoelectrical properties. NPs of the alkali and noble metals i.e. Cu, Ag and Au have a broad absorption band in the visible zone of the electromagnetic solar spectrum. The facet, size and shape controlled synthesis of metal NPs is important in present day cutting-edge materials. Due to their advanced optical properties, metal NPs find applications in many research areas. Gold NPs coating is widely used for the sampling of SEM, to enhance the electronic stream, which helps in obtaining high quality SEM images Metal oxide nanoparticle are synthesized mainly due to their increased reactivity and efficiency. These nanoparticles have exceptional properties when compared to their metal counterparts. Examples of metal oxide based nanoparticles are aluminium oxide, cerium oxide, iron oxide, magnetite, silicon dioxide, titanium oxide, zinc oxide (Dreaden *et al.*, 2012).

2.2.1.3 Carbon Based Nanoparticles

These nanoparticles are completely made up of carbon and hence they are known as carbon based nanoparticles. These carbon based nanoparticles can be classified into fullerenes, graphene, carbon nanotubes, carbon nanofibres, carbon black and sometimes carbon in nanosize. Fullerenes and carbon nanotubes (CNTs) represent two major classes of carbon-based NPs. Fullerenes contain nanomaterial that are made of globular hollow cage such as allotropic forms of carbon. They have created noteworthy commercial interest due to their electrical conductivity, high strength, structure, electron affinity, and versatility (Astefanei *et al.*, 2015).

Figure 2: Carbon Based Nanoparticle



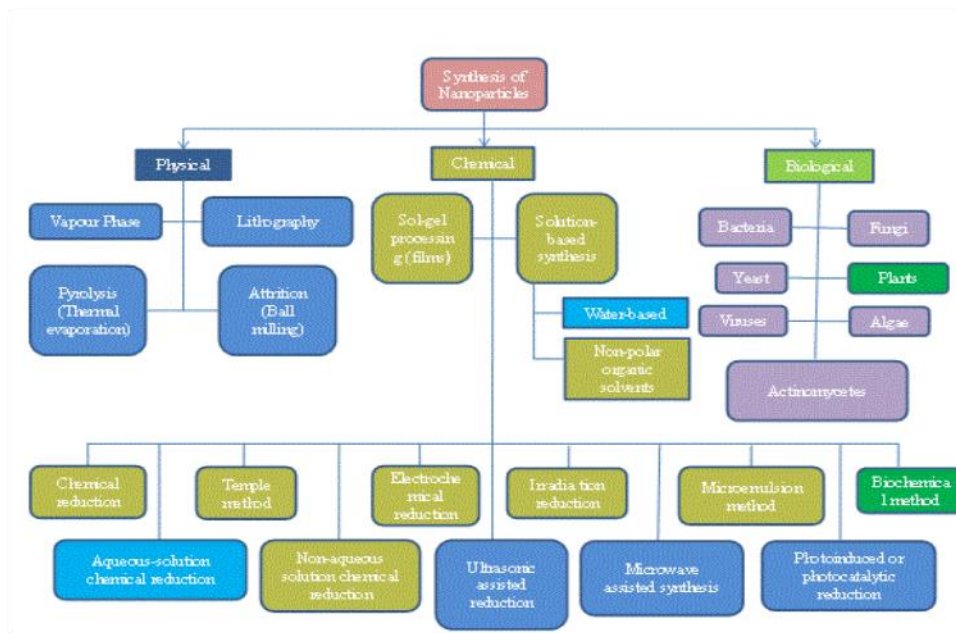
(Patel *et al.*, 2019)

These are structurally resembling to graphite sheet rolling upon itself. The rolled sheets can be single, double or many walls and therefore they named as single-walled (SWNTs), double-walled (DWNTs) or multi-walled carbon nanotubes (MWNTs), respectively. They have been synthesized via chemical vapor deposition (CVD) technique (Elliott *et al.*, 2013). Due to their unique physical, chemical and mechanical characteristics, these materials are not only used in pristine form but also in nanocomposites for many commercial applications such as fillers, efficient gas adsorbents for environmental remediation (Ngoy *et al.*, 2014), and as support medium for different inorganic and organic catalysts (Saeed and Khan, 2016).

2.3 SYNTHESIS OF NANOPARTICLES

Nanoparticles have multifunctional properties and it has very interesting applications in various fields such as medicine, nutrition and energy. Additionally it has broad application in agriculture, industry and in plant sciences. Silver metal is used to control bodily infection, prevent food spoilage, wound healing agent and in ulcer treatment. Now a days the colloidal silver nanoparticles are used as antimicrobial agent, wound dressing material, bone and tooth cement and in water purification. The different methods which are used for the synthesis of the nanoparticles such as the physical, chemical and biological are illustrated below.

Figure 3: Methods of Synthesis of Nanoparticles



(Ayesha, 2017)

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2.3.1 Physical Methods

The physical methods include, plasma arcing, ball milling, thermal evaporation, spray pyrolysis, ultra thin films, pulsed laser desorption, lithographic techniques, sputter deposition, layer by layer growth, molecular beam epitaxial and diffusion flame synthesis of nanoparticles. The physical method can be used as a nanoparticle generator for long term experiments for inhalation toxicity studies and as a calibration device for nanoparticle measurement equipment (Reddy, 2015).

In physical method, the metal nanoparticles are generally synthesized by evaporation - condensation that could be carried out by atmospheric pressure. Nanoparticles of various materials such as Ag, Au, PbS and fullerene have been produced

using evaporation- condensation technique. However the generation of the silver nanoparticles have several drawbacks because it occupies a large space and consumes a great deal of energy while raising the environmental temperature around the source material and requires a lot of time to achieve a thermal stability (Haider and Kang, 2015).

2.3.2 Chemical Methods

The chemical methods have been used to synthesize the nanoparticles by electrodeposition, sol-sol process, chemical solution deposition, chemical vapour deposition, soft chemical method, Langmuir Blodgett method, catalytic route, hydrolysis, co-precipitation method and wet chemical method. Chemical methods provide an easy way to synthesize silver nanoparticles in solution. Each method has its own advantages and disadvantages with common problems like costs, scalability, particle sizes and size distribution. The silver nanoparticles are synthesized in large quantities by reducing silver nitrate with ethylene glycol in the presence of PolyVinyl Pyrrolidone (PVP), this process is called polyol process. In this case, ethylene glycol served as both reductant and solvent. It showed the presence of PVP and its molar ratio relative to silver nitrate played important role in determining the geometric shape and size of the product. The metallic nanoparticles are traditionally synthesized by wet chemical synthesis techniques where the chemicals used are quite often toxic and flammable. Generally the chemical synthesis of the silver nanoparticles in solution employs the following three main components as metal precursors, reducing agents and stabilizing or capping agents. The formation of colloidal solutions from the reduction of silver salts involves two stages namely the nucleation and subsequent growth. It is also revealed that the size and the shape of the synthesised silver nanoparticles are strongly dependent on these stages (Trans *et al.*, 2013).

2.3.3 Biological methods

The synthesis of the nanoparticles by physical and chemical methods can cause considerable environmental defect, technically laborious and economically expensive. In general, the physical and chemical methods involves the use of hazardous chemicals or expensive physical methods. However the biological methods make their ways in between and proving their advantages over them. Recently, the biological synthesis of the nanoparticles using the plant extracts appears to be an attractive alternative to conventional chemical synthesis. Moreover the combination of molecules found in plant

extract perform as both reducing and stabilizing agents during the nanoparticle synthesis (Kulkarni and Muddapur, 2014).

The biological methods uses microorganisms and enzymes that had been suggested as possible eco-friendly alternative. It was found to be more pharmacologically active than physicochemical synthesized nanoparticles. The biological synthesis of nanoparticles is a single step bio reduction method and less energy is used to synthesize eco- friendly nanoparticles. These methods use resources such as plant extracts, bacteria, fungi, micro algae such as cyanobacteria, diatom macro algae and enzymes. The development of the biologically inspired experimental processes for the synthesis of the nanoparticles is evolving into an important branch of nanotechnology. Nanoparticles can be synthesised from a wide variety of biological entities. These entities differ from the degree of the biochemical processing abilities that can be effectively used to synthesize particular metallic or metallic oxide nanoparticles. Not all biological entities can synthesize nanoparticles due to their enzyme activities and intrinsic metabolic processes. So a careful selection of the appropriate biological entity is necessary to produce nanoparticles with well-defined properties such as size and morphology (Malik *et al.*, 2014).

Biosynthesis of nanoparticles as an merging highlight of the intersection of nanotechnology and biotechnology have been increased attention due to growing need to develop environmentally benign technologies in material synthesis. A great deal of effort have been put into the biosynthesis of inorganic material, especially metal nanoparticle using microorganisms and plants (Khalil *et al.*, 2014).

It has emerged as an attractive method when compared to traditional synthesis for producing nanoparticles. The biological synthesis of the nanoparticles offers a clean, nontoxic and good method of synthesizing nanoparticles with a wide range of sizes, shapes, compositions and physicochemical properties. It has the ability to act as a template in the synthesis, assembly and organization of nanometer scale materials to fabricate well defined micro and macro scale structures. These properties acts as efficient biological factories capable of significantly reducing the environmental pollution and reclaiming metals from the industrial waste (Shah *et al.*, 2015).

2.4 SILVER NANOPARTICLES AND THEIR SIGNIFICANCE

Nanoparticles cover a broad area of interest including cosmetics, food industry, electronics, medicine and environmental applications. Surface modification of the nanomaterials have strong effect on the interaction of these nanomaterials with cells in addition to this it also helps to convert toxic nanomaterials to the more toxic to less toxic nanomaterials. The metallic property of the metal nanoparticles exhibit the photo electric effect (Khan *et al.*, 2019).

Silver is well known from the ancient times mainly due to its medicinal and preservative properties and it is one of the basic elements that make up the planet. It has efficient antimicrobial agents when compare to the other salts because of its large surface area. Silver nanoparticles find use in many fields and the major applications include their use as catalyst, as optical sensors, in textile engineering and most importantly in the medical field as a bactericidal and as a therapeutic agent. Wide use of silver nanoparticles are found in pharmaceutical industries and medicine, inkjet printing application, biological labelling, nanodevice fabrication, biosensing, photonics and optoelectronics (Jawaad *et al.*, 2014).

Silver nanoparticles has been proved to be most effective because of its good medicinal efficacy. Silver nanoparticle size ranges between 1 to 100 nm. They are incorporated into the wide range of medical devices. The ionic silver in right amount is suitable in treating wounds. In fact silver nanoparticles are now replacing silver sulfadiazine as an effective agent in the treatment of wounds. Due to their effective physiochemical properties these nanomaterial has been received considerable attention (Abdelghany *et al.*, 2018).

The silver nanoparticles are widely used as food additives and bioplastics mainly to eliminate the presence of the microorganisms. Many types of silver based compounds have been identified. They were reported to show better healing capacity and scar less healing when tested using an animal model. It has its unique role in various fields because of its distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activities. An important method of biosynthesis of nanoparticles is by the application of plant extract to the biosynthesis reaction. Synthesis of silver nanoparticles had been studied with the help of chemical and physical methods, but the development of

reliable technology to produce nanoparticles is an important branch of nanotechnology (Logeswari *et al.*, 2015).

2.4.1 Synthesis of silver nanoparticles

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

Most commonly used biomolecules arise from plant extracts. Apart from the plant extract, biomolecules from microbes (bacteria, fungi, yeasts and actinomycetes), algal and cyanobacterial inception have also been successfully used for the synthesis of nanoparticles. These biological molecules extracted from different origin are externally added to metal salts to make nanoparticles. To synthesize silver nanoparticles, biological extract *in situ* reduces silver salts (Ag^+) to metallic silver, Ag^0 . In the method of nanoparticle synthesis in a green way, biological molecules not only reduce the metal salts but also cover the formed nanoparticles or acts as *in situ* reducing and capping agent. This capping is advantageous over as it acts as multifunctional way; (i) prevents the agglomeration of the nanoparticles, (ii) reduces the toxicity, and (iii) improves antimicrobial activity (Roy *et al.*, 2019).

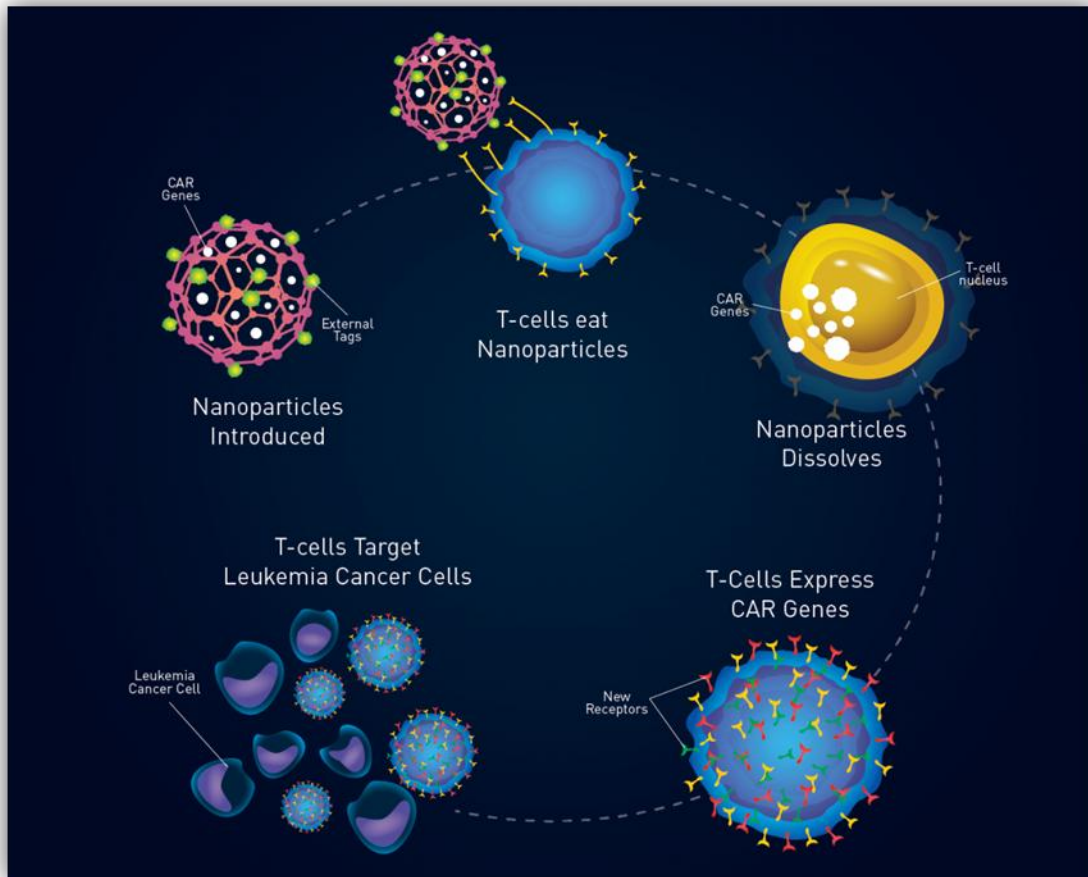
2.5 SILVER NANOPARTICLE AND CANCER

An important feature of Ag NPs is their involvement in cancer treatment. Cancer is a disease characterized by uncontrolled cellular growth and spread, during which cells become unresponsive to the usual check-points, leading to tumor growth and metastasis (Rai *et al.*, 2014). However, chemotherapy does not specifically target drugs to cancerous sites, therefore exposing healthy cells to undesirable effects. Moreover, a large dose is required owing to its rapid elimination, nonspecific distribution, specificity and sensitivity in cells (Jurj *et al.*, 2017). AgNPs are a promising tool as anticancer agents in diagnostics and probing (Huang *et al.*, 2017) with strong effects against different cancer cell lines offering many advantages (Conde *et al.*, 2012). Their better penetration, and the possibility to track Ag NPs in the body make them a more efficient tool in cancer treatment with less risk compared to standard therapeutic procedures (Bhattacharrya *et al.*, 2010).

The unique AgNP properties, such as easy surface functionalization, optical properties, reproducible synthetic routes and high surface: volume ratio, makes them suitable for cancer treatment (Sau *et al.*, 2010). The optical properties can be tuned to have an absorption at specific wavelengths that is useful for imaging and photothermal applications in cells and tissue. Ag NPs can also be functionalized with different molecules such as DNA/RNA to selectively target different cells and antibodies or polymers. These agents are important to extend the half-life time for *in vivo* circulation that is critical in drug and gene delivery applications. In addition, AgNPs are used as an

ablation tool for cancer cells due their ability to convert radiofrequencies into heat (Peloz *et al.*, 2015).

Figure 4: Nanoparticles in Cancer Treatment



(Rose, 2019)

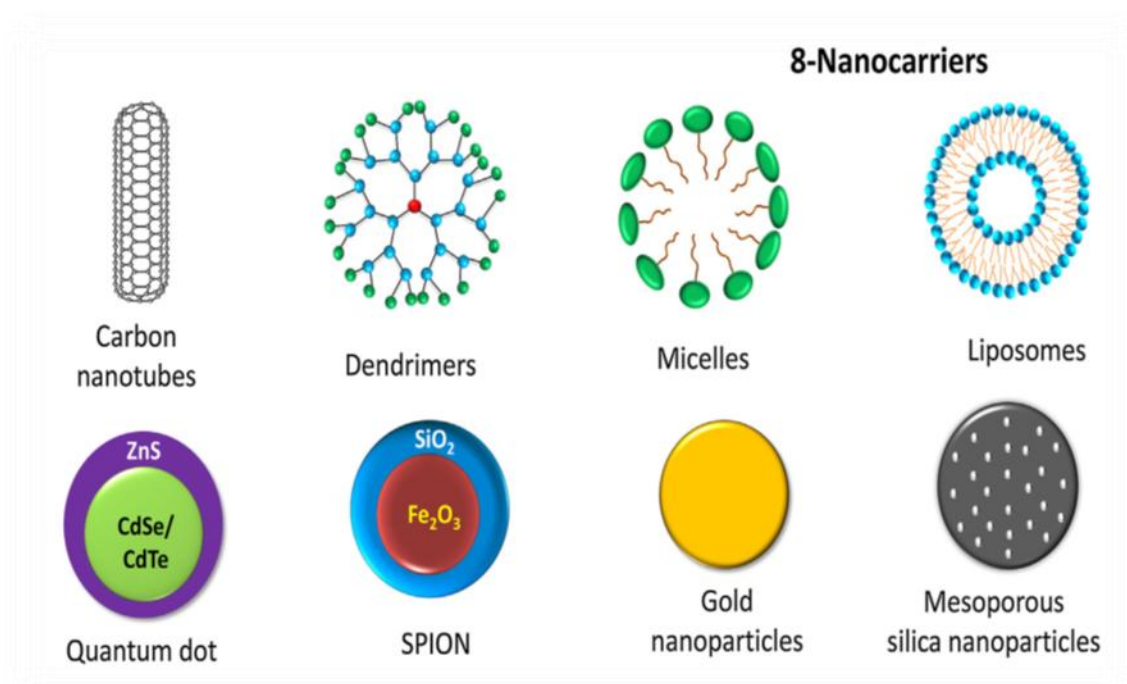
2.6 NANOCARRIERS

Nonspecific distribution and uncontrollable release of drugs in conventional drug delivery systems have led to the development of smart nanocarrier-based drug delivery systems, which are also known as Smart Drug Delivery Systems. Smart Drug Delivery Systems can deliver drugs to the target sites with reduced dosage frequency and in a spatially controlled manner to mitigate the side effects experienced in Conventional system. Chemotherapy is widely used to treat cancer, which is the second leading cause of death worldwide. Site-specific drug delivery led to a keen interest in the Smart Drug Delivery Systems as an alternative to chemotherapy. Smart nanocarriers, nanoparticles used to carry drugs, are at the focus of Smart Drug Delivery Systems. A smart drug

delivery system consists of smart nanocarriers, targeting mechanisms, and stimulus techniques (Hossen *et al.*, 2019).

When nanoparticles are used as transport modules for other substances, they are called nanocarriers. Conventional nanocarriers do not have the ability to carry and release drugs at the right concentration at the targeted site under external or internal stimulation. First, smart nanocarriers should avoid the cleansing process of the body's immune system. Second, they should be accumulated at the targeted site only. Third, smart nanocarrier should release the cargo at the targeted site at the right concentration under external or internal stimulation (Liu *et al.*, 2016).

Figure 5: Nanocarriers



(Hossen *et al.*, 2019)

Nanocarriers are the base of Smart Drug Delivery Systems. Unfortunately, not all types of nanocarriers are reliable as drugs carriers in Smart Drug Delivery Systems. There are different types of nanocarriers used as drug delivery system. They are liposomes, micelles, dendrimers, meso-porous silica nanoparticles, gold nanoparticles, super paramagnetic iron, carbon nanotubes and quantum dots. Smart Drug Delivery Systems utilizes the physiochemical differences between cancer cells and healthy cells to identify cancer sites. To exactly identify the cancer cell site, there are two major approaches: passive targeting and active targeting. Passive targeting utilizes the Enhanced

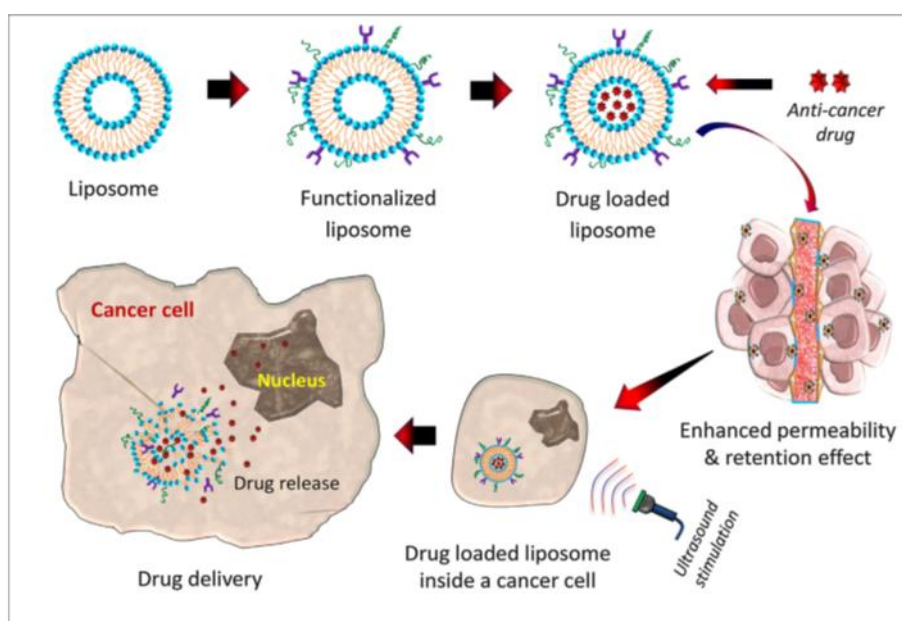
Permeability effect to specify the cancer site indirectly. Active targeting uses overexpressed cell surface receptors of cancer cells to target cancer cells directly like a guided missile (Bae *et al.*, 2011).

2.6.1 Liposomes

The possibility of liposomal drug-delivery scaffold has reformed in the pharmaceutical field. Alec Bangham, in 1961, first developed the concept of liposomes. As the basic and active investigations in the field of liposomes have been completed, their applications are presently concentrated in different areas, for example, biomolecules and gene delivery. Liposomes are round vesicles depicted by a lipids bilayer with an internal aqueous center (Kaul *et al.*, 2016).

The other parts of the structure are phospholipids or engineered amphiphiles consolidated with sterols (such as cholesterol) to facilitate film penetrability. Thin-film hydration is the most generally utilized formulation strategy for liposomes, in which lipid parts with or without a drug are broken down in an organic solvent. Alternate techniques include switch-stage vanishing, solidify drying, and ethanol injection . Strategies such as layer expulsion, sonication, homogenization as well as freeze-thawing are being exploited to regulate the size and size distribution. Liposomes can be planned and handled to vary in estimate, charge, and lamellarity (Manchanda *et al.*, 2020).

Figure 6: Liposome-based smart drug delivery system for cancer therapy



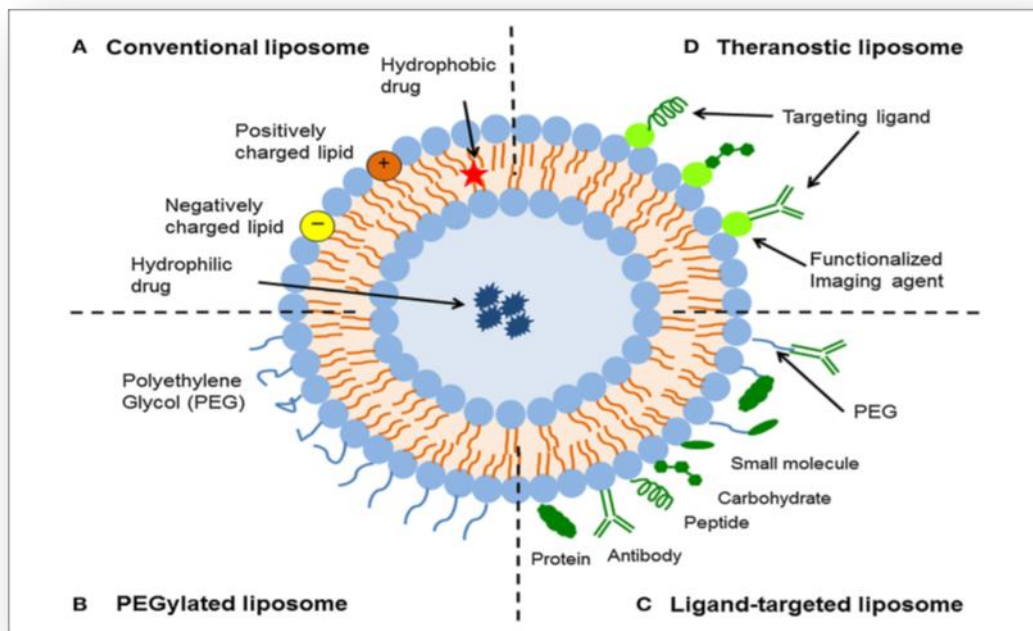
(Hossen *et al.*, 2019)

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There are four different types of liposomal drug delivery system. They are as follows:

- Conventional Liposome
- PEGylated Liposome
- Ligand Targeted Liposome
- Theranostic Liposome

Figure 7: Different types of liposomal drug delivery systems



(Hossen *et al.*, 2019)

2.6.1.1 Conventional liposome

Conventional liposome - Liposomes consist of a lipid bilayer that can be composed of cationic, anionic or neutral (phosphor) lipids and cholesterol, which encloses an aqueous core. Both the lipid bilayer and the aqueous space can incorporate hydrophilic and hydrophobic compounds, respectively. Conventional liposome can possess different lipid compositions, however the most commonly used lipids are phosphatidylcholines and cholesterol. Conventional liposomes, the first generation of liposomes, are a family of vesicular structures based on lipid bilayers surrounding aqueous compartments (figure 1). These particles are typically composed of only phospholipids (neutral and/or negatively charged) and/or cholesterol without modification. With its hydrophobic lipid bilayer and the hydrophilic aqueous space, various types of drug compounds could be incorporated accordingly. Off-the-shelf particles are normally supplied as lyophilized or pre-liposome powder form. Custom made liposomal formulation can be rationally designed with high versatility to increase drug delivery efficiency, by being very flexible systems through changing their physicochemical properties such as size, lipid composition, surface charge and number and fluidity of the phospholipid bilayer. Compared to free drugs, conventional liposomal formulations enhance drug delivery by reducing the toxicity of compounds, improving drug biodistribution, modifying pharmacokinetics (Sercombe *et al.*, 2015).

2.6.1.2 PEGylated Liposome

PEGylated liposome - Liposome characteristics and behaviour *in vivo* can be modified by addition of a hydrophilic polymer coating, polyethylene glycol (PEG), to the liposome surface to confer steric stabilization. PEGylation, the covalent linking of polyethylene glycol (PEG) chains, is currently considered as an effective approach to increase stability and prolong liposomes *in vivo* circulation time. PEGylation has been reported to hinder the adsorption of protein opsonins in the circulation onto liposome surfaces, which results in increased clearance of liposomes by the mononuclear phagocytic cells in the liver and spleen. Consequently, PEGylation improves the residence time of liposome, as well as encapsulated therapeutic agents, in the circulation. In the drug delivery field, PEGylated and non-PEGylated liposomes have received a number of clinical approvals. The most successful example of a PEGylated liposomal formulation is Doxil (PEGylated liposomes encapsulating the anticancer drug

doxorubicin (DXR)), approved for Kaposi's sarcoma, ovarian cancer, breast cancer and multiple myeloma (Mohamed *et al.*, 2019)

2.6.1.3 Ligand-targeted Liposome

Ligand targeted liposome- liposomes can be used for specific targeting by attaching ligands (e.g., antibodies, peptides, and carbohydrates) to its surface or to the terminal end of the attached PEG chains. Ligand-targeted liposomes (LTLs) have the potential to revolutionize the treatment of cancer. Antibodies were the first ligands that were coupled to liposomes and the resulting formulations were named immunoLiposomes. More recently, other ligands, such as peptides that can bind to tumor specific antigens have also been used. Collectively, liposomes that have had a ligand coupled to their surface are referred to as 'ligand targeted liposomes', or abbreviated to 'targeted-liposomes'. The development of these formulations has focused on the achievement of certain characteristics that constitute the 'ideal targeted-liposome'. The first requirement is a rapid and simple method to couple ligands to the liposome surface (Khidir and Saeed, 2020)

2.6.1.4 Theranostic liposomes

It is a single system consist of a nanoparticle, a targeting element, an imaging component, and a therapeutic component. Theranostic liposomes are effective drug delivery systems for the management of infections. With added features of targeting and stealth, theranostic liposomes can be made more effective. The targeted drug delivery systems for infections are devised to improve the therapeutic window of encapsulated drugs by increasing their delivery to the target area and minimizing the drug-associated toxicity (Kaul *et al.*, 2016).

2.6.1.5 Lecithin - Conventional liposome

Lecithins are a mixture of phospholipids where phosphatidylcholines are the main components (up to 98% w/w). Egg or soy lecithin as well as purified phospholipids is used for pharmaceutical purposes as dispersing, emulsifying, and stabilizing agents included in intramuscular and intravenous injectables or parenteral nutrition. Lecithins have been used to form liposomes, mixed micelles, and submicron emulsions for

pharmaceutical purposes. Moreover, aqueous lecithin dispersions (water-lecithin-dispersion (WLD) alone or in combination with cationic molecules have been proposed as carriers of lipophilic drugs and even as oligonucleotides delivery systems for cancer treatment. Actually, nanoparticles designed from lecithin-in-water emulsions were successfully used to deliver docetaxel to tumor cells *in vitro* and even in a tumor model in mice. Moreover, lecithin-based nanoparticles have demonstrated to deliver siRNA to breast cancer cells (Khidir *et al.*, 2020)

Phosphatidylcholines, the main components of lecithins, are glycerophospholipids that incorporate choline as the head group. The fatty acids bound to the glycerophosphatidic acid can vary but generally one of them is unsaturated and the other one is saturated. Phosphatidylcholine (PC) is a major constituent of the cell membranes which is more commonly found in the exoplasmic or outer leaflet of the plasma membrane. PC also plays a role in membrane-mediated cell signaling. The phospholipase D-mediated catabolism of PC yields phosphatidic acid (PA) and choline, which are important lipid second messengers involved in several signaling pathways. PA binds to Raf-1 and promotes its recruitment to the plasma membrane where it is activated by direct interaction with Ras. Ras-mediated Raf-1 activation leads to mitogen-activated protein kinase (MAPK) and PI3K/Akt activation. Therefore, PA would have a pivotal role in the amplification of signaling cascades required for survival and growth. PA also binds the mammalian target of rapamycin (mTOR), a protein kinase that regulates cell cycle progression and cell growth regulating several cellular events like translation, transcription, membrane trafficking, and protein degradation (Mohammed *et al.*, 2017)

2.7 CHARACTERIZATION OF SILVER AND ZINC OXIDE NANOPARTICLES

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

2.7.1 UV-Visible absorbance spectroscopy

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

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

2.7.2 Fourier transform infrared (FT-IR)

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

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

2.7.3 Scanning Electron Microscopy (SEM)

The conventional Everhart-Thornley (E-T) and the In-Lens detector of modern SEMs are capable to image sample surface morphology at high dimensional resolution to a sub-nm scale. Imaging that means of transmitted electrons in an SEM exploits the high signal-to-noise ratio resulting from the dominant “forward” electron scattering within a nanoscale sample. In addition, the transmitted electrons suffer reduced scattering events inside thin sample, leading to an intense electron signal in transmission mode. Surface effects, like charging and contamination are not as critical in comparison with the secondary electron (SE) imaging mode. Hence, the SEM in transmission mode (T-SEM) provides a science tool for dimensional (lateral) measurements of nanoparticles that is particle size distribution, however is additionally well suited for “in-depth” observation of structured nanoparticles (Hodoroaba *et al.*, 2016).

2.7.4. Energy Dispersive X-ray (EDX)

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

2.7.5 X-ray diffraction (XRD)

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

2.8 HERBAL MEDICINE - *Camellia sinensis*

Green tea is produced by processing of leaves of the plant *Camellia sinensis* belonging to the family Theaceae and is popularly consumed worldwide. Green tea has been shown to have beneficial effects on human health such as anti-cancer, anti-obesity, anti-diabetic, anti-cardiovascular, anti-infectious and anti-neurodegenerative (Hayakawa *et al.*, 2018). Epigallocatechin gallate (EGCG) is the most abundant catechin in green tea. Several epidemiological studies have shown the anti-cancer effects of consumption of tea. A survey case controlled studies which had been reported by 2008 revealed that green tea consumption showed risk-reduction in a total of 39 cases of breast, colon, esophagus, kidney, bladder, lung, ovary, pancreas, prostate, stomach cancers, whereas 46 cases showed no risk-reduction (Suzuki *et al.*, 2016). These findings suggest that green tea have a preventive effect in some types of cancer. For most of the site-specific cancers, a decreased relative risk was found. EGCG decreased the numbers of intestinal tumors in rats treated with dimethylhydrazine. Authors suggested that anti-cancer effects may be not due to EGCG itself but to its intracellular metabolites in view of EGCG's low bioavailability. EGCG is a prominent anti-oxidant and quenches reactive oxygen species (ROS), which facilitate oxidative DNA damage, mutagenesis, and tumor promotion, leading to anti-cancer effects (Lambert *et al.*, 2013). EGCG can exhibit anti-oxidant activity through several mechanisms including catalytic metal chelation, hydrogen atom transfer, and electron transfer. Chemically, the anti-oxidant activity of EGCG can be interpreted by the existence of the polyhydroxyl structure and the gallate group which play key roles to scavenge free radicals and by the presence of phenolic groups with sensitivity to be oxidized, resulting in generation of a quinone (Shirakami *et al.*, 2018).

Figure 8: *Camellia sinensis*



Green tea is obtained from the leaves of the plant *Camellia sinensis*, and it is most popular beverages worldwide, particularly in Asia. Green tea is recognized to have health-promoting effects that are attributed to green tea polyphenols (GTPs), such as epicatechin, epigallocatechin, epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG) . In addition, its antioxidant, anti-inflammatory, and antibacterial properties have been reported to provide various benefits to its consumers. Several investigators have reported that the addition of GTPs to drinking water can prevent carcinogenesis and the development of various types of malignancies. GTPs exhibit anticancer effects via the regulation of different cancer-related processes and factors, including DNA methylation, histone modification, micro-RNA, and proteins. They also play an important role via the regulation of apoptosis, growth, invasion, and angiogenesis in various types of malignancies. A recent study showed that GTPs can influence the pathological roles of not only cancer cells, but also cancer stem cells. These findings have led to a hypothesis that GTPs have strong anticancer effects *in vivo* and *in vitro* (Mohammed *et al.*, 2017).

2.9 HERBAL MEDICINE - *Durio zibethinus*

Durian (*Durio zibethinus* Murr.) is an energy-dense seasonal tropical fruit grown in Southeast Asia. It is one of the most expensive fruits in the region. It has a creamy texture and a sweet-bitter taste. The unique durian flavour is attributable to the presence of fat, sugar, and volatile compounds such as esters and sulphur-containing compounds such as thioacetals, thioesters, and thiolanes, as well as alcohols. Experimental animal models have shown that durian beneficially reduces blood glucose and cholesterol levels. Durian extract possesses anti-proliferative and probiotics effects in *in vitro* models. These effects warrant further investigation in human interventional studies for the development of functional food (Husin *et al.*,2018)

Durio zibethinus belongs to family Bombacaceae, genus Durio. It is a seasonal tropical fruit grown in Southeast Asian countries such as Malaysia, Thailand, Indonesia, and Philippines. There are nine edible Durio species, namely, *Durio lowianus*, *Durio graveolens*, *Durio kutejensis*, *Durio oxleyanus*, *Durio testudinarum*, *Durio grandiflorus*, *Durio dulcis* and also *Durio zibethinus* (Idris, 2011). In Thailand, Durian species were registered based on local names such as Monthong, Kradum, and Puang Manee. There are similar varieties between Malaysian and Thailand but with different name as follows: D123 and Chanee, D158 and Kan Yao, and D169 and Monthong (Husin *et al.*, 2018).

Similar to Thailand, Durian varieties in Indonesia are registered based on their local names, such as Pelangi Atururi, Salisun, Nangan, Matahari, and Sitokong (Tirtawinata *et al.*, 2016). The durian fruit shape varies from globose, ovoid, obovoid, or oblong with pericarp colour ranging from green to brownish. The colour of edible aril varies from one variety to the others and fall in between the following: yellow, white, golden-yellow or red (Ho *et al.*, 2015). It is eaten raw and has a short shelf-life, from two to five days. Fully ripened durian fruit has a unique taste and aroma, and is dubbed “King of Fruits” in Malaysia, Thailand, and Singapore. The unique taste and aroma is attributed to the presence of volatile compounds like esters, aldehydes, sulphurs, alcohols, and ketones (Belgis and Wijaya, 2016). Durian is also rich in polyphenols such as flavonoids (flavanones, flavonols, flavones, flavanols, anthocyanins), phenolic acids (cinnamic acid and hydroxybenzoic acid), tannins, and other bioactive components such as carotenoids and ascorbic acid (Park *et al.*, 2015). Current epidemiological studies have suggested that polyphenols decrease the risk of chronic diseases such as cardiovascular diseases, cancers and diabetes (Mostofsky *et al.*, 2017).

Currently, there are limited studies exploring the health benefits of bioactive components in durian. Hundreds of volatile compounds have been identified in Durian varieties such as esters (ethyl propanoate, methyl-2-methylbutanoate, propyl propanoate), sulphur compounds (diethyl disulphide, diethyl trisulphide and ethanethiol), thioacetals (1-(methylthio)-propane), thioesters (1-(methylthio)-ethane), thiolanes (3,5-dimethyl-1,2,4-trithiolane isomers), and alcohol (Chin *et al.*, 2007).

Figure 9: *Durio zibanthinus* (A)- Durian Fruit, (B)- Durian Leaves



(A)



(B)

The propionate ester (400 mmol/L) increased peptide and glucagon-like peptide 1 in primary cultured human colonic cells. This study also showed that 10 g/day of inulin-propionate ester reduced energy intake (14%) compared with the control (inulin) (Chamber et al 2015). Durian is also rich in polyphenols such as flavonoids (flavanones, flavonols, flavones, flavanols, anthocyanins), phenolic acids (cinnamic acid and hydroxybenzoic acid), tannins, and other bioactive components such as carotenoids and ascorbic acid (Wisutiamonkul *et al.*, 2015).

Current epidemiological studies have suggested that polyphenols decrease the risk of chronic diseases (e.g., cardiovascular diseases, cancers and diabetes). Polyphenols might act synergistically with other phytochemicals (Costa *et al.*, 2017).

With these literatures cited the methodology of the present study was discussed in following chapter.

3.0 METHODOLOGY

Camellia sinensis and *Durio zibenthinus* were found to have extensive traditional usage for medicinal purposes. Therefore the present study aims at evaluating the efficacy of liposome carrier loaded with the nanoparticles synthesized using the candidate plants.

3.1 Collection of Plant Species

The Durian Leaves were collected from Kallar region of Coimbatore District, Tamil Nadu. The Green tea Leaves were collected from Yedakkadu region of Nilgiri District, Tamil Nadu. The leaves of the selected plants were washed thoroughly 2-3 times with running tap water. Then the leaves were shade dried and coarsely powdered and stored in well closed bottles for further analyse in laboratory.

3.2 Extraction of the Plant Materials

The coarse powder (10g) of leaves of the *Camellia sinensis* and *Durio zibenthinus* were then subjected to successive extraction in 100ml of aqueous and ethanol solvent by using Soxhlet apparatus. The solvents present in collected extracts were evaporated, stored and then used for further analysis. The DMSO (Dimethyl sufloxide) is used as dissolved solvents for these extracts.

3.3 Qualitative Phytochemical Analysis

Preliminary phytochemical analysis was carried out as per standard methods described by Brain and Turner (1975) and Evans (1996).

3.3.1 Detection of Alkaloids

Aqueous and ethanol extracts of *Camellia sinensis* and *Durio zibenthinus* were dissolved individually in diluted hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

Mayers test:

Filtrates were treated with Mayers reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Wagner s test:

Filtrates were treated with Wagners reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

3.3.2 Detection of Flavonoids

Lead acetate test:

Aqueous and ethanol extracts of *Camellia sinensis* and *Durio zibenthinus* were treated individually with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

H₂SO₄ test:

Aqueous and ethanol extracts of *Camellia sinensis* and *Durio zibenthinus* were treated individually with few drops of H₂SO₄. Formation of orange colour indicates the presence of flavonoids.

3.3.3 Detection of Steroids

Two ml of acetic anhydride was added to five ml of the aqueous and ethanol extracts of *Camellia sinensis* and *Durio zibenthinus* , along with two ml of H₂SO₄. The colour was changed from violet to blue or green in some samples indicates the presence of steroids.

3.3.4 Detection of Terpenoids

Salkowskis Test

Five ml of the aqueous and ethanol extracts of *Camellia sinensis* and *Durio zibenthinus* was mixed with two ml of chloroform and concentrated H₂SO₄ (3ml) was carefully added to form a layer. An appearance of reddish brown colour in the inner face indicates the presence of terpenoids.

3.3.4 Detection of Anthroquinones

Borntrager's Test

About five ml of the aqueous and ethanol extracts of *Camellia sinensis* and *Durio zibenthinus* was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of CHCl_3 was added to the filtrate. Few drops of 10% NH_3 was added to the mixture and heated. Formation of pink colour indicates the presence of anthroquinones.

3.3.5 Detection of Phenols

Ferric chloride test:

10ml of aqueous and ethanol extracts of *Camellia sinensis* and *Durio zibenthinus* were treated individually with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

Lead acetate test:

10ml of aqueous and ethanol extracts of *Camellia sinensis* and *Durio zibenthinus* were treated individually with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of phenol.

3.3.6 Detection of Saponins

About 0.5ml of the aqueous and ethanol extracts of *Camellia sinensis* and *Durio zibenthinus* were shaken with five ml of distilled water. Formation of frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

3.3.7 Detection of Tannins

About 0.5ml of the aqueous and ethanol extracts of *Camellia sinensis* and *Durio zibenthinus* were mixed with water and heated on a water bath. The mixture was filtered and ferric chloride was then added to the filtrate. A dark green colour was formed. It indicates the presence of tannins.

3.4 SYNTHESIS OF SILVER NANOPARTICLES

The silver nitrate at a concentration of 1mM was prepared using deionised water and used for the synthesis of silver nanoparticle (Harbon, 1998). To 20 ml aqueous and ethanol extracts of *Camellia sinensis* and *Durio zibenthinus*, 80 ml of aqueous solution of 1mM of silver nitrate was added and when exposed to bright sunlight the change in color took place within few minutes from yellowish to reddish brown color as described in Sulaiman *et al.* (2013). Thus, silver nanoparticles were obtained by repeated centrifugation at 12000 rpm for 20 min, and then, to remove the water-soluble biomolecules, the pellet was dispersed in deionized water, repeated thrice. The purified pellets were then dried on petri plates at 60°C under vacuum for 24 h. Then the dried silver nanoparticles were used for further study (He *et al.*, 2017).

3.5 CHARACTERIZATION OF GREEN SYNTHESIZED SILVER NANO PARTICLES

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

3.5.1 UV visible spectrum (Premasudha *et al.*, 2015)

The formation of silver nanoparticle was primarily observed by monitoring the change in colour of the ethanol extracts of *Camellia sinensis* and *Durio zibenthinus* treated individually with silver nitrate (1mM). The bio-reduction of Ag ions in aqueous extract was monitored with the UV-visible spectra of the solutions after diluted a small aliquot (0.1 ml) of the sample to 10 times with double distilled H₂O. UV-visible spectra were recorded as a spectrophotometer at a wavelength range of 300-700nm at room temperature. Double distilled water was used as reaction blank

3.5.2 Scanning Electron Microscopy (SEM) (Nabikhan *et al.*, 2010)

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

3.5.3 EDAX spectrum measurements (Scimega et al., 2018)

The presence of elemental silver was confirmed through energy dispersive spectroscopy (EDS). EDX analysis was carried out for dried nanoparticles exposed were coated on to carbon film and performed on Scanning electron microscopy instrument equipped with Thermo EDX attachments. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

3.5.4 Fourier Transform IR (FT-IR) Analysis (Mittal et al., 2014)

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

3.5.5 X-Ray diffraction (XRD) studies (Priyaragini et al., 2013)

Crystalline silver nanoparticles were determined by X-ray diffraction analysis. The crystalline dried powder of silver nanoparticles onto glass slides was carried out on an instrument operating at 30 Kv, 40 mA, with Cu K α radians at 2 θ angle. The pattern was recorded by Cu K α radiation with λ of 1.5406 Å and nickel monochromator. The scanning was done in the region of 2 θ from 10° to 90°. The size of the nanoparticles was calculated through the Scherer's equation.

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

Where D is the average crystal size, λ is the X-ray wave length ($\lambda=1.5406$ Å), θ is Bragg's angle (2 θ), β the full width at half maximum (FWHM) in radians.

3.6 RADICAL SCAVENGING ASSAY

3.6.1 DPPH Radical Scavenging Assay (Brand-Williams et al., 1995)

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhyorazyl (DPPH) free radical.

DPPH 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,

Reagents

1. DPPH - (2, 2-diphenyl-2-picryl hydrazyl hydrate) (0.1mM in methanol)
2. Methanol
3. Ascorbic acid standard

Procedure

0.5 ml of 0.1mM DPPH solution in methanol was mixed with 0.5ml of aqueous and ethanol extracts of *Camellia sinensis* and *Durio zibenthinus* solution of varying concentrations (100,200, 300, 400 and 500 µg/ml). Corresponding blank sample was prepared and L-Ascorbic acid (25-500µg/ml) was used as reference standard. Mixture of 0.5ml methanol and 0.5ml DPPH solution was used as control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 520nm after 30 minutes incubation in dark condition using UV-Vis spectrophotometer. The inhibition % was calculated using the following formula.

$$\text{Inhibition \%} = \frac{\text{Ac}-\text{As}}{\text{Ac}} \times 100$$

Where Ac is the absorbance of the control As is the absorbance of the sample.

3.6.2 ABTS radical scavenging assay (Shirwaikar et al., 2006)

The antioxidant effect of extracts were determined using ABTS radical cation decolorization assay. ABTS is a chromogen which changes into a coloured mono cation radical form (ABTS) has an absorption peak at 745 nm. Antioxidants reduce (ABTS) into its colourless form and the extent of decolourisation is proportional to the percent reduction of ABTS.

Reagents

- i. ABTS - 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
- ii. Ammonium persulfate
- iii. Ethanol

Procedure

The ABTS radical cation (ABTS⁺) was produced by reacting ABTS solution (7mM) with (2.45mM) ammonium persulfate and the mixture was allowed to stand in dark at room temperature for 16 hours before use. 0.7ml of aqueous and ethanol extracts of *Camellia sinensis* and *Durio zibenthinus* of varying concentrations (100, 200, 300, 400 and 500 µg/ml) 0.3mL of ABTS solution was added. The absorbance (A) was read at 745nm and the percentage inhibition was calculated as follows.

$$\% \text{ scavenging activity} = \frac{A_c - A_s}{A_c} \times 100$$

Where A_c is the absorbance of the control and A_s is the absorbance of the sample.

3.6.3 Hydrogen peroxide scavenging activity (Ruch et al., 1989).

The hydrogen peroxide scavenging assay was carried out by the following the method of Ruchetal (1989). For this aim, a solution of H₂O₂ (43 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). 2.4ml of synthesized silver nanoparticle of varying concentrations (100, 200, 300, 400 and 500 µg/ml) was added to 0.6 ml of H₂O₂ solution (0.6 ml, 43 mM). Blank solution contained sodium phosphate buffer without H₂O₂ was measured. The absorbance value of the reaction mixture was recorded at 230 nm and the percentage inhibition was calculated as follows,

$$\% \text{ scavenging activity} = \frac{A_c - A_s}{A_c} \times 100$$

Where A_c is the absorbance of the control and A_s is the absorbance of the sample

3.6.4 Hydroxyl radical scavenging activity (Klein at al., 1991)

The scavenging activity of sample extracts on hydroxyl radical was measured according to the following method. Various concentrations (100, 200, 300, 400 and 500 µg/ml) of synthesized silver nanoparticle were added with 1.0 ml of iron-EDTA solution, 0.5 ml of EDTA solution (0.018%), and 1.0 ml of DMSO (0.85% DMSO (v/v) in 0.1 M phosphate buffer, pH 7.4) sequentially. The reaction was initiated by adding 0.5 ml of ascorbic acid (0.22%) and incubated at 80-90 °C for 15 min in a water bath. After incubation the reaction was terminated by the addition of 1.0 ml of ice-cold TCA (17.5% w/v). Three ml of Nash reagent was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the colour formed

was measured spectrophotometrically at 412 nm against reagent blank. The hydroxyl radical scavenging activity was calculated by the following formula:

$$\% \text{ scavenging activity} = \frac{A_c - A_s}{A_c} \times 100$$

Where A_c is the absorbance of the control and A_s is the absorbance of the sample.

3.6.5 Reducing Power assay (Yildirim et al., 2001)

Briefly, Various concentrations (100, 200, 300, 400 and 500 $\mu\text{g/ml}$) of synthesized silver nanoparticle were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$] (1%), and then the mixture was incubated at 50°C for 30 min. Then, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the upper layer solution was mixed with 2.5 ml of distilled water and 0.5 ml of FeCl_3 (0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power.

3.6.6 Nitric oxide radical scavenging assay (Sreejayan and Rao, 1997)

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction as described previously. Sodium nitroprusside (5 mM) in standard phosphate buffer saline solution (0.025 M, pH : 7.4) was incubated with different concentration of extracts (100, 200, 300, 400 and 500 $\mu\text{g/ml}$) dissolved in standard phosphate buffer saline solution (0.025 M, pH : 7.4) and the tubes were incubated at 25°C for 5 hr. Control experiments without test compounds but with equivalent amounts of buffer were conducted in an identical manner. After 5 hr, 0.5 ml of incubation mixture was removed and diluted with 0.5 ml of Griess reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthyl ethylene diamine was read at 546 nm and the percentage inhibition was calculated as follows,

$$\% \text{ scavenging activity} = \frac{A_c - A_s}{A_c} \times 100$$

Where A_c is the absorbance of the control and A_s is the absorbance of the sample.

3.7 PREPARATION OF SILVER NANOPARTICLE LOADED NANOLIPOSOMES (Maryana *et al.*, 2016)

The thin layer hydration method coupled with homogenization and sonication was used to produce leaf extract loaded nanoliposome. Initially 60mg phosphatidylcholine was dissolved in 8ml 99% ethanol. The resulting clear solution was evaporated in a rotary evaporator at 50°C and 110mm Hg until a thin film was formed (60min). Then 0.033ml of the synthesized silver nanoparticle was added and it was mixed with the phosphatidylcholine layer using the rotary evaporator for 5 min at 68°C. For hydration of the synthesized silver nanoparticle, 10 ml of water and glycerol (6:4) were added to the flask and mixed at 68°C for 25min. In this phase, glycerinated liposomes were formed. To reduce the size of the liposomes to a nanoscale, high shear homogenization was done for 15 min at 20000 rpm at a temperature between 50 and 60C. Finally sonication was done using an ultrasonic probe sonicator for 10 cycles of 1 min ultrasonic processing with 1 min intervals. The ultrasonic probe sonicator was set at 70% of amplitude with 0.5 cycles/sec (200W, 24kHz).

3.8 ANTICANCER ACTIVITY

In order to determine the cytotoxic effect of the silver nanoparticle loaded nanoliposomes. MTT assay was performed. The cell viability was tested using SRB assay.

3.8.1 MTT dye reduction assay (Igarashi and Miyazawa, 2001)

Cell viability was evaluated by the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-deiphenyltetrazolium bromide (MTT).

Principle:

MTT is a water soluble tetrazolium salt that is reduced by metabolically viable cells to a colored water insoluble formazan salt. Live cells convert MTT into its formazan derivative, the number of surviving cells can be determined by the amount of MTT formazan produced which is measured in a microtiter plate reader.

Reagent

1. PBS (Phosphate buffer saline)-pH-7.4
2. MTT-3mg/ml in PBS
3. Isopropanol in 0.04N HCL (Acid propanol)

Procedure

The cells were centrifuged and the medium was removed and then incubated with 50µl of MTT at 37°C for 3 hours. After incubation, 200µl of PBS was added to all the samples and the liquid was then carefully aspirated. Acid propanol of 200µl was added and left overnight in the dark. The absorbance was read at 650nm in a micro titer plate reader. The optical density of the control cells was fixed to be 100% viable and the per cent viability of the cells in the treatment groups were calculated accordingly. Cytotoxicity was expressed as the concentration of extract inhibiting cell growth by 50% (IC50 value)

3.8.2 Sulphorhodamine B Assay (Skehan et al., 1990)

The cytotoxic effect exerted towards the molt-3 cell line and PBL by the extracts was studied by the SRB assay as elucidated.

Principle

Sulphorhodamine B (SRB) is a bright pink amino xanthenes dye with two sulphonic acid groups. Under mild acidic conditions, SRB binds to protein basic amino acids in TCA fixed cells to provide a sensitive index of cellular protein content, which is directly proportional to cell viability. The SRB assay is useful in measuring drug-induced cytotoxicity.

Reagents

1. TCA (40% and 1%)
2. Sulphorhodamine B (SRB) - (0.4% in 1% TCA)
3. Acetic acid (1%)
4. Tris (10Mm, pH-10.5)
5. PBS

Procedure

After treatment with silver nanoparticle loaded nanoliposomes for 24 hours, cells were collected by centrifugation and washed with PBS. An aliquot of 350µl of ice cold 40% TCA was layered on the top of the treated cells and incubated at 4°C for one hour after which they were washed 5 times with 200µl of ice cold PBS. The buffer was removed and SRB (350µL) was added to each tube and left in contact with the cells for 30 minutes at room temperature. The unbound dye was removed by washing four times with 350µl portion of 1% acetic acid. Then 350µl of 10Mm tris (pH10.5) was added to each tube to stabilize the protein bound dye. The pellets were shaken gently for 20 minutes on a gyratory shaker. The debris was spun down and the absorbance of the tris layer in each group was transferred to a 96 well plate & read using microplate reader at 490nm. The cell survival was measured as the percentage absorbance compared to the control (untreatable cells).

The results of the various biochemical parameters analysed in the present study is presented and discussed in the next chapter.

4.0 RESULTS AND DISCUSSION

In India, medicinal plants are used as a natural source of medicine from ancient times and a large number of people are dependent on traditional medicinal practices for their efficiency, safety and to meet health care needs. Secondary metabolites of medicinal plants have tremendous use in modern and traditional medicine, and are the sources of important drugs (Benarba and Pandiella, 2020).

In recent times, the focus on medicinal plant research has increased all over the world and a large body of evidence and knowledge has accumulated in the literature, which shows the immense potential of medicinal plants used in various medical, pharmaceutical, cosmetic and agrochemical applications. With the increasing acceptance of herbal medicines as an alternative form of health care, the screening of medicinal plants for bioactive compound is imperative (Keshari *et al.*, 2020).

“Green synthesis” or “Green Nanotechnology” is a new platform to design novel products that are beneficial to human and environment health and has 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. 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. Many reports have come on nanoparticles synthesized from plants, microbes or other natural resources (Datkhile *et al.*, 2019).

Camellia sinensis possess antioxidant, antidiabetic, anti-inflammatory, antibacterial, antiviral and above all cancer protective properties. It also acts positively on neurodegenerative diseases such as Parkinson and Alzheimer disease (Vishnoi *et al.*, 2018). The study shows that *Camellia sinensis* possess anticancer activity against cancer cell lines (Vijay, 2017). Due to higher antioxidant and anti-inflammatory potency of durian, the functional activities of durian pulp warrant confirmation *in vivo* (Chingsuwanrote *et al.*, 2016). Chloroform, hexane, ethyl acetate and methanol extracts

from durian fruit pulp has potential antioxidant and antiproliferative effect on human leukemia (HL-60) cells (Sitheek *et al.*, 2020).

The present study was formulated to synthesize and characterize silver nanoparticles of leaves of *Camellia sinensis* and *Durio zibenthinus* and to find out their efficacy on antioxidant and anticancer activities. The results obtained are discussed as follows.

4.1 Preliminary phytochemical analysis of aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*.

Preliminary phytochemical studies are helpful in the determination of chemical constituents present in the plant material. Extraction of phenolic compounds from plant materials is typically depends upon different extraction techniques, such as solid-to liquid ratio, solvent composition, time of extraction, particle size of plant materials, temperature and pH (Harika *et al.*, 2017).

Aqueous extractions of green tea was prepared to evaluate antioxidant and anti-cancer activity. The extract possessed higher radical scavenging activity and also inhibited the growth of breast cancer cell line, it has potent anticancer activity (Armoskaite *et al.*, 2011). The toxicity and antioxidant activity of aqueous, ethyl acetate, n-hexane extracts from the leaves of *Durio zibenthinus* possessed potential antioxidant activity. Among the extracts tested aqueous extract showed higher antioxidant activity (Aruan *et al.*, 2019). Aqueous and ethanolic extract of *Durio zibenthinus* was tested for antioxidant and anti-cancer activity and the results reveal that Durian fruit can be utilized as a potential source of bioactive compounds which act as radical scavenger and inhibit the growth of cancer cells (Swaminathan and Doraiswami, 2020).

According to these literatures cited, aqueous and ethanolic crude extracts of *Camellia sinensis* and *Durio zibenthinus* possessed potential antioxidant and anticancer activity. Therefore, in the present investigation, aqueous and ethanolic extracts of these two plant leaves were prepared by Soxhlet method and used for further biochemical processing and preparations.

In the present study, the candidate plants extracted with aqueous and ethanol solvents were screened for their phytochemical constituents and the results showed the

presence of several secondary metabolites including alkaloids, flavonoids, phenols, anthroquinones, saponins, terpenoids, tannins and steroids. The results are tabulated in Table 1. The results indicated that the aqueous and ethanolic extract of both *Camellia sinensis* and *Durio zibethinus* leaves are rich in phytochemical constituents. The results also showed that the concentration of phenols are found to be higher when compared to the other secondary metabolites.

Table 1
Phytochemical analysis of aqueous and ethanolic extract of leaves of
Camellia sinensis and *Durio zibethinus*.

S. No	Phytochemical constituent	<i>Camellia sinensis</i>		<i>Durio zibethinus</i>	
		AQUEOUS	ETHANOL	AQUEOUS	ETHANOL
1.	Alkaloids				
	Meyer's Test	++	++	++	++
	Wagner's Test	++	++	++	++
	Dragendroff's Test	++	++	++	++
2.	Flavonoids				
	Aqueous NaOH test	++	++	++	++
	Conc.Sulphuric acid test	++	++	++	++
	Schinado's test	++	++	++	++
3.	Phenols				
	Ferric chloride test	+++	+++	+++	+++
	Lead acetate test	+++	+++	+++	+++
4.	Steroids				
	Leibermann-Burchard Test	++	++	++	++
	Salkowski Test	++	++	++	++
5.	Anthroquinone				
	Borntrager's Test	+	+	+	+
6.	Saponins				
	Froth Test	-	-	-	-
7.	Tannins				
	Braemer's Test	+	+	+	+
8.	Terpenoids				

	Burchard Test	+	+	+	+
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(+++) **Present in high intensity**

(++) **Present in moderate intensity**

(+) **Present in low intensity**

(-) **Absence**

Several studies are reported that reiterates the presence of multiple phytochemicals in their extracts. The hydroalcoholic extracts of leaves of *Ephedra intermedia* were screened for their phytoconstituents. The results showed the presence of tannins, glycosides, phenolics, flavonoids and alkaloids (Gul *et al.*, 2014).

Among the extracts tested, the methanolic and aqueous extracts of *Syzygium australe* and *Syzygium leuhmannii* contained most of the phytochemicals such as phenolics, saponins, triterpenes, polysterols, flavonoids and tannins, compared to the ethyl acetate, chloroform and methanol extracts (Mohanta *et al.*, 2017).

Ganapathy and Karpagam, (2016) assessed the phytochemicals present in the leaves and fruits of *Aegle marmelos*, that showed the presence of tannins, flavonoids, saponins and alkaloids. Phytochemical screening of ethanolic extract of *Citrus paradise* showed that it is abundant in secondary metabolites such as alkaloids, carbohydrates, saponins, reducing sugars, flavonoids, phenols, proteins, tannins, terpenoids and glycosides (Roghini and Vijayalakshmi, 2018). In agreement with above reports, the present study shows that the *Camellia sinensis* and *Durio zibenthinus* contains phytochemical constituents such as phenols, alkaloids, flavonoids, terpenoids, tannins and steroids in the aqueous and ethanol extract

4.2 Synthesis of silver nanoparticles of aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*

Nanoparticles have been abundantly used in nanochemistry to enhance the immobilization and activity of catalysts in pharmaceutical nanoengineering for delivery of therapeutic agents in chronic disease conditions. Silver nanoparticles have received considerable attention due to their good conductivity, chemical stability, catalytic property, photonics and optoelectronics, unique antibacterial, antifungal and UV filtering properties. It also being considered for use in next generation biological applications including antimicrobial agents, drug delivery and bioimaging probes (Tavabe *et al.*, 2020). The silver nanoparticles of aqueous and ethanolic extracts of *Camellia sinensis* and *Durio zibenthinus* synthesized upon exposure to sunlight for 20 minutes. *Camellia sinensis* and *Durio zibenthinus* were found to act as the stabilizing agent for the

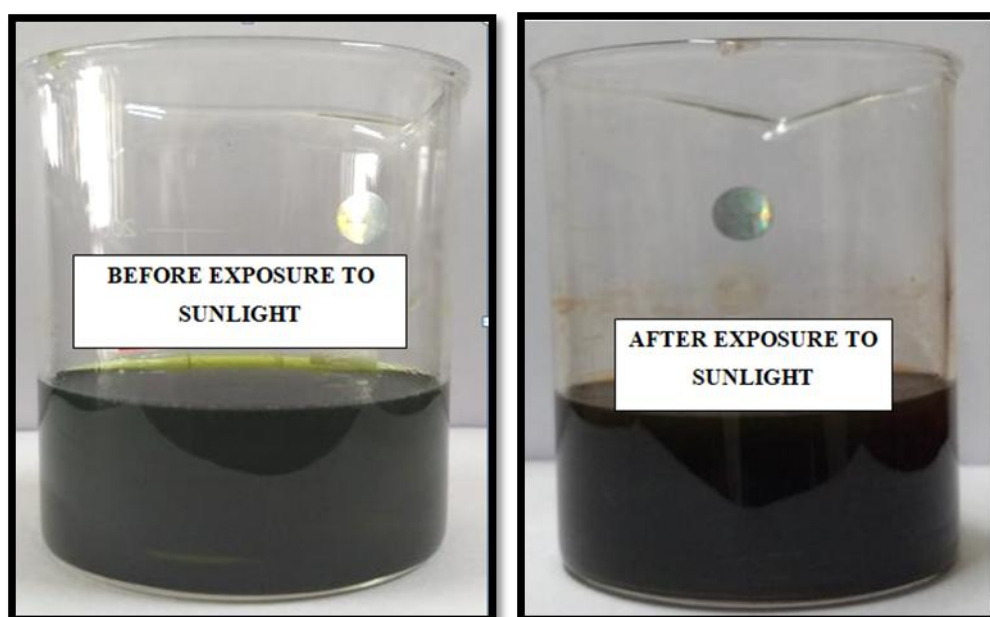
reduction of Ag^+ to Ag^0 . As the silver nanoparticles are formed, the color of the solution changes from green to reddish brown which is an indication of the formation of silver nanoparticles as shown in Figure 10. The change in color was due to the change in surface plasmon resonance of silver nanoparticles during the formation.

Figure 10

Green synthesized silver nanoparticles showing change of colour from green to reddish brown



A



B

A - *Camellia sinensis* extract B- *Durio zibenthinus* extract

Green synthesis of silver nanoparticles was carried out by mixing 1 mM AgNO₃ and different volumes of neem leaf extract that is 5 ml, 10 ml, 15 ml and 20 ml under sunlight exposure for 5, 10, 15 and 20 min respectively. With increasing sunlight exposure period, the color changed from yellow to reddish brown. The intensity of color increases in proportion to time due to reduction of Ag (Mankad *et al.*, 2018).

Synthesis of silver nanoparticles from natural sources has gained importance in recent days. The synthesis exhibited a 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 (Aritonang *et al.*, 2019). In the presence of sunlight, polyphenol rich aqueous leaf extract of medicinally important *P.integrifolia* was effectively used to synthesise stable AgNPs. The change in color is due to the excitation of Surface Plasmon Resonance in solution (Singh *et al.*, 2018).

On the addition of aqueous extract of neem leaves to silver nitrate solution, a color change from pale green to brown was observed on placing the reaction mixture in direct sunlight (Mankad *et al.*, 2018). Similarly aqueous extract of Durian seeds was utilized to reduce Ag⁺ to Ag⁰ where the observed color change from green to reddish brown confirms the reduction. The maximum absorbance exhibited by the reaction solution at 420 nm is the characteristic surface plasmon resonance peak of *Durio zibenthinus* silver nanoparticles (Smitha *et al.*, 2018).

In agreement with above reports, the candidate plants of the present study *Camellia sinensis* and *Durio zibenthinus* also possess reduction of Ag⁺ to Ag⁰, which was observed by the colour change from green to reddish brown in both the extracts. The change in color is due to the excitation of Surface Plasmon Resonance (SPR). Thereby, it confirms green synthesized nanoparticles using *Camellia sinensis* and *Durio zibenthinus*.

After synthesizing the nanoparticles, characterization of the nanoparticles was carried out to confirm the green synthesis using *Camellia sinensis* and *Durio zibenthinus* leaf extracts.

4.3 Characterization of synthesized silver nanoparticles of leaves of *Camellia sinensis* and *Durio zibenthinus*

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

4.3.1 UV-visible spectroscopy of synthesized silver nanoparticle of leaves of *Camellia sinensis* and *Durio zibenthinus*

The reduction of silver ions to silver nanoparticles was reflected in spectral data obtained using a UV-Visible spectrometer. The synthesis of silver nanoparticles was analyzed using UV-Visible (UV-Vis) spectrometry. The absorbance of nanoparticles was measured in the range 300-700nm. The UV-visible absorption spectrum of synthesized silver nanoparticles of aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus* are shown in the Figures 11, 12, 13 and 14

Figure 11

UV-Visible spectroscopy of synthesized silver nanoparticles of aqueous extract of leaves of *Camellia sinensis*

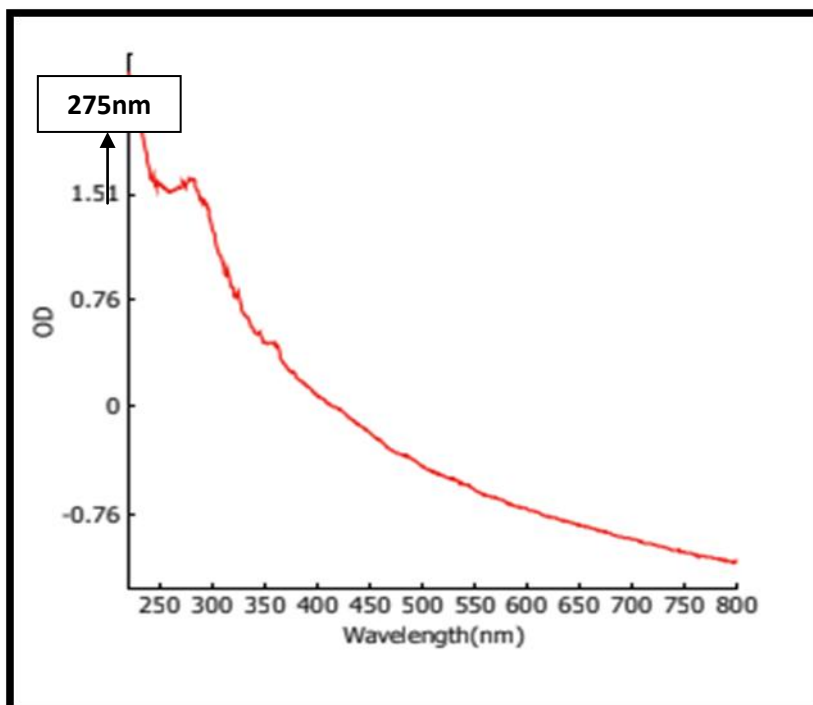


Figure 12

UV-Visible spectroscopy of synthesized silver nanoparticles of aqueous extract of leaves of *Durio zibenthinus*

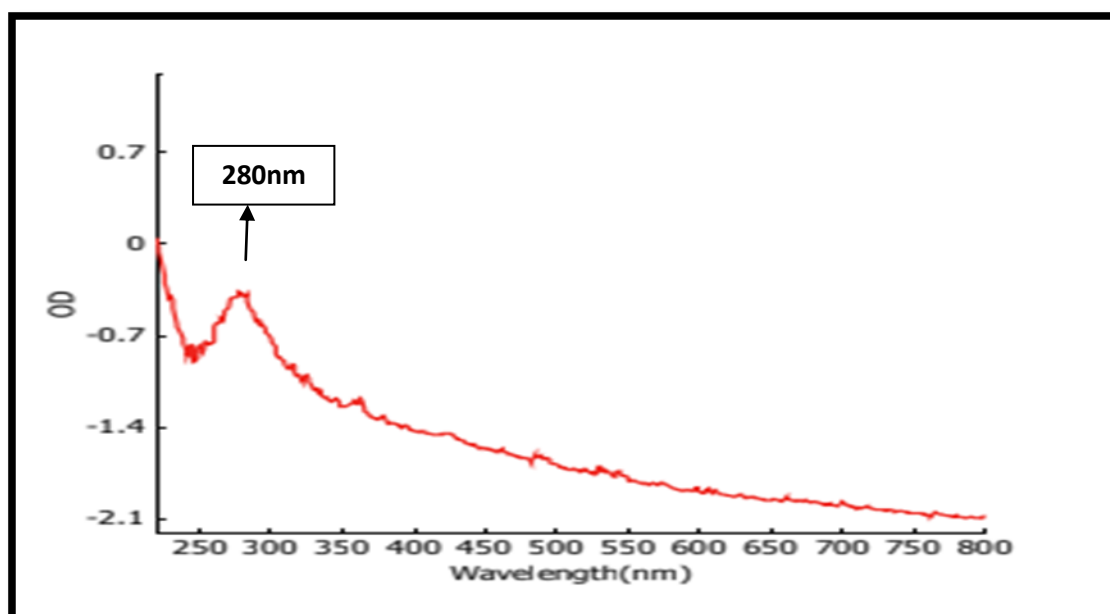


Figure 13

UV-Visible spectroscopy of synthesized silver nanoparticles of ethanolic extract of leaves of *Camellia sinensis*

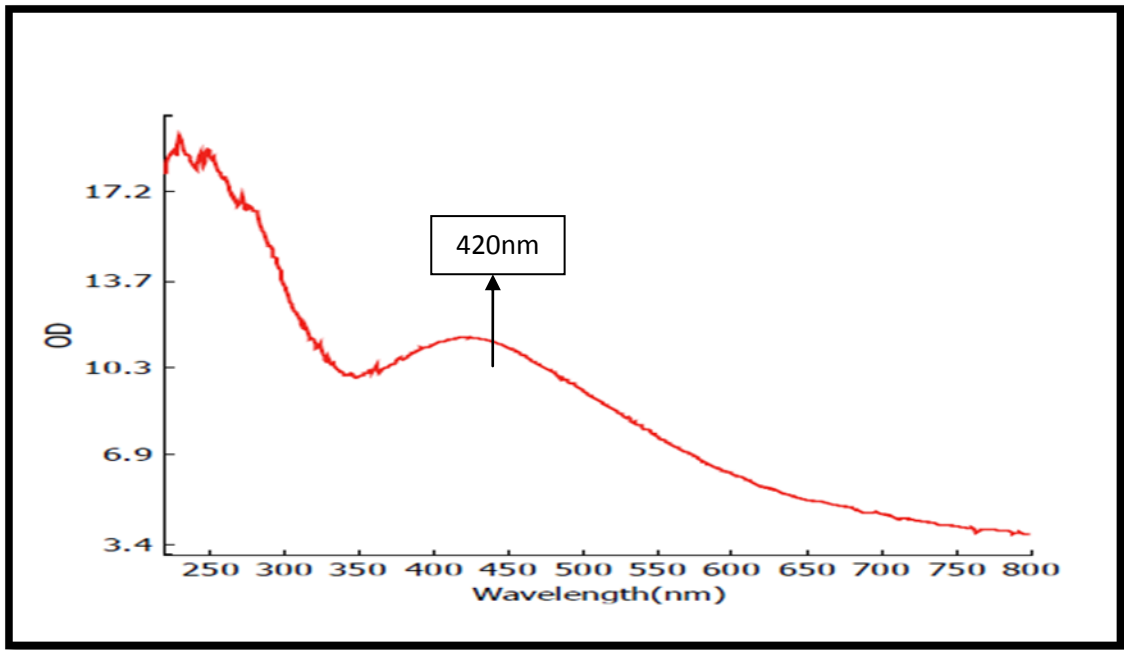
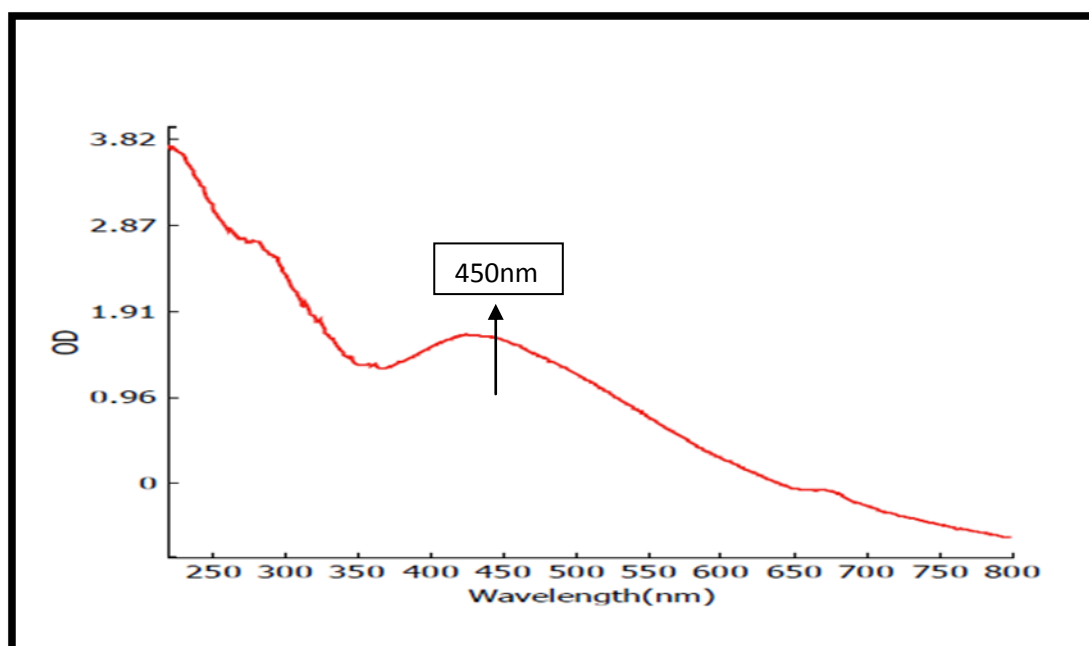


Figure 14

UV-Visible spectroscopy of synthesized silver nanoparticles of ethanolic extract of leaves of *Durio zibenthinus*



From the Figure 11 and Figure 12, the silver nanoparticles synthesized from the aqueous extract of leaves of *Camellia sinensis* and *Durio zibenthinus* formed in the reaction media has absorption peaks observed at 275nm and 280nm respectively. The peak characteristics reveal that nanoparticles synthesized were not pure and presence of aggregates were possible.

From the Figure 13, it is clear that the silver nanoparticles synthesized from the ethanolic extract of the leaves of *Camellia sinensis* formed in the reaction media has absorption spectra of maximum at 400 - 460 nm and the remarkable broadening of peak indicated that the particles are well and polydispersed. The absorption peak of *Camellia sinensis* was found at 420nm. This clearly indicates that there is an interaction between silver nanoparticles and bimolecules present in the leaves of *Camellia sinensis*.

From the Figure 14, it is evident that the silver nanoparticles synthesized from the ethanolic extract of the leaves of *Durio zibenthinus* formed in the reaction mixture has attained an absorption spectra in the wavelength range at 400 - 460 nm and the peak observed indicated that the particles are polydispersed. The absorption peak of

Durio zibenthinus was found at 450nm. This clearly indicates that there is an interaction between silver nanoparticles and bimolecules present in the leaves of *Durio zibenthinus*

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

S. podophyllum silver nanoparticles synthesis were measured by observing Surface Plasmon Resonance by UV spectroscopy. The SPR peaks were observed at 455 nm for the synthesized silver nanoparticles (Yasir *et al.*, 2017)

The synthesized silver nanoparticles from the leaves of Medicinal Plants (*Impatiens balsamina* and *Lantana camara*) was monitored by recording the absorption spectra at a wavelength range from 300-800 and shows intense peak at 414nm that confirmed the synthesis of AgNPs. The peak located between 410-450nm has observed for AgNPs and might be attributed to spherical nanoparticles (Aritonang *et al.*, 2019).

The biosynthesized silver nanoparticle through green chemistry method by using *Pelargonium sidoides* ethanolic extract. The colour of the ethanolic extract of *Pelargonium sidoides* was pale yellow before its treatment with silver nitrate solution, but after the reaction it turned to dark brown indicating the formation of AgNPs. This colour is attributed to surface Plasmon Resonance which is a size-dependent property of NPs. The UV-Vis spectrum of colloidal solutions of silver nanoparticle synthesized using *Cydonia oblonga* extract showed absorbance peak at 415nm (Kgatshe *et al.*, 2019).

The results of the present study confirms the formation of spherical nanoparticles by both the *Camellia sinensis* and *Durio zibenthinus* ethanolic extracts as it evident from UV-Vis spectral peak obtained at 420nm and 450nm respectively. In compliance with these results, the synthesized AgNPs of ethanolic extract of *Camellia sinensis* and *Durio zibenthinus* were used for further characterization and analysis.

4.3.2 Scanning Electron Microscope (SEM) and energy dispersive spectroscopy of synthesized silver nanoparticles of ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*.

The shape and size of the silver nanoparticles synthesized from the leaves of *Camellia sinensis* and *Durio zibenthinus* were identified using SEM and the elemental components present in the nanoparticles was confirmed by the EDX analysis. The SEM images revealed that the synthesized silver nanoparticles were in the range of 26.80 - 44.40 nm as shown in Figure 15. It shows a clear image of highly dense silver nanoparticles of leaf extracts of *Camellia sinensis*.

A spherical shaped nanoparticle of different size was obtained with variation in the volume of silver nitrate colloidal solution was observed. The Figure 16 shows the EDX quantitative analysis graph, which confirms the nanostructure of the silver nanoparticles of leaf extracts of *Camellia sinensis*. The silver nanoparticles synthesized from leaf extracts of *Camellia sinensis* contains about 36.32 weight per cent Ag, 38.18 weight per cent carbon, 9.36 weight per cent Cl and about weight per cent of oxygen.

The SEM images of *Durio zibenthinus* revealed that the synthesized silver nanoparticles were found to be in the range of 2.50 - 56.70 nm as shown in Figure 17. It shows a clear image of highly dense silver nanoparticles of leaf extracts of *Durio zibenthinus*. SEM images revealed the agglomeration of nanoparticles and individual nanoparticles are less spherical and cubic or diamond in shape. The Figure 18 shows the EDX quantitative analysis graph, which confirms the nanostructure of the silver nanoparticles of leaf extracts of *Durio zibenthinus*. The silver nanoparticles synthesized from leaf extracts of *Durio zibenthinus* contains about 5.99% weight per cent Ag, 60.72% of carbon, 4.14 weight per cent K, 0.55% weight per cent Cl and about 28.67 weight per cent of oxygen.

The SEM analysis of seeds of *Tectona grandis* clearly shows the silver nanoparticles were oval, spherical in shape. Most of the nanoparticles were aggregated and few individual particles were also observed. Elemental mapping of AgNPs by FESEM-EDX shows the presence of 4 per cent of Ag and 6 per cent of oxides (Rautela *et al.*, 2019)

Figure 15

SEM images of synthesized silver nanoparticles of ethanolic extract of leaves of *Camellia sinensis*

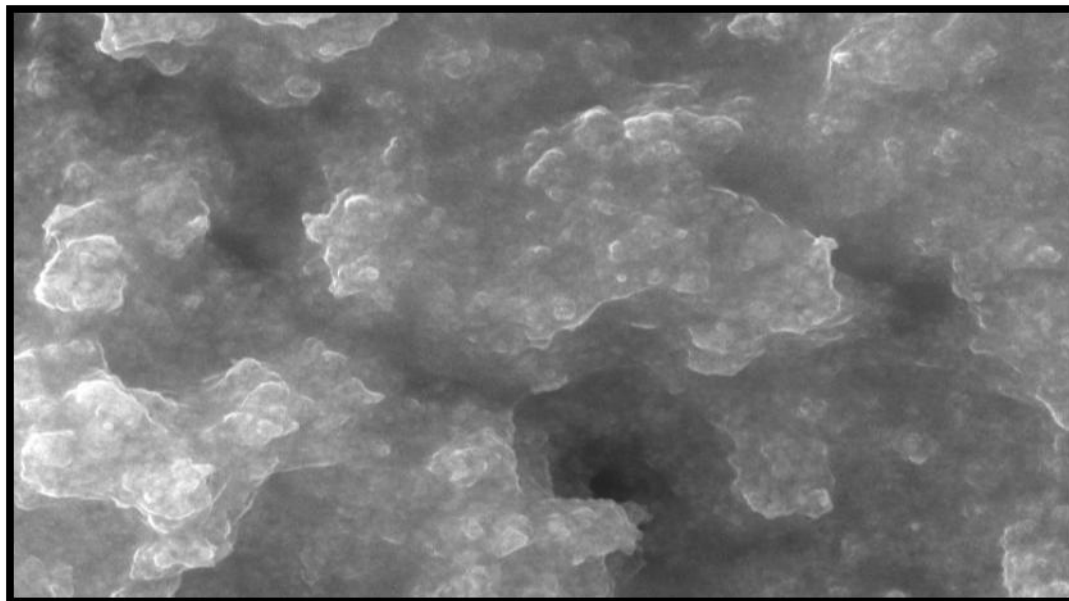


Figure 16

EDX images of synthesized silver nanoparticles of ethanolic extract of leaves of *Camellia sinensis*

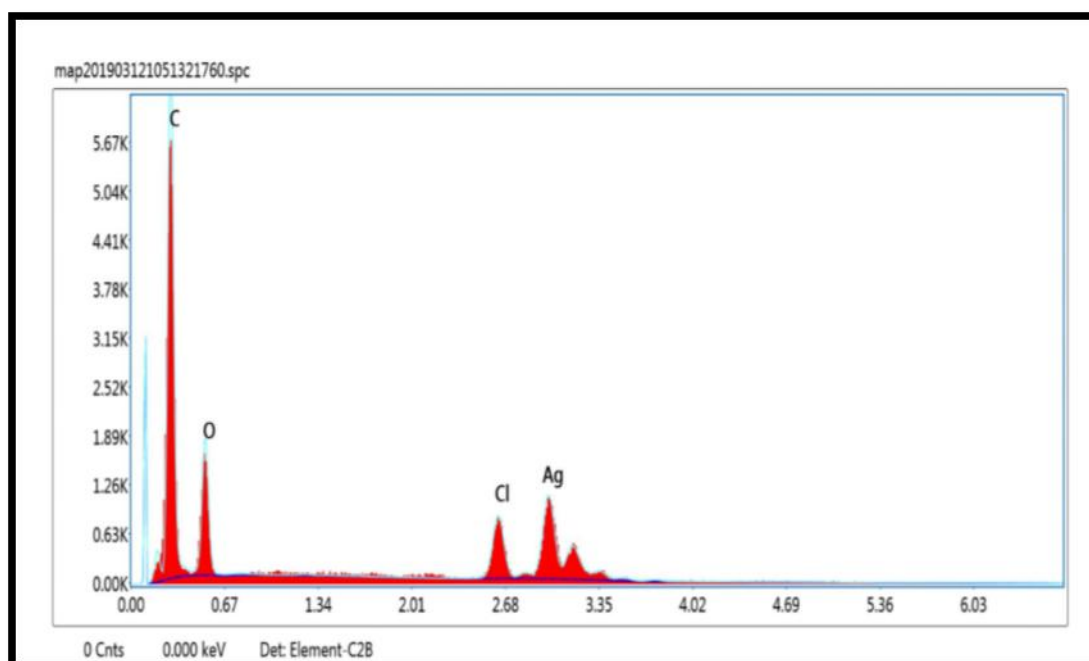


Figure 17

SEM images of synthesized silver nanoparticles of ethanolic extract of leaves of *Durio zibenthinus*

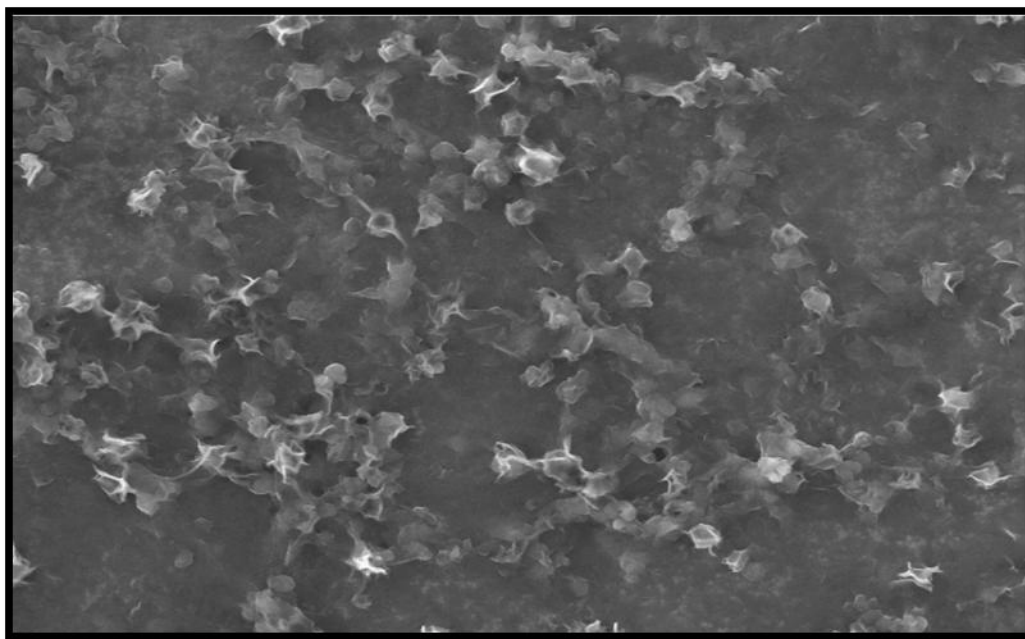
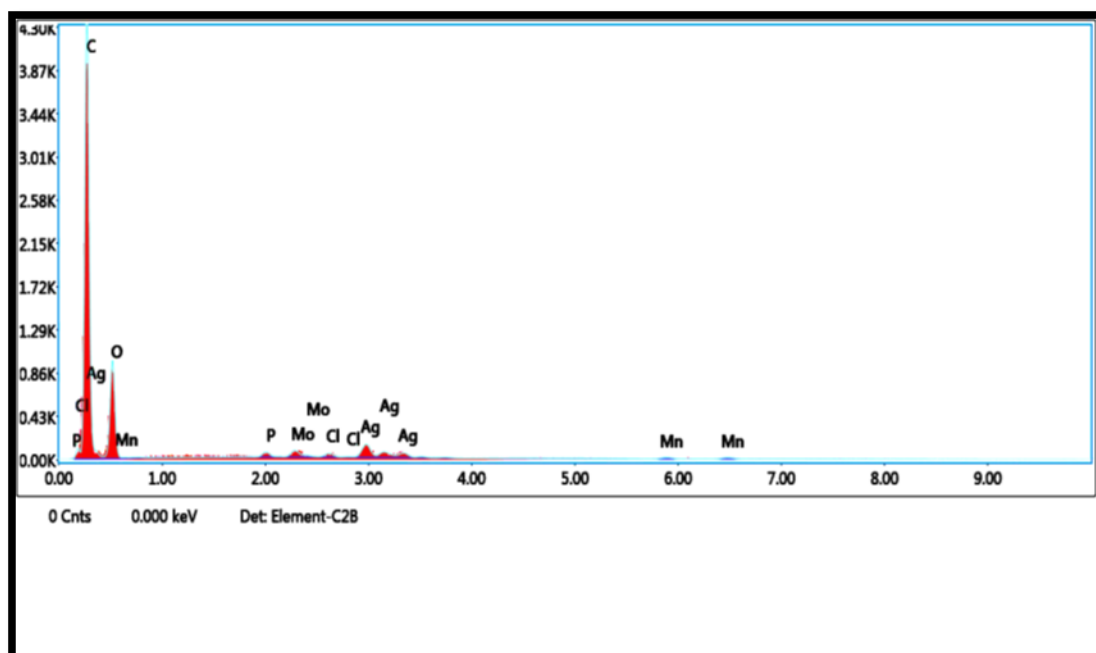


Figure 18

EDX images of synthesized silver nanoparticles of ethanolic extract of leaves of *Durio zibenthinus*



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

Fadel and Lam (2016), reported that the silver nanoparticle of peel extract of *Raphanus sativus L*, are spherical with range between 34nm to 50nm. *Allophylus serratus* leaf have the diameter of 40-200nm with spherical shape and some are irregular and the EDX analysis shows higher percent of silver nanoparticle (Jemal *et al.*, 2017).

Energy dispersive X-ray spectroscopy determine the chemical nature of the synthesized silver nanoparticles using *Fraxinus excelsior* leaf extract. The EDX profile displayed a strong peak for silver about 71.06 wt% and also some of the weak peaks for C,O,Cl were observed which may have originated from the biomolecules bound to the surface of the silver nanoparticles (Paveen *et al.*, 2016).

The present study results are in agreement with the above cited literatures and it can be inferred that both the plant extracts were able to support the nanoparticles formation. *Camellia sinensis* plant extract readily supported with the Ag component 36.32% weight as against 6% weight by *Durio zibenthinus*. However both the plant extracts *Camellia sinensis* and *Durio zibenthinus* were able to synthesize spherical nanospheres in the range of 28-44nm and 2.50 - 56.70 nm respectively.

4.3.3 FT-IR of synthesized silver nanoparticles of ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*

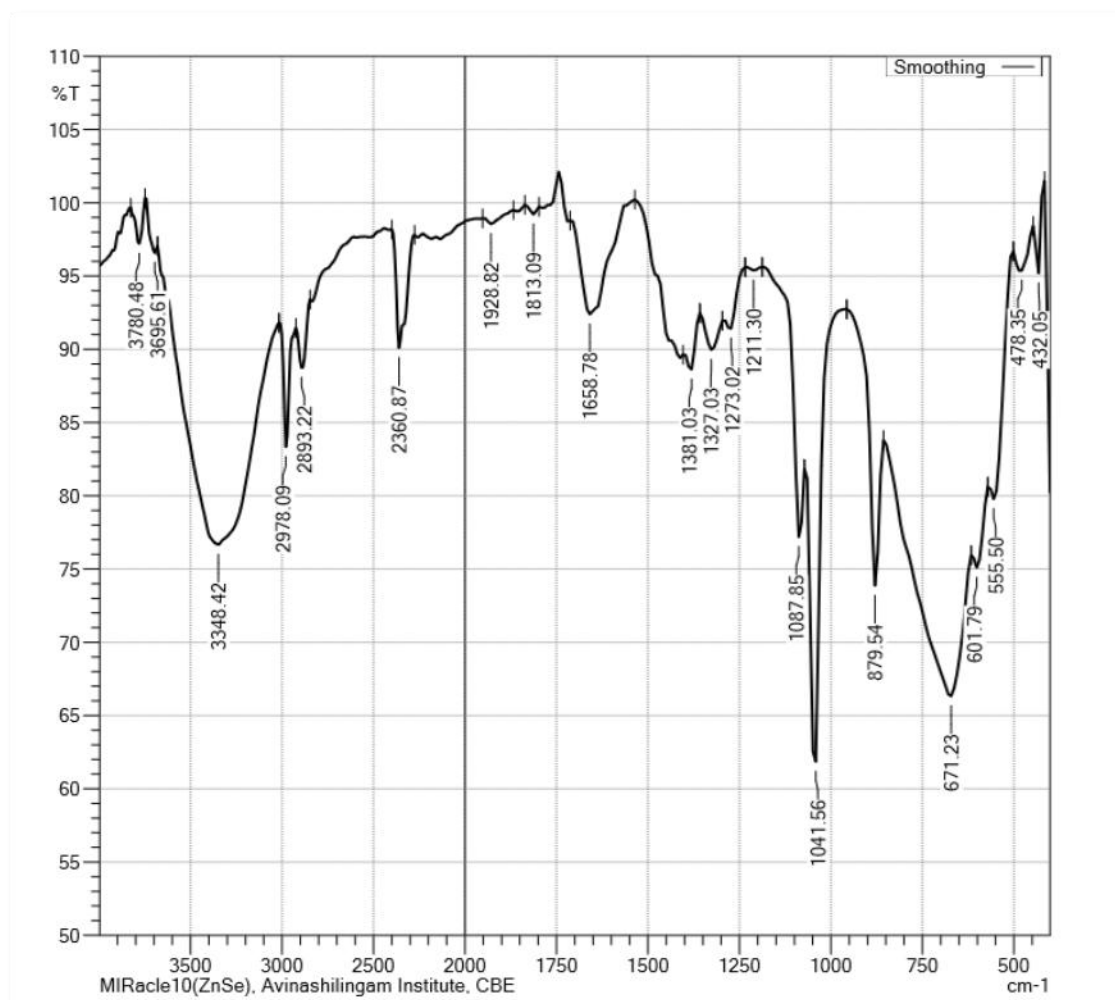
Fourier Transform Infrared Spectrophotometer (FT-IR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed the characteristics of the chemical bond as can be seen in the annotated spectrum. By interpreting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

the chemical bonds in a molecule can be determined (Ashokkumar and Ramaswamy, 2014).

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

Figure 19

FT-IR spectrum of synthesized silver nanoparticles of ethanolic extract of leaves of *Camellia sinensis*

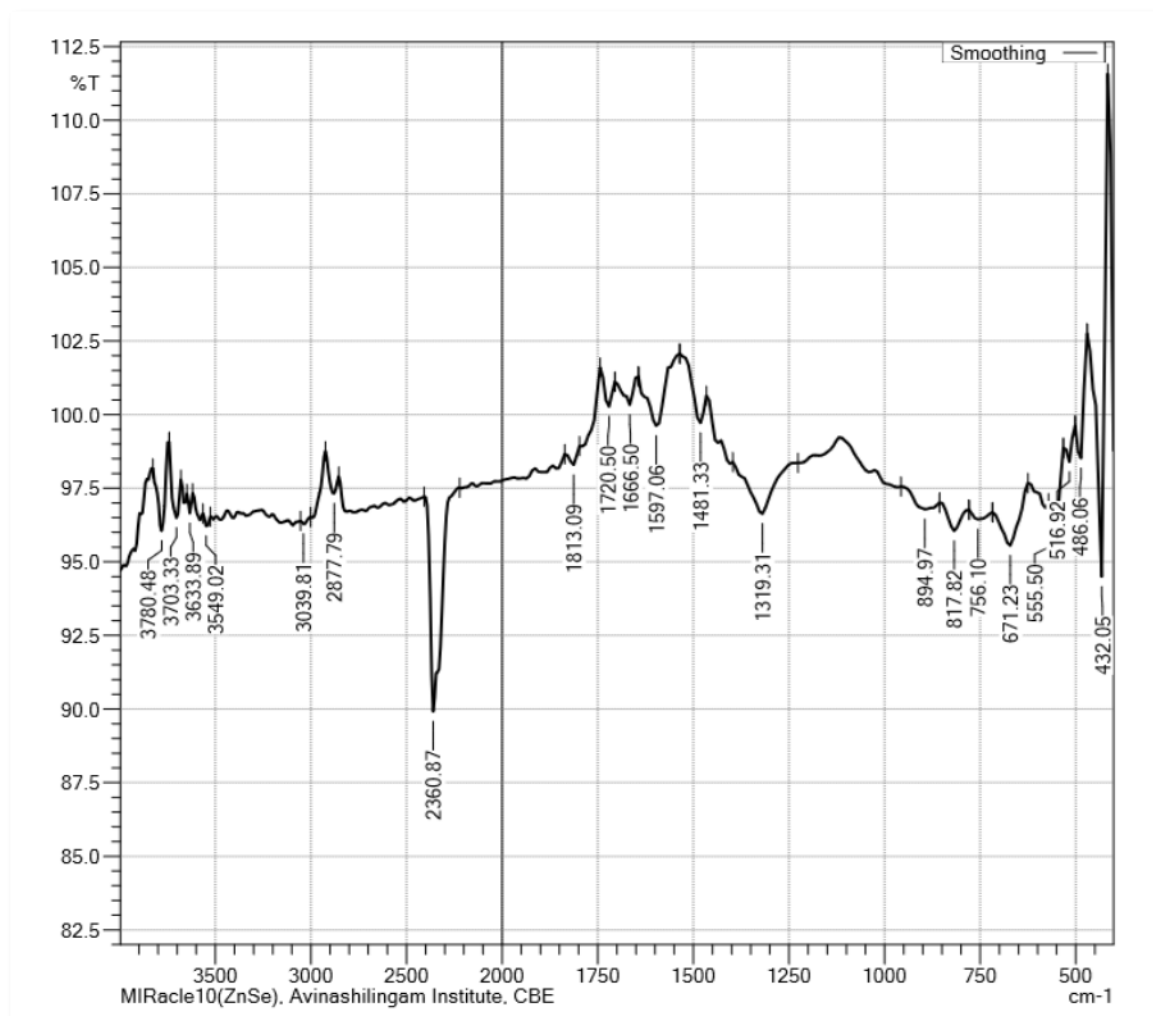


The FT-IR spectrum of synthesized silver nanoparticles of *Camellia sinensis* is presented in the above Figure 19. The peaks at 3780.48 - 3348.42 cm⁻¹ reveal O-H stretching of alcohols, 2978.09 - 2893.22 cm⁻¹ represents C-H stretching mode in alkenes. The distinct peaks 2356.87 cm⁻¹ which is attributed to O=C=O stretching vibrations of carbon dioxide. The peak 1928.82-1658.78 cm⁻¹ reveals C-H bending of

aromatic compounds, 1381.03 - 1327.03 cm^{-1} represents O-H bending of phenol, 1273.02- 1211.30 cm^{-1} C-O stretching of alkyl aryl ether, the peak 1087.85-1041.56 cm^{-1} reveals C-O stretching of primary alcohol, 879.54 - 671.23 cm^{-1} represents C=C bending of alkenes, 601.79-432.05 cm^{-1} represents C-Br stretching of halo compounds.

Figure 20

FT-IR spectrum of synthesized silver nanoparticles of ethanolic extract of leaves of *Durio zibenthinus*



The FT-IR spectrum of synthesized nanoparticles of *Durio zibenthinus* are represented in the above Figure 20. The peak 3780.48 - 3549.02 cm^{-1} reveal O-H stretching of alcohols. 3039.81 cm^{-1} , 2877.79 cm^{-1} , 2360.87 cm^{-1} , 1813.09 cm^{-1} , 1666.50 cm^{-1} represents N-H stretching of alcohols, C-H stretching of alkane, O=C=O stretching vibrations of carbon dioxide, C=O stretching of acid halide respectively. The

peak 1597-1481.33 cm^{-1} represents N-O stretching nitro compounds. The distinct peaks 1319.31 cm^{-1} and 894.97 which is attributed to C-O stretching of aromatic compounds and C=C bending of alkenes respectively. The peak 817.82 - 432.06 cm^{-1} represents C-Br stretching of halo compounds.

According to Jyoti *et al.* (2016), leaves of *Urtica dioica* shows the bands at 3422 cm^{-1} in the spectra corresponds to O-H stretching vibration indicating the presence of alcohol and phenol. Bands at 2921 and 2856 cm^{-1} region arising from C-H stretching of aromatic compound was observed. The band at 1743 cm^{-1} was assigned for C-C stretching (non-conjugated). The band at 1631 cm^{-1} in the spectra corresponds to C-N and C-C stretching indicating the presence of proteins. The band at 1450 cm^{-1} was assigned for N-H stretch vibration present in the amide linkages of the proteins. The bands at 1450 cm^{-1} and 1043 cm^{-1} were assigned for N-H and C-N (amines) stretch vibration of the proteins respectively. The band at 1377 cm^{-1} exemplifies the N=O symmetry stretching typical of the nitro compound. The band at 1240 cm^{-1} corresponds to C-N stretching of amines. The band at 596 cm^{-1} region could be attributed to C-Br stretching, which is characteristic of alkyl halides.

The FTIR spectrum of the synthesized silver nanoparticles of *Cestrum nocturnum* shows peaks at 3852, 3750 and 3469 cm^{-1} corresponds to the O-H stretching of hydroxyl groups, IR absorption of polysaccharide and C-H pulsation of aldehyde. The obtained results suggest the presence of various functional group in the leaf extract as well as in synthesized AgNPs (Keshari *et al.*, 2020).

The FT-IR spectra of synthesized silver nanoparticles of *Pelargonium sidoides* leaf extract shows 3432.94 cm^{-1} N-H stretch, 2777.28 cm^{-1} single aldehyde, 2676.19 cm^{-1} C-H; O-H, 2071.75 cm^{-1} $\text{C}\equiv\text{C}$, 1637.58 cm^{-1} $\text{C}=\text{C}$ and 1121.56 cm^{-1} $\text{C}=\text{O}$. The peaks near 3440 cm^{-1} , 2924 cm^{-1} and 2854 cm^{-1} assigned to OH stretching and aldehydic C-H stretching respectively. The band at 1629 cm^{-1} corresponds to amide arising due to carbonyl stretch in proteins. The peak at 1041 cm^{-1} corresponds to C-N stretching vibration of the amine. The peak near 1741 cm^{-1} corresponds to $\text{C}=\text{C}$ stretching. The peak near 833 cm^{-1} assigned to $\text{C}=\text{CH}_2$ and the peaks near 677 cm^{-1} and 651.96 cm^{-1} assigned to CH out of plane bending vibrations are substituted ethylene systems CH=CH (cis). FT-IR spectra of silver nanoparticles exhibited prominent peaks at 2,927, 1,631, and

1,383 cm^{-1} . The spectra showed sharp and strong absorption band at 1,631 cm^{-1} assigned to the stretching vibration of (NH) C=O group. The band 1,383 developed for C-C and C-N stretching; presence of the sharp peak at 2,927 cm^{-1} was assigned to C-H and C-H (methoxy compounds) stretching vibration, respectively (Kgatshe *et al.*, 2019).

The FT-IR spectral results of *Camellia sinensis* and *Durio zibenthinus* confirms the presence of non-conjugated aromatic compounds such as alcohol or phenol as observed from the supporting literatures cited. Further analysis need to be carried out to understand and elucidate the exact nature and structure of the green synthesized silver nanoparticles.

4.3.4 X-Ray Diffraction (XRD) pattern of synthesized silver nanoparticles of ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*

The nanoparticle shape and morphology are very much important in nanoparticles preparation. The important tool to study the characteristics of nano materials is X -ray diffraction. The size, shape, lattice parameter determination and phase fraction analysis of any compound can be determined easily by XRD. The silver nanoparticles synthesized from the leaves of *Camellia sinensis* and *Durio zibenthinus* were dried and small amount of dried silver nanoparticles can be used for XRD analysis. XRD pattern was analysed to confirm the crystalline nature of silver nanoparticles of *Camellia sinensis* and *Durio zibenthinus* and the results are given in Figure 21.

The XRD pattern of silver nanoparticles synthesized from the leaves of *Camellia sinensis* indicated that the structure of nanoparticles may be face centered cubic (FCC). The XRD spectrum showed eleven different peaks. These diffraction peaks observed at 2θ include 31.89°, 34.59°, 36.42 °, 47.72°, 56.66°, 62.92°, 67.95 °, 69.13° and 77.01° respectively and corresponds to the planes (100), (002), (101), (102), (110), (103), (112), (201) and (202) indexed according to the facets of silver.

Figure 21
XRD pattern of synthesized silver nanoparticles of ethanolic extract of leaves of *Camelia sinensis*

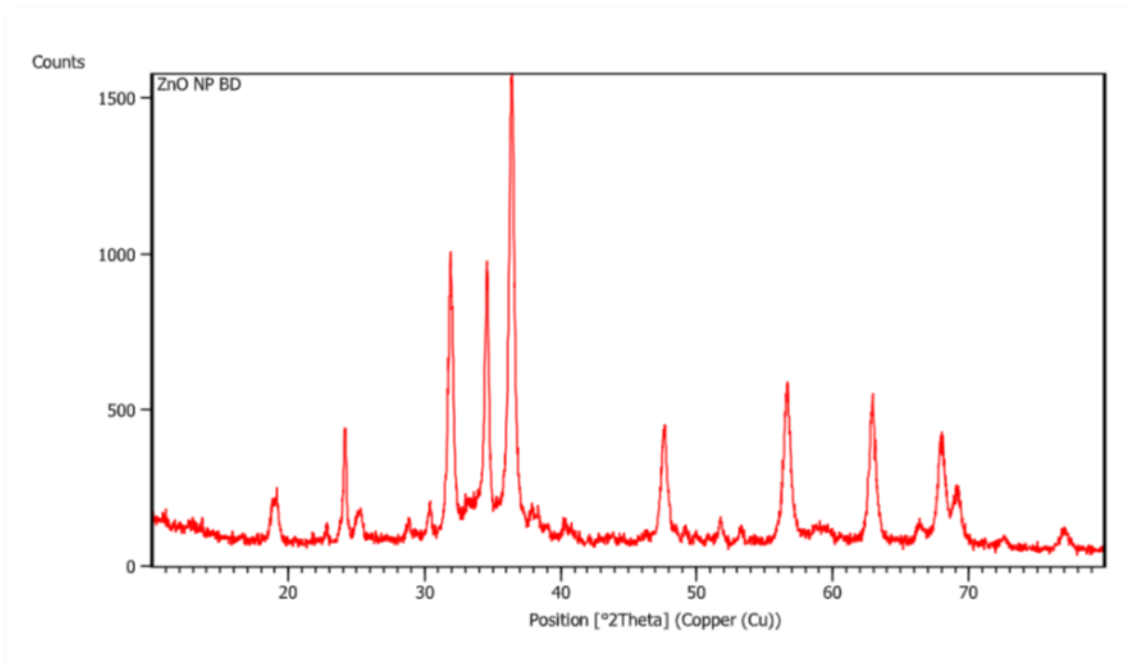
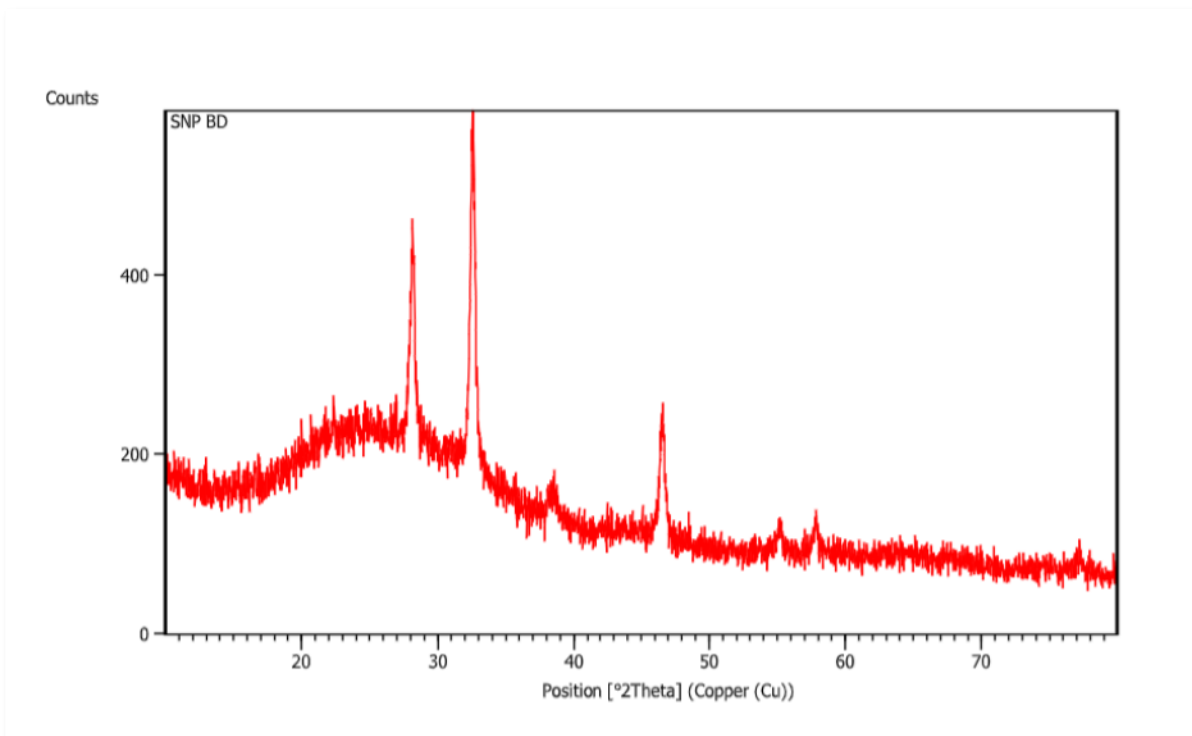


Figure 22
XRD pattern of synthesized silver nanoparticles of ethanolic extract of leaves of *Durio zibenthinus*



The XRD pattern of silver nanoparticles synthesized from the leaves of *Durio zibenthinus* indicated that the structure of nanoparticles may be simple cubic. The XRD spectrum showed eleven different peaks as shown in Figure 22. These diffraction peaks observed at $2\theta = 28.16^\circ$, 32.52° and 46.56° respectively and corresponds to the planes (111), (200) and (222) indexed as facet of silver crystal respectively.

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

It can be inferred that the *Camellia sinensis* could show the Face centered cubic structure as per the facets of silver. However, *Durio zibenthinus* could show only simple cubic structure.

4.4 Free radical scavenging activity of the crude extract, synthesized silver nanoparticles of aqueous and ethanolic extract of leaves of *Camellia sinensis*, *Durio zibenthinus*

Free radicals can chemically react with DNA, lipid or protein and steal their electrons so as to become neutral. Reactive Oxygen Species are toxic by-products of aerobic metabolism. They play a major role in the development of many chronic and neurodegenerative disorders, such as Parkinson's and Alzheimer's disease, heart and blood vessel disorders, muscle degeneration, gene mutation and cancer (Keskin *et al.*, 2017).

ROS plays an important role in cell signaling, controlling processes such as growth and development. They are very lethal and cause extensive damage to protein, DNA and lipids and thereby affect normal cellular functioning. Antioxidant machinery such as enzymatic components like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and non-enzymatic antioxidants like ascorbic acid, reduced glutathione, α -tocopherol, carotenoids, flavonoids and the osmolyte proline are present to ensure cell survival (Das and Roychoudhury, 2014).

Antioxidants are substances that remove, prevent or delay oxidative damage to a target molecule. Therefore, an antioxidant may act to control the level of free radicals to

counteract oxidative damage. Medicinal plants mostly combat oxidative stress with their antioxidant activities. Medicinal plants are rich sources of polyphenols, which might attribute to their antioxidant activity. There is a lot of evidence on the protective and curative effects of medicinal plants on various complications, which includes antimicrobial, anticancer, antidiabetic, anti-atherosclerotic, immunomodulatory, and even renoprotection or hepatoprotective effects (Baradaran *et al.*, 2014).

4.4.1 DPPH radical scavenging activity of crude extract, synthesized silver nanoparticles of aqueous and ethanolic extract of leaves of *Camellia sinensis*, *Durio zibenthinus*

DPPH radical scavenging assay is one of the most commonly used method for measuring antioxidant activity. DPPH is a stable free radical and has a strong absorption band in the range of 515-520nm. In the presence of antioxidants, the purple color of DPPH is decolourized or reduced to pale yellow. This is due to the transfer of hydrogen atom between DPPH radical and antioxidant molecules (Szerlauth *et al.*, 2019).

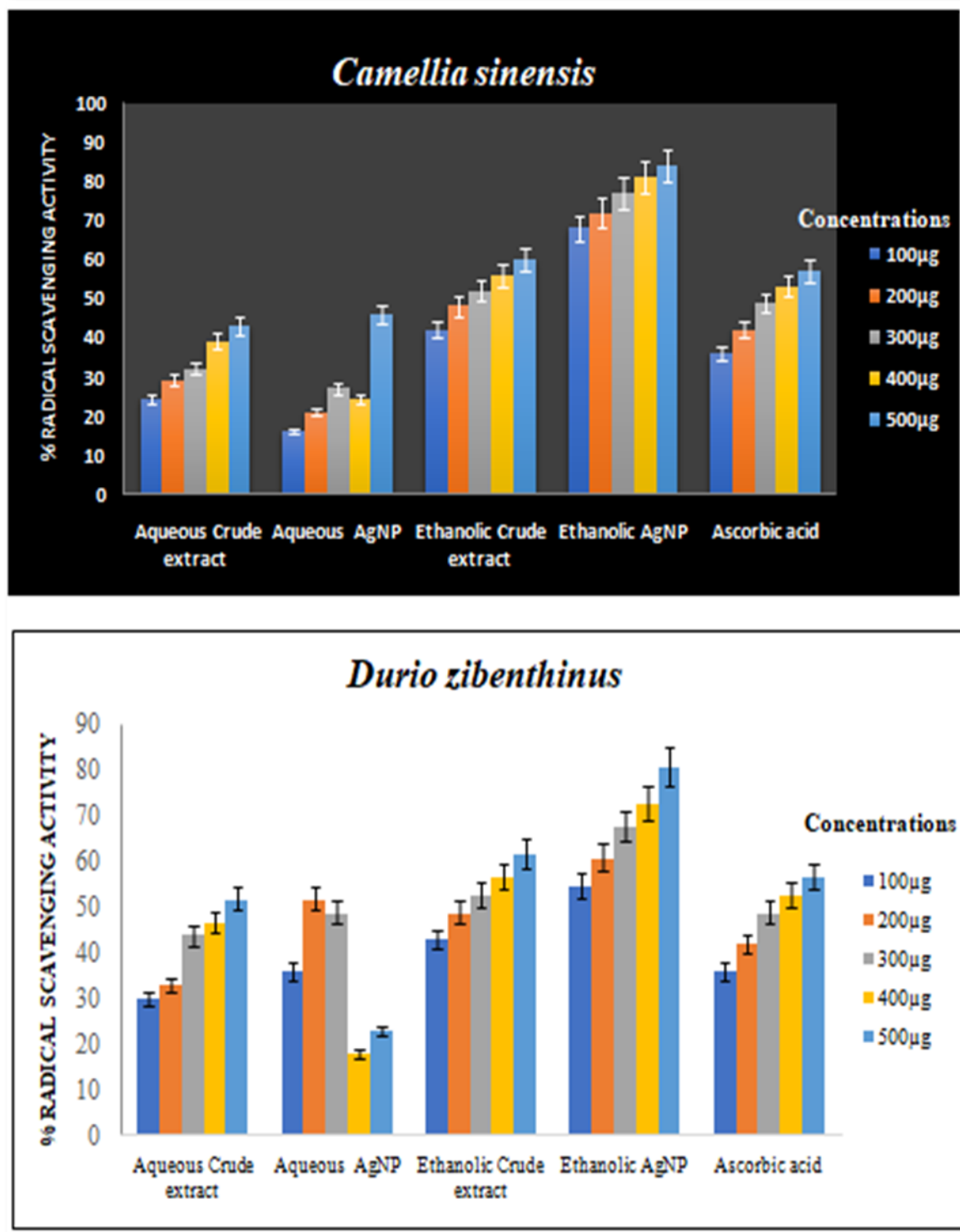
In the present study, upon addition of crude extract, synthesized silver nanoparticles (aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*) and ascorbic acid to the DPPH solution. There was a rapid decrease in the optical density at 515nm. The degree of discoloration indicated the scavenging potential of the extracts tested. The results of the DPPH assay is presented in Figure 23.

Based on the literature, the assay was performed using varying concentrations of crude extract and synthesized silver nanoparticles of aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus* ranging from 100, 200, 300, 400, 500 μg and ascorbic acid ranging from 100, 200, 300, 400, 500 μM .

The results revealed that crude extract, synthesized silver nanoparticles and Ascorbic acid scavenged DPPH radicals at all the concentrations tested. All the extracts were able to scavenge DPPH radical even at lower concentration of 100 μg and 100 μM . Upon increasing the concentration of the samples, the scavenging activity was also increased. However, the activity was maximum at 500 μg and 500 μM which indicates the dose dependent activity of crude extract, synthesized silver nanoparticles of aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus* and Ascorbic acid.

Figure 23

DPPH radical scavenging activity of the crude extract, synthesized silver nanoparticles of aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*



(The values are represented in \pm SD for duplication in each category)

Among the different extracts tested, the ethanol crude extract and *synthesized AgNPs* of both *Camellia sinensis* and *Durio zibenthinus* showed better scavenging activity compared to aqueous extracts, while ascorbic acid showed moderate activity. The results demonstrated that ethanolic crude extract and synthesized silver nanoparticles (ethanolic extract of *Camellia sinensis* and *Durio zibenthinus*) and ascorbic acid were able to neutralize the DPPH radical indicating the antioxidant potential. The synthesized silver nanoparticles from crude extracts with the mixture of phytoconstituents might be attributed for the strong scavenging potential exhibited by *Camellia sinensis* and *Durio zibenthinus*.

In line with our observations, Raj *et al.* (2016) reported that snake gourd seed, bottle gourd pulp and brinjal peel showed good radical scavenging activity among 10 vegetables tested while in fruit variety, litchi peel, passion fruit pulp and rose apple seed showed better scavenging activity against ascorbic acid as positive standard.

The aqueous and ethanolic extracts of *A.pierreana* and *M.denticulata* were prepared and tested for their antioxidant activity. The ethanolic extract of both *A.pierreana* and *M.denticulata* exhibited superior radical scavenging activity towards DPPH when compared to aqueous extract (Gudise *et al.*, 2019).

The antioxidant methods confirmed that the synthesized silver nanoparticles of *C.nocturnum* possess more radical scavenging activity as compared to the vitamin C (Keshari *et al.*, 2020).

The antioxidant activity of synthesized silver nanoparticle of spice blend formulation was evaluated by DPPH radical scavenging assay, while rutin used as a standard. The sample exhibited potential radical scavenging activity against DPPH in a dose dependent manner (Otunola *et al.*, 2017).

In accordance with these supporting studies, the free radical scavenging activity might be due to the presence of various phenols and other phytochemicals.

4.4.2 ABTS radical scavenging activity of the synthesized silver nanoparticles of aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus* Ascorbic acid

The ABTS assay is widely used method for the assessment of the antioxidant capacities of natural products, based on quenching of stable colored radicals (ABTS) and show the radical scavenging ability of antioxidants even when present in complex biological mixtures such as plant or food extracts. In the ABTS radical scavenging assay (an electron transfer-based assay), the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonate) radical cation (ABTS), which has a dark blue color, is reduced by an antioxidant into colorless ABTS (Sujarwo *et al.*, 2019).

In ABTS radical scavenging assay, the synthesized silver nanoparticles of ethanol extract of *Camellia sinensis* and *Durio zibenthinus* showed good scavenging activity towards ABTS radical. Varying concentrations ranging from 100 to 500 μ g of crude extracts and synthesized AgNPs and 100 to 500 μ M of ascorbic acid were used to test the scavenging potential. When the crude extract, synthesized AgNPs and ascorbic acid were allowed to react with ABTS radical, there was a change in the optical density, which was observed spectrometrically and the results are presented in Figure 24. The results revealed that at lower concentration, there was a moderate radical scavenging activity with the increase in concentration, the ABTS radical was scavenged to a greater extent. Among the groups tested, synthesized AgNPs (ethanolic extract of *Camellia sinensis* and *Durio zibenthinus*) showed significant antioxidant activity. The possessed antioxidant activity might be due to the transfer of electrons between the antioxidant compounds and ABTS radical.

In yet another recent report, the fruit pulp extracts of *Caryocar villosum* were analyzed for their antioxidant potential *in vitro* using ABTS assay. When compared, the shell extracts of the fruit showed better free radical scavenging capacity than the pulp or seed extracts (Yamaguchi *et al.*, 2000).

Lee *et al.* (2015) compared the antioxidative potential of flesh and peel of two Asian pears (*Pyrus pyrifolia* Nakai cv. *Niitaka* and *Chuhwangbae*). The results showed that the peels exhibited higher ABTS scavenging than the flesh. The high antioxidative

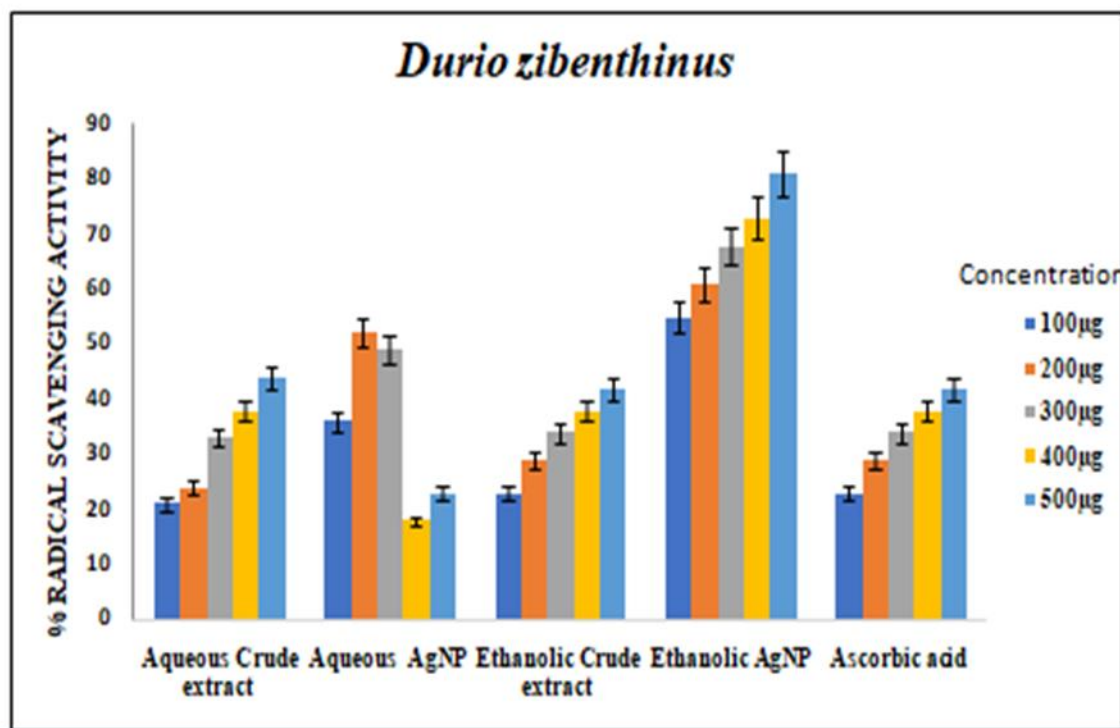
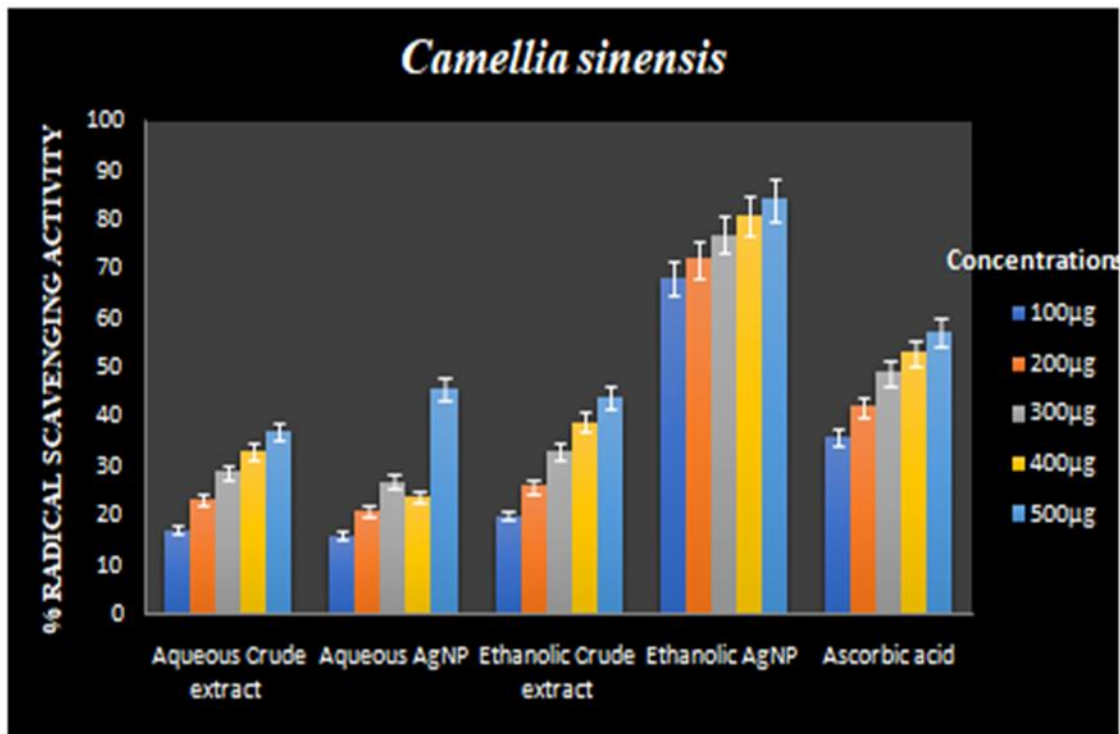
activities of the pear fruit peels were attributed to phenolic compounds including flavonoids.

The synthesized AgNPs from aqueous extract of *Erythrina suberosa* possessed highest scavenging activity when reacted with ABTS due to both lyophilic and hydrophilic constituents (Mohanta *et al.*, 2017).

All the assays confirmed significant antioxidant activity of green synthesized AgNPs which indicates the direct role of secondary metabolites namely, phenolic compounds, terpenoids and so on in removing exposed radicals. The aqueous and ethanolic extract of *D.bipinnata* showed good scavenging activity towards ABTS radical which might be due to the transfer of electrons between hydrophilic compounds and ABTS radical (Guntur *et al.*, 2018).

Figure: 24

ABTS radical scavenging activity of the synthesized silver nanoparticles of aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*



(The values are represented in \pm SD for duplication in each category)

In compliance with these cited literature, the result of the current investigation shows that the flavonoids present in the synthesized silver nanoparticles of ethanolic

extract of *Camellia sinensis* and *Durio zibenthinus* might be responsible for the ABTS scavenging potential.

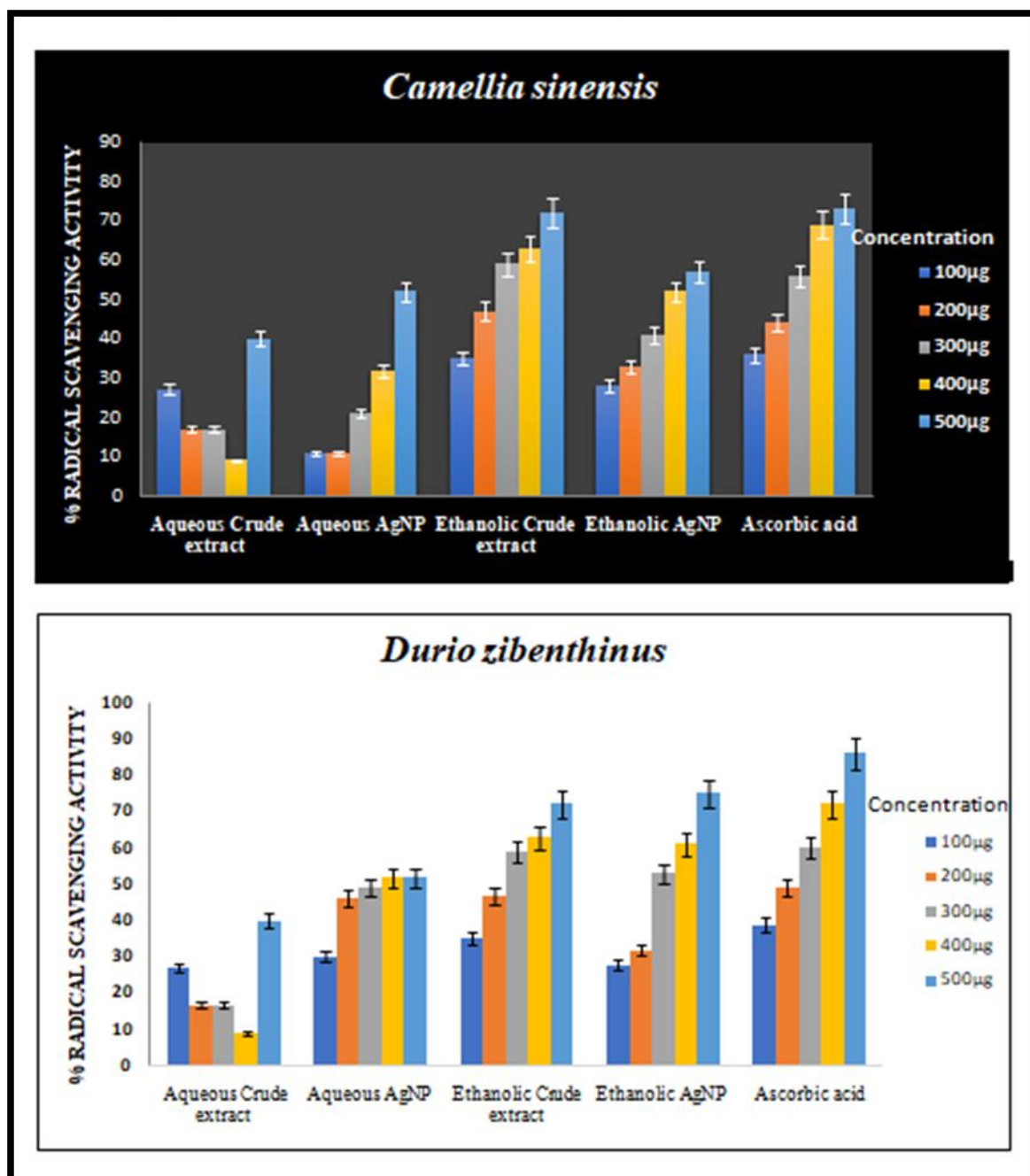
4.4.3 Reducing ability of the synthesized silver nanoparticle of aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Reducing power assay is based on reduction, caused by antioxidant, of ferric ions into ferrous ions, which is monitored by recording absorbance at 700nm. Dietary antioxidant such as ascorbic acid was used for comparison. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Benslama and Harrar, 2016).

The reducing capacity of the crude extract, synthesized AgNPs nanoparticle (aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*) and *Ascorbic acid* were measured by their ability to transform Fe^{3+} to Fe^{2+} at various concentrations. The results revealed that ethanolic crude extract, synthesized AgNPs nanoparticle (ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*) and *Ascorbic acid* were able to reduce Fe^{3+} ions even at a lower concentration of 100 μ g and 100 μ M, respectively. The activity increased with increase in the concentration of the extracts and ascorbic acid which indicates the dose dependent response of the extract groups and the results are given in Figure 25. Thus the result demonstrated that ethanolic crude extract, synthesized AgNPs nanoparticle (ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*) and *Ascorbic acid* possess good antioxidant potential when compared to aqueous extract.

Figure 25

Reducing ability of the synthesized silver nanoparticle of aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*



(The values are represented in \pm SD for duplication in each category)

In another study, the antioxidant activity of green synthesized AgNPs using *Prosopis farcta* fruit extract possessed better reducing activity due to the presence of high phenolic content in the extract (Salari *et al.*, 2019).

The ethanol extracts of *Caesalpinia bonducella seed* are subjected to the synthesis of AgNPs. *The samples* were tested for their ability to reduce Fe^{3+} to Fe^{2+} and the results revealed that the extracts exhibited good reducing capability, gained by the presence of polyphenolic compounds. These polyphenols are capable of chelating metal ions, such as iron and copper thereby avoids the formation of free radicals (Christobet *et al.*, 2020)

Polyphenols are the compounds with common flavone structure and their derivatives includes flavonoids, catechins, flavonones, isoflavones, anthocyanins, proanthocyanidins, anthocyanidins, phenolic acids, phenolic alcohols, polyphenolic amides, stilbenes, chalcones and lignans (Baradaran *et al.*, 2014).

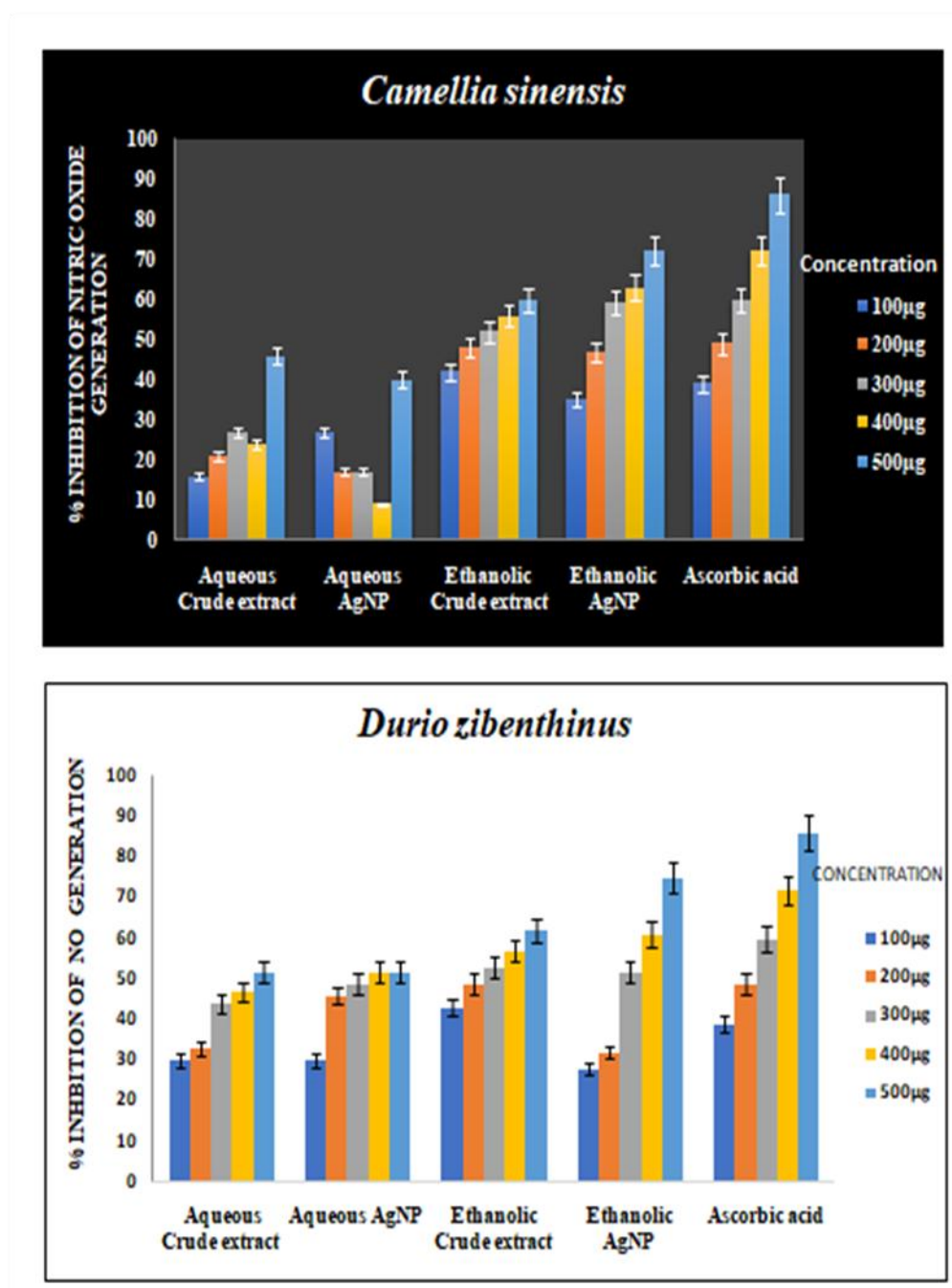
In concord with this report, the results of the present study showed that ascorbic acid and synthesized AgNPs of ethanolic extract of *Camellia sinensis* and *Durio zibenthinus* exhibited better reducing activity due to the presence of flavonoid compound. This might be able to directly interact with the ferric ions and reduces ferrous ions proving their antioxidant capacity to fight against radical mediated diseases.

4.4.4 Inhibition of nitric oxide generation by the synthesized silver nanoparticle of aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*

Nitric oxide (NO) is a signaling molecule that plays a significant role in the prolongation of inflammation and immunological responses. During inflammation, NO is released and sustained at very high levels, leading to inflammation of the joints, lung and gut. The synthesis and release of NO promotes inflammation; therefore, extracts that could act as scavengers of NO or inhibitors of its production, especially with corresponding low cytotoxicity, could be used to mitigate the propagation of inflammation by NO. The mechanism of action of the bioactive extracts or their constituents may be via the inhibition of the activity of inducible nitric oxide synthase or by free radical scavenging activities. Nitric oxide and singlet oxygen are among the free radicals that are involved in lipid peroxidation and implicated in the pathogenesis of chronic inflammation (Adebayo *et al.*, 2019)

Figure 26

Inhibition of nitric oxide generation by the synthesized silver nanoparticle of aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*



(The values are represented in \pm SD for duplication in each category)

The crude extract, green synthesized AgNPs (aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*) and Ascorbic acid were tested for their inhibitory potential against NO generation *in vitro* in the present study.

The synthesized AgNPs of ethanol extract of *Camellia sinensis* & *Durio zibenthinus* and ascorbic acid showed maximum inhibition, respectively at a concentration of 500µg and Ascorbic acid shows an inhibition at 500µM concentration. The crude extract, synthesized AgNPs (aqueous and ethanolic extract of leaves of *Camellia sinensis* & *Durio zibenthinus*) and ascorbic acid inhibited the generation of nitric oxide in a concentration dependent manner and the results are shown in Figure 26. When the results were compared, *ethanolic extract of Camellia sinensis and Durio zibenthinus* exhibited better inhibition against NO generation than the aqueous extract.

Nitric oxide is a pro-inflammatory mediator and is found to be involved in various physiological events. It is extremely important to defend the body. However, its overproduction leads to tissue damage and activation of pro-inflammatory mediators associated with acute and chronic inflammation (Njoya *et al.*, 2017).

Nitric oxide inhibition was performed with different concentrations of *Baccaurea ramiflora* fruit juice. The results showed that nitric oxide radical formed was significantly inhibited by the *Baccaurea ramiflora* fruit juice in a dose dependent manner (Saha *et al.*, 2009).

Silver nanoparticles of *Cestrum nocturnum* were tested for their antioxidant potential. Among the groups tested, the ethanolic extract inhibited the nitric oxide generation better than the other extracts (Keshari *et al.*, 2020).

The study on the antioxidant potential of the silver nanoparticle synthesized from spice blend formulations was studied. The results revealed that the synthesized AgNPs exhibited higher antioxidant activity for nitric oxide inhibition activity (Otunola *et al.*, 2018). In the present investigation, crude extracts render better inhibitory potential against NO generation. However, green synthesized nanoparticles could offer its inhibition only at a higher concentration of the plant extracts chosen for the study.

4.4.5 H₂O₂ scavenging activity of the synthesized silver nanoparticle of aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*

Hydrogen peroxide is a weak oxidizing agent and possibly inactivates a few enzymes by the oxidation of essential thiol groups. Hydrogen peroxide can cross cell membranes rapidly and react with Fe²⁺, and possibly Cu²⁺ ions, to form hydroxyl radicals,

which then become powerful oxidising agents. H_2O_2 is highly important because of its ability to penetrate into biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radicals in the cells. Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to H_2O_2 , thus neutralizing it to water (Saha and Verma, 2015).

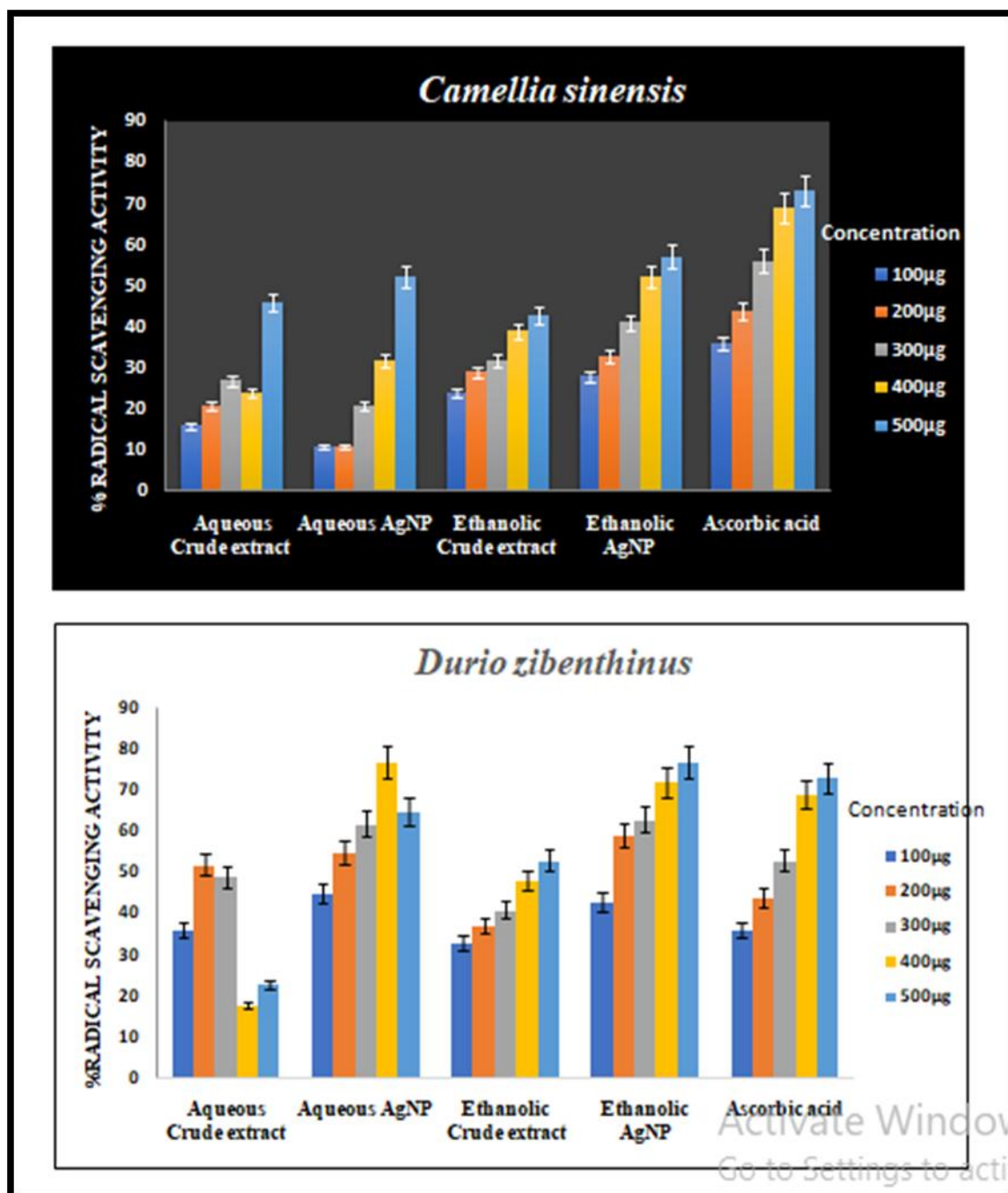
In the present study, crude extract, the green synthesized AgNPs (aqueous and ethanolic extract of leaves of *Camellia sinensis* & *Durio zibenthinus*) and Ascorbic acid were subjected to oxidative stress by adding H_2O_2 . The samples were able to scavenge H_2O_2 to a considerable extent. Among the samples tested, aqueous crude extract of *Camellia sinensis* and *Durio zibenthinus* possessed lower antiradical scavenging activity. The other extracts and ascorbic acid showed increased activity with increasing concentration. The results are provided in Figure 27. Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to H_2O_2 , thus neutralizing it to water. It is clearly evident from the results that synthesized silver nanoparticle and ascorbic acid were able to neutralize H_2O_2 to water to a considerable extent which confirms the enhanced antioxidant potential of the green synthesized plant extracts in comparison with the crude extracts of *Camellia sinensis* and *Durio zibenthinus*.

The antioxidant and antifungal activities of polyphenol-rich extracts of the driedfruit pulp of *Garcinia pedunculata* (GP) and *Garcinia morella* (GM) was evaluated (Sarma *et al.*, 2016). The results indicated that the cold water extracts of GP and GM were potent scavengers of H_2O_2 than the hot water extracts.

Three selected Tunisian cultivars of *Phoenix dactylifera* L. (Allig, Deglet, Nour, and Bejo) were analysed from their antioxidant activities using H_2O_2 scavenging assay. The extracts showed strong and concentration-dependent activity. The best antioxidant activity was obtained in Allig, followed by Bejo and Deglet Nou which can be positively correlated with the total phenolic and flavonoid content of the sample (Kchaou *et al.*, 2014).

Figure 27

H₂O₂ scavenging activity of the synthesized silver nanoparticle of aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*



(The values are represented in \pm SD for duplication in each category)

Similar results were observed in the present study also. The results clearly suggest the presence of phenols and flavonoids in the samples. Thus, the presence of significant amount of phenols and flavonoids might be attributed to the free radical scavenging potential.

From the above radical scavenging assays of crude extract and synthesized silver nanoparticles of ethanolic extract of *Camellia sinensis* and *Durio zibenthinus*, it can be inferred that the potential plant extracts could enhance their antioxidant potential by the green synthetic process as evidenced by the increased radical quenching ability acquired by the AgNPs of the plant extracts.

4.4.6 Hydroxyl radical scavenging activity of the synthesized silver nanoparticles of aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*

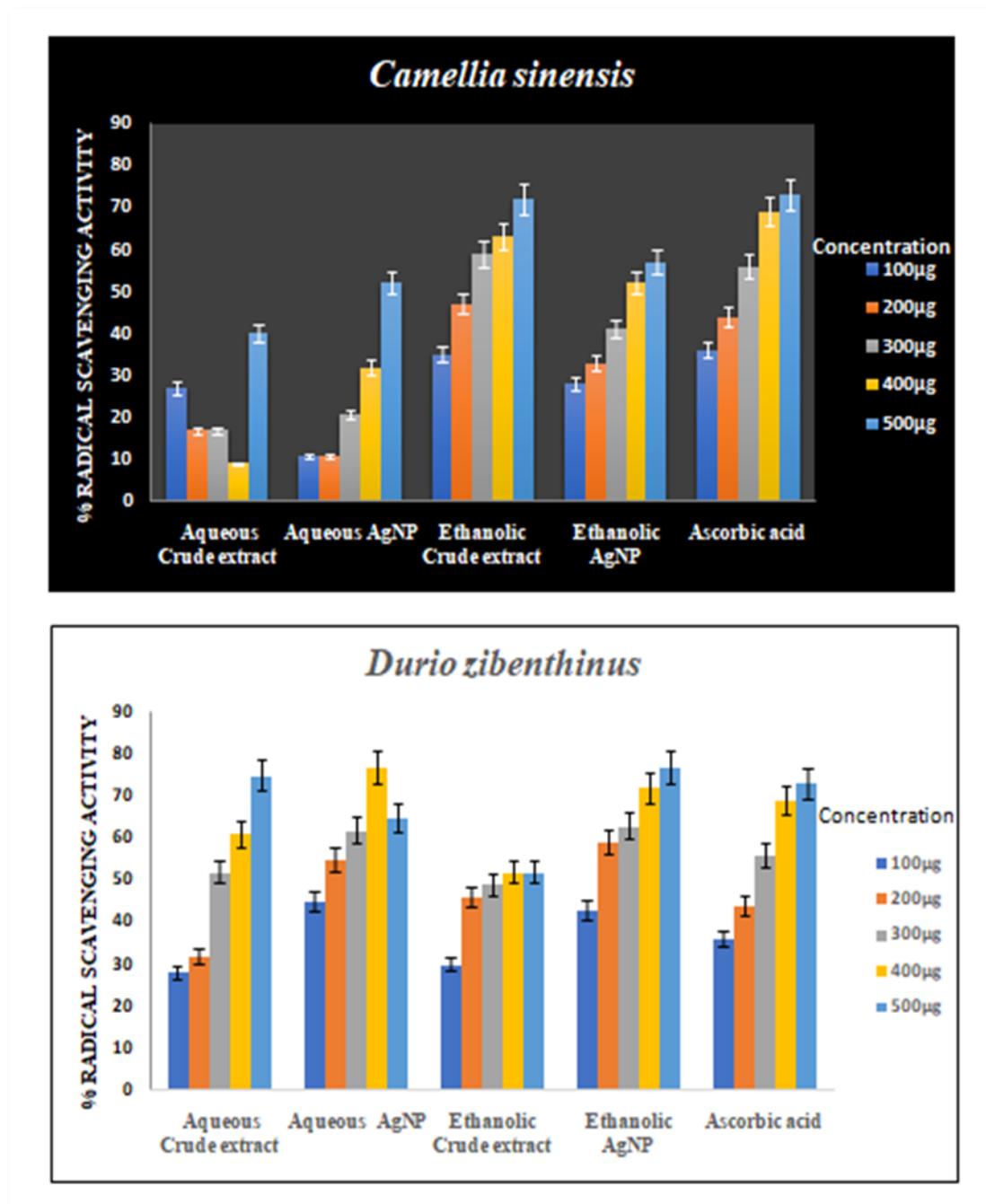
Hydroxyl radical is the most reactive oxygen centered species and causes severe damage to adjacent biomolecule. Hydroxyl radical scavenging activity was estimated by generating the hydroxyl radicals using ascorbic acid-iron EDTA. The hydroxyl radicals were formed by the oxidation reaction with the dimethylsulphoxide (DMSO) to yield formaldehyde, which provides a convenient method to detect hydroxyl radicals by treatment with Nash reagent (Pavithra and Vadivukarasi, 2015).

In hydroxyl radical scavenging assay, the crude extract, the synthesized silver nanoparticles possess better scavenging activity towards hydroxyl radical. Aqueous crude extract and synthesized AgNPs of *Camellia sinensis* and *Durio zibenthinus* showed lesser activity when compared with the other extracts. Ascorbic acid also showed good scavenging activity. Varying concentrations ranging from 100 to 500 μ g of synthesized AgNPs and 100 to 500 μ M Ascorbic acid were used to test the scavenging potential. When the samples were allowed to react with hydroxyl radical, there was a change in the optical density, which was observed spectrometrically and the results are presented in Figure 28. The results revealed that at lower concentration, there was a moderate radical scavenging with the increase in concentration, the hydroxyl radical was scavenged to a greater extent. Among the groups tested, ethanolic extract of both crude extract and synthesized AgNPs of *Camellia sinensis* and *Durio zibenthinus* showed significant antioxidant activity.

The possessed antioxidant activity might be due to the presence of hydrogen donating ability of secondary metabolites.

Figure 28

Hydroxyl radical scavenging activity of the synthesized silver nanoparticles of aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus*



(The values are represented in \pm SD for duplication in each category)

The ethanol, hydroethanol and aqueous extracts of *K.foetidissima* when added to the reaction mixture, they scavenge hydroxyl radicals in a concentration dependent manner. The scavenging of the hydroxyl radicals may be due to the presence of hydrogen donating ability of phenolic compounds in the extracts (Sasikumar and Kalaisezhiyan, 2019).

The AgNPs of *Lippia nodiflora* scavenged OH radical in a concentration dependent manner. The scavenging ability was significant at all tested concentrations with maximal inhibition. The experimental result of AgNPs to scavenge or inhibit the OH radical signifies that the AgNPs could significantly inhibit lipid peroxidation because OH radicals are well concerned in peroxidation process (Sudha *et al.*, 2017).

The synthesized silver nanoparticles from leaves of *Cardiospermum halicacabum* possess high radical scavenging activity. The scavenging activity of silver nanoparticles exhibit high inhibition due to the large surface area (Sundararajan *et al.*, 2016).

The antioxidant activity of synthesized AgNPs of *Cestrum nocturnum* was evaluated. The free and bound phenolic extract significantly scavenged hydroxyl radical which was comparable with standard ascorbic acid (Keshari *et al.*, 2020).

In the present study, ethanolic extracts of *Camellia sinensis* and AgNPs of *Durio zibenthinus* extracts were able to scavenge the hydroxyl radicals indicating the presence of phenol rich compounds in the plants.

4.5 Anticancer activity of the ethanolic extract of crude, synthesized silver nanoparticles and silver nanoparticles loaded nanoliposomes of *Camellia sinensis* and *Durio zibenthinus*

Cancer is one of the most life-threatening diseases, with more than 100 different types occurring due to some molecular changes within the cell. It is the third leading cause of death worldwide following cardiovascular and infectious diseases (Shaikh *et al.*, 2014).

Natural compounds are good sources for the development of new remedies for different diseases. Several medicinal plants and herbal ingredients have been reported to have anticancer effects. Phytochemicals constitute a heterogeneous set of bioactive

compounds classified by chemical structure and include polyphenols, alkaloids, carotenoids and nitrogen compounds that are naturally found in fruits, vegetables, grains and other plant products and are often responsible for distinct plant characteristics such as color pigmentation and smell. Moreover, many are integral for host protection against viruses, parasites and other externally damaging agents. Several studies have revealed that these compounds are able to affect cell proliferation and cell cycle regulation, and usually participate in multiple signaling pathways, which are often disrupted in tumor initiation, proliferation and propagation (Hosseini and Ghorbani, 2015).

4.5.1 Cytotoxic effect of the crude ethanolic extract, synthesized silver nanoparticles and silver nanoparticles loaded nanoliposomes of *Camellia sinensis* and *Durio zibenthinus* as determined by MTT assay

The MTT Assay is a sensitive, quantitative and reliable colorimetric assay which measures viability, proliferation and activation of cells. Viable cells are capable of reducing the yellow MTT under tetrazolium ring cleavage to a water-insoluble purple-blue formazan derivative which precipitates in the cellular cytosol and can be dissolved after cell lysis, whereas cells being dead following a toxic damage, cannot transform MTT. Thus the formation of formazan product is proportionate to the viable cell number and inversely proportional to the degree of cytotoxicity (Senthilraja and Kathiresan, 2015).

4.5.1.1 MTT Assay of *Camellia sinensis*

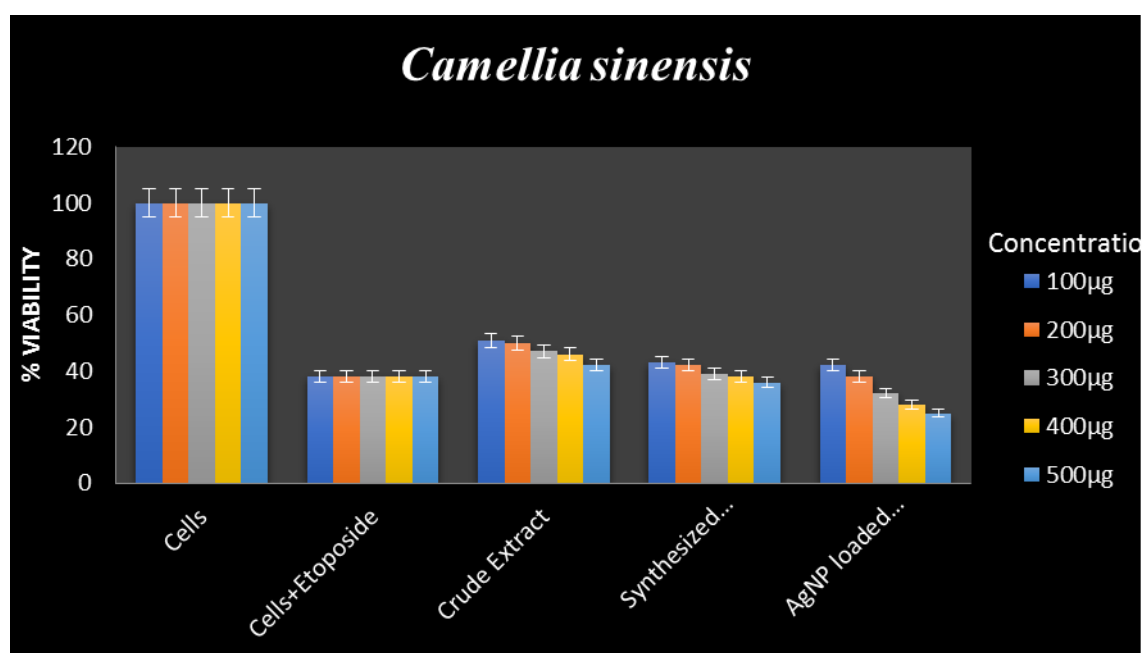
In order to determine the cytotoxic effect of the crude ethanol extract and synthesized AgNPs, and AgNPs loaded nanoliposomes of *Camellia sinensis*, MTT assay was performed in Molt -3 cell line. The experiment was carried out using varying concentrations of *Camellia sinensis* leaves ranging from 100, 200, 300, 400 and 500 µg.

The results revealed that the extracts of Molt-3 cell line and Etoposide were able to induce cell death even at low concentrations 100 µg. when molt-3 leukemia cell line was treated with increasing concentrations of crude extracts, the viability was found to be decreased further. Almost all the further variants of the *Camellia sinensis* extract tested (Crude extracts, AgNPs and nanoliposomes loaded AgNPs) showed better activity. Since the crude extract of *Camellia sinensis*, synthesised nanoparticles and AgNP loaded nanoliposomes enhance the cell death even at low concentrations. The percent viability of

the cells at a lower concentration of 100 μg were found to be 51% for crude extract of *Camellia sinensis*, 43% for synthesised silver nanoparticles and 42% for AgNPs loaded Nanoliposomes. The results revealed that leaves of *Camellia sinensis* were able to induce cell death and therefore can be used for the treatment of oxidative stress associated diseases including cancer. Further studies have to be carried out to confirm the results obtained.

Figure 29

Cytotoxic effect of crude ethanolic extract, synthesized silver nanoparticles and silver nanoparticles loaded nanoliposomes of *Camellia sinensis* by MTT assay



(The values are represented in \pm SD for duplication in each category)

4.5.1.2 MTT Assay of *Durain zibenthinus*

In order to determine the cytotoxic effect of the methanol extract of *Durain zibenthinus*, MTT assay was performed in molt -3 cell line. The experiment was carried out using varying concentrations of *Durain zibenthinus* ranging from 100, 200, 300, 400 and 500 μg .

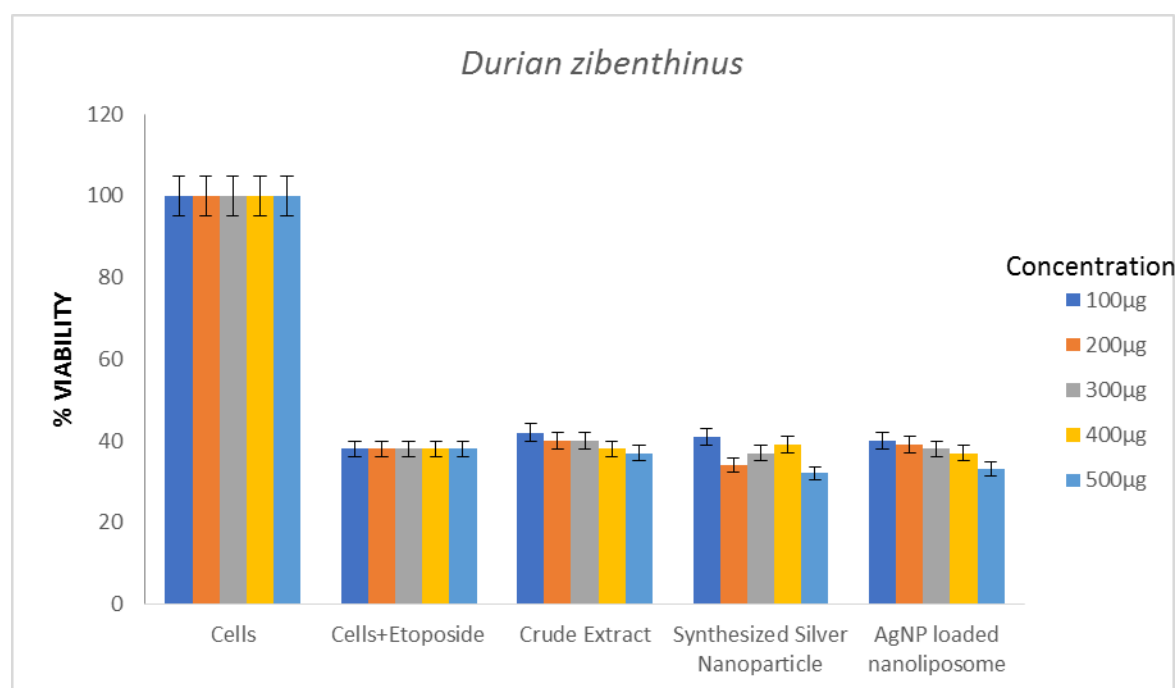
The viability of the control cells were fixed at 100% and the values of the other treatment groups were calculated accordingly. The results reveal that the ethanol crude extract, synthesized AgNPs and AgNPS loaded nanoliposomes were able to induce cell

death even at the lower concentration of 100µg as shown in Figure 30. When Molt-3 leukemic cell line was treated with increasing concentration of extracts, the viability was found to be decreased when compared to the control group. Further, all the three groups showed cytotoxic effect at its higher concentration (500µg). The per cent viability of the cells at a lower concentration of 100µg were found to be 52%, 41% and 40% for crude extract, AgNPs and AgNPs loaded nanoliposomes which was increased further with increasing concentration, indicating its dose dependent cytotoxicity.

Semsri *et al.* (2015) studied the cytotoxic effect of crude ethanolic extract of *Moringa oleifera* Lam. leaves on K562 (leukemic) cells using MTT assay. The results showed that that the crude ethanolic extract of *Moringa oleifera* leaves inhibited cell proliferation in a dose- and time-dependent manner thereby exhibiting distinct cytotoxic effect. The methanol and aqueous extracts of bark and fruits of *Zanthoxylum heitzii* were analyzed for their cytotoxic properties. Among the four extracts tested, only the methanol extract of fruits and barks significantly inhibited cell proliferation of HL-60 cells (Pieme *et al.*, 2014).

Figure 30

Cytotoxic effect of crude extract, synthesized silver nanoparticles and silver nanoparticles loaded nanoliposomes of *Durio zibenthinus* by MTT assay



(The values are represented in \pm SD for duplication in each category)

In vitro cytotoxic effect of methanol extract of *Withania coagulans* (WC) dried fruits (berries) was evaluated in human breast cancer cell line MDA-MB-231 and Vero cell line. The methanol extract of WC showed significant anticancer activity against MDA-MB-231 cell line but did not show any significant cytotoxic effect on the normal VERO cell line at concentrations that were cytotoxic to human breast cancer cells (Ahmad *et al.*, 2017). Hoshyar *et al.* (2015) investigated the alcoholic and aqueous extracts from the fruit of *Berberis vulgaris* for their cytotoxicity. Both extracts significantly inhibited proliferation of breast cancer cells (MCF-7) but were not toxic to normal human breast epithelial cells (MCF10-A).

The MTT assay confirms that the tumor inhibition rate of *Scutellaria baicalensis* loaded nanoliposomes was much higher than *Scutellaria baicalensis in vitro* and *in vivo*. Hence, the study suggests that *Scutellaria baicalensis* loaded nanoliposomes may serve as a promising approach for cancer therapy (Li *et al.*, 2014)

The study showed that *Nepeta deflersiana* AgNPs have the capacity of inducing apoptosis and necrosis cell death of HeLA cells through SubG1 cell cycle arrest confirmed by MTT assay and apoptotic assay. It suggest the anticancer potential of biosynthesized *Nepeta deflersiana* -AgNPs against human cervical cancer cells and could play an important role in the development of new therapeutic agent for the treatment of cancer (Al-Sheddi *et al.*, 2018).

Thus the results reveal that the variant preparations (Crude extracts, silver nanoparticles and nanoparticle loaded liposomes)of the candidate plant extracts were found to be suitable for therapeutic preparations against cancer.

4.5.2 Cytotoxic effect of the ethanol crude extract, synthesized silver nanoparticles and silver nanoparticles loaded nanoliposomes of *Camellia sinensis* and *Durio zibenthinus* as determined by SRB assay

The sulforhodamine B assay is a technique used for measuring cell mass. This assay measures the cell growth by direct quantification of total protein mass but not

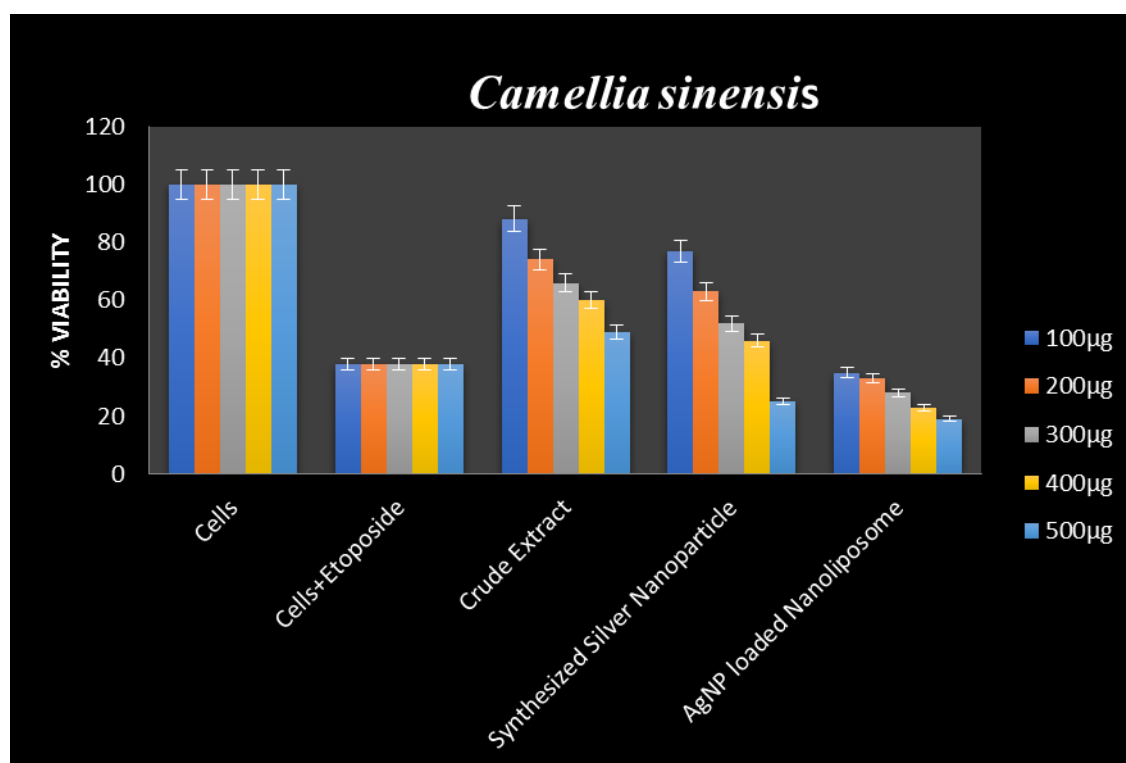
by relying on the direct determination of the metabolic function or activity of living cells. It is based on binding of the SRB dye to basic amino acids in cellular proteins which can be measured spectrophotometrically (Silva *et al.*, 2016).

4.5.2.1 SRB Assay of *Camellia sinensis*

In SRB assay, the results demonstrated that all the three different extract preparations exhibited significant cytotoxicity effect against molt-3 leukemia cell line. When molt -3 leukemia cell were treated with Etoposide alone, cell viability declined. When the cells were treated with different concentrations of crude extract of *Camellia sinensis*, it induced cell death as shown in Figure 31. Among the group tested, AgNPs loaded nanoliposomes of *Camellia sinensis* rendered good toxicity followed by synthesised silver nanoparticles and crude extracts. Thus, the results indicated that the AgNPs loaded nanoliposomes of *Camellia sinensis* showed a higher cell death even at lower concentration.

Figure 31

Cytotoxic effect of crude ethaolic extract, synthesized silver nanoparticles and silver nanoparticles loaded nanoliposomes of *Camellia sinensis* by SRB assay



(The values are represented in \pm SD for duplication in each category)

4.5.2.2 SRB Assay of *Durio zibenthinus*

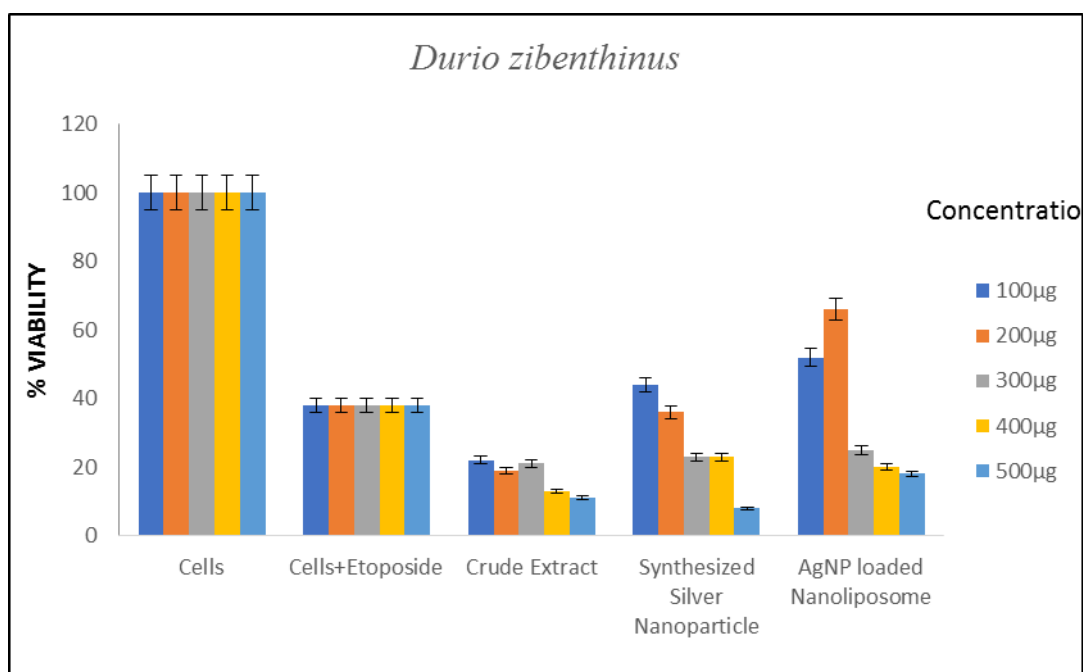
In SRB assay, the results demonstrated that all the three different extract preparations exhibited low cytotoxicity effect against molt-3 leukemia cell line. When molt -3 leukemia cell were treated with Etoposide alone, cell viability declined as shown in Figure 23. When the cells were treated with different concentrations of extracts of *Durio zibenthinus* induced cell death. Among the groups tested, crude extract of *Durio zibenthinus* showed comparatively higher cell death even at lower concentration. The data altogether indicates that *D. zibenthinus* crude extract has profound effect in controlling molt-3 cell line proliferation.

MCF-7, MDA-MB-231, SKOV-3 cancer cell lines and normal mammary epithelial cells (MCF-10A) were treated with hexane, chloroform, ethyl acetate and methanol extracts of two mango varieties (*Mangifera indica*) to evaluate cytotoxic effects by SRB assay. The methanol extracts showed less cytotoxicity to normal mammary epithelial cells but exhibited antiproliferative effects against all three cancer cell lines in a dose-dependent manner (Ediriweera *et al.*, 2016).

Chaudhary *et al.* (2017) observed that the leaf extract of *Memecylon umbellatum* was found to possess significant dose-dependent cytotoxicity against colon carcinoma cells (HCT 116) in addition to selectivity towards cancer cells as compared to normal cells (Vero).

Figure 32

Cytotoxic effect of ethanolic crude extract, synthesized silver nanoparticles and silver nanoparticles loaded nanoliposomes of *Durio zibenthinus* by SRB assay



(The values are represented in \pm SD for duplication in each category)

Anticancer potential of silver nanoparticles biosynthesized from *Olea chrysohylla* and *Lavandula dentate* leaf extracts were investigated against HCT 116 colon cancer cells by SRB assay. The results showed that *Lavandula dentate* showed higher antiproliferative potential compared to *Olea chrysohylla* (Sufyani *et al.*, 2019). HeLa cells were treated with aqueous extract of *Salvia aramiensis* loaded nanoliposomes to evaluate cytotoxic effects by SRB assay. The results were found to possess significant dose-dependent cytotoxicity (Karatoprak *et al.*, 2020)

Thus, The results of both the cytotoxic assays suggested the presence of various bioactive components including phenols in the crude extracts and synthesized silver nanoparticles and nanoparticles loaded nanoliposomes. Hence, presence of these phenols and other secondary metabolites might be responsible for protective effect rendered by the extract against leukemic (Molt-3) cells. The above findings strongly hold up our results that crude extracts and synthesized silver nanoparticles and nanoparticles loaded

nanoliposomes shows selective cytotoxicity towards Molt-3 leukemic cells. Nanoparticles loaded nanoliposomes of *Camellia sinensis* showed better activity compared to other extracts, which might be due to the potent drug delivery property of nanocarriers, prepared using *Camellia sinensis*. However an alternate green synthetic procedure can be opted for *Durio zibenthinus* as the incorporation of silver in the AgNPs of *Durio zibenthinus* in the present method were found to be less compared to *Camellia sinensis*. Therefore, it can be concluded that AgNPs loaded nanocarriers, prepared using *Camellia sinensis* rendered good response and hence can be much suitable candidate for pharmaceutical preparations used for the treatment of oxidant mediated diseases including cancer.

5.0 SUMMARY AND CONCLUSION

Free radicals are unstable molecules, which contains an unpaired electron spinning on the outermost layer around the nucleus. Due to the excessive production of reactive species, induced by exposure to external oxidants or a failure in the defense mechanisms, damage to cell structures, DNA, lipids and proteins occur which increases the risk of different diseases. These radical reactions are implicated in the pathology of many human diseases including atherosclerosis, ischemic heart disease, ageing, hepatotoxicity, inflammation, diabetes, immunosuppression, and neurodegenerative conditions.

Antioxidants are compounds that scavenge these free radicals by accepting or donating a pair of electrons and make them less reactive. A redox balance between the free radicals and antioxidants should be maintained. If this is not maintained either due to decreased antioxidant content or excessive free radicals, oxidative stress condition prevails, which is involved in the pathogenesis of various diseases including cancer.

Cancer is one of the most fatal diseases in human population and one of the most frequent causes of death worldwide. Cancer is caused by excessive free radical damage, which ultimately causes damage to the DNA, protein and lipids. This process of DNA damage leads to mutations that cause normal cells to transform into a cancer cells. To prevent cancer, synthetic and natural sources are used either alone or in combination. Interest in the pharmacological effects of bioactive compounds on cancer treatments and prevention has increased dramatically over the past two decades.

Medicinal plants are the important source of antioxidants that contain organic compounds which are essential for health. This bioactive substance includes tannins, carbohydrates, alkaloids, flavonoids, terpenoids, phenolics, glycosides and saponins. Alkaloids constitute a wide variety of nitrogenous compounds which could be quenchers of singlet oxygen. They are usually found in plant sources. Flavonoids are a group of natural products with many biological and pharmacological activities like antibacterial, antiviral, antioxidant, anti-inflammatory and antimutagenic effects. Phenolic compounds, terpenoids and tannins are widely present in food and nutraceuticals which possess

biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation and anti-atherosclerosis. More studies have been reported on the plant extracts and their isolated compounds which protects against free radical induced oxidative damage. They are effective antioxidants for improving human health and prevent carcinogenesis, aging and cardiovascular diseases.

Nanotechnology has been playing a prominent role in the nanomedicine. Bionanomedicine have enormous potential to positively impact the health care system. People all over world are increasing their attention to natural products and researchers looking for new leads to develop better drugs against various diseases. The *Camellia sinensis* and *Durio zibenthinus* contains phytochemical constituents such as phenols, alkaloids, flavonoids, terpenoids, tannins and steroids in the aqueous and ethanol extract.

The inference from the results of the present study can be *Camellia sinensis* and *Durio zibenthinus* possess ability to reduce Ag^+ to Ag^0 , which was visualised by the colour change from green to reddish brown in both the extracts. The change in color is due to the excitation of Surface Plasmon Resonance. Thereby, it confirms green synthesized nanoparticles using *Camellia sinensis* and *Durio zibenthinus*. The formation of spherical nanoparticles by both the *Camellia sinensis* and *Durio zibenthinus* ethanolic extracts is evident from the UV-Vis spectral peak obtained at 420nm and 450nm respectively. In compliance with these results, the synthesized AgNPs of ethanolic extract of *Camellia sinensis* and *Durio zibenthinus* were used for further characterization and analysis.

The SEM and EDX results showed that the plant extracts were able to support the nanoparticles formation. *Camellia sinensis* plant extract readily supported with the Ag component 36.32% weight as against 6% weight by *Durio zibenthinus*. However both the plant extracts *Camellia sinensis* and *Durio zibenthinus* were able to synthesize spherical nanospheres in the range of 28-44nm and 2.50 - 56.70 nm respectively.

The FT-IR spectral results of *Camellia sinensis* and *Durio zibenthinus* confirms the presence of non-conjugated aromatic compounds such as alcohol or phenol. Further analysis need to be carried out to understand and elucidate the extract nature and structure of the green synthesized silver nanoparticles. The XRD results inferred that the *Camellia*

sinensis could show the Face centered cubic structure as per the facets of silver. However, *Durio zibenthinus* could show only simple cubic structure.

The DPPH results revealed that crude extract, synthesized silver nanoparticles and Ascorbic acid scavenged DPPH radicals at all the concentrations tested. All the extracts were able to scavenge DPPH radical even at lower concentration of 100 μ g and 100 μ M. Upon increasing the concentration of the samples, the scavenging activity was also increased. However, the activity was maximum at 500 μ g and 500 μ M which indicates the dose dependent activity of crude extract, synthesized silver nanoparticles of aqueous and ethanolic extract of leaves of *Camellia sinensis* and *Durio zibenthinus* and Ascorbic acid. The study shows that the free radical scavenging activity might be due to the presence of various phenols and other phytochemicals. The result of the ABTS scavenging assay shows that the flavonoids present in the synthesized silver nanoparticles of ethanolic extract of *Camellia sinensis* and *Durio zibenthinus* might be responsible for the ABTS scavenging potential. The results of the present study showed that ascorbic acid and synthesized AgNPs of ethanolic extract of *Camellia sinensis* and *Durio zibenthinus* exhibited better reducing activity due to the presence of flavonoids which might be able to directly interact with the ferric ions and reduces ferrous ions proving their antioxidant capacity to fight against radical mediated diseases. The crude extracts render better inhibitory potential against NO generation. However, green synthesized nanoparticles could offer its inhibition only at a higher concentrations of the plant extracts chosen for the study.

The radical scavenging of crude extract and synthesized silver nanoparticles of ethanolic extract of *Camellia sinensis* and *Durio zibenthinus*, inferred that the potential plant extracts could enhance their antioxidant potential by the green synthetic process as evidenced by the increased radical quenching ability acquired by the AgNPs of the plant extracts. The ethanolic extracts of *Camellia sinensis* and AgNPs of *Durio zibenthinus* extracts were able to scavenge the hydroxyl radical indicating their presence of phenol rich compounds in the plants.

The results of MTT and SRB cytotoxic assays suggested the presence of various bioactive components including phenols in the crude extracts and synthesized silver nanoparticles and nanoparticles loaded nanoliposomes . Hence, presence of these phenols

and other secondary metabolites might be responsible for the protective effect rendered by the extract against leukemic (Molt-3) cells. The above findings strongly hold up our results that crude extracts of the candidate plants, synthesized silver nanoparticles and nanoparticles loaded nanoliposomes show cytotoxicity towards Molt-3 leukemic cells. Nanoparticles loaded nanoliposomes of *Camellia sinensis* showed better activity compared to other extracts, which might be due to the potent drug delivery property of nanocarriers, prepared using *Camellia sinensis*. However an alternate green synthetic procedure can be opted for *Durio zibenthinus* as the incorporation of silver in the AgNPs of *Durio zibenthinus* in the present method were found to be less compared to *Camellia sinensis*. Therefore, it can be concluded that AgNPs loaded nanocarriers, prepared using *Camellia sinensis* rendered good response and hence can be much suitable candidate for pharmaceutical preparations used for the treatment of oxidant mediated diseases including cancer.

BIBLIOGRAPHY

- ✍ Ahmad, A., Ghouri, M.Z., Khan, Z.N. and Khan, H. (2020), Nanotechnology: Transformation of agriculture and food security, American journal of biotechnology and bioscience. 3:19, 1-20.
- ✍ Ahmad, R., Fatima, A., Srivastava, A.N. and Khan, M.A. (2017), Evaluation of apoptotic activity of *Withania coagulans* methanolic extract against human breast cancer and Vero cell lines, Journal of ayurvedic integrated medicine.
- ✍ Ahmed, S., Saifullah., Ahmad, M., Swami, B. L. and Ikram, S. (2016), Green synthesis of silver nanoparticles using *Azadirachata indica* aqueous leaf extract, Journal of radiation research and applied sciences, 9: 1-7.
- ✍ Al-Sheddi, E. S., Al-Zaid, N. A., Al-Oqail, M. M., Al-Massarani, S. M., El-Gamal, A. A., & Farshori, N. N. (2019). Evaluation of cytotoxicity, cell cycle arrest and apoptosis induced by *Anethum graveolens* L. essential oil in human hepatocellular carcinoma cell line. Saudi pharmaceutical journal, 27(7), 1053-1060.
- ✍ Ashokkumar, R. and Ramaswamy, M. (2014), Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian medicinal plants, Journal of Current Microbiology and Applied Sciences, 3(1): 395-406.
- ✍ Aslam, M.S. and Ahmad, M.S. (2016), Worldwide importance of medicinal plants current and historical perspectives, Recent advances in biology and medicine, 2:88-93.
- ✍ Aziz. N.A.A. and Jalil, A.M.M. (2019), Bwacive compounds, Nutritional value and potential health benefits of indigenous durian: Review, Recent developments in bioactive molecules evaluation, 9(9):785.
- ✍ Barrios, V. A. E., Mendez, J. R. R., Aguilar, N. V. P., Espinosa, G. A. and Rodríguez, J. L. D. (2012), FTIR-An Essential Characterization Technique for Polymeric Materials. In Infrared Spectroscopy-Materials Science, Engineering and Technology. Intech Open, 196-211.

- ✍ Bhattacharya, P., Lin, S., Turner, J. and Ke, P. C. (2010), Physical adsorption of charged plastic nanoparticles affects algal photosynthesis, *The Journal of physical chemistry*, 114: 16556.
- ✍ Brain, K.R. and Turner, T.D. (1975), Practical evaluation of phyto pharmaceuticals, *Wrightsciencetchnia*,144.
- ✍ Chambers, E.S., Viardot, A., Psichas, A., Morrison, D.J., Murphy, K.G., Zac-Varghese, S.E.K., McDougall, K., Preston, T., Tedford, C. and Finlayson G.S. (2015), Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults, *Gut*, 64:1744-1754.
- ✍ Chaudhary, S., Chandrashekar, K.S., Pai, K.S.R., Setty, M.M., Devkar, R.A., Reddy, N.D. and Biswas, S. (2017), Screening of Anticancer Activity of Selected Medicinal Plants Indigenous to Western Ghats: *Argyreia nervosa*, *Memecylon malabaricum* and *Memecylon umbellatum*, *Advanced science*, 23, 1781-1784.
- ✍ Chauhan,R.,Yadav,H.O.S and sehrawat,N.(2020),Nanobioremediation:Anem and a versatile tool for sustainable environmental clean up-overview,*Journal of materials and environmental sciences*, 11:4
- ✍ Chin S.T., Nazimah S.A.H., Quek S.Y., Man Y.C., Rahman R.A., Dzulkifly M.H. Changes of volatiles' attribute in durian pulp during freeze- and spray-drying process. *Int. J. Food Sci. Technol.* 2008;41:1899-1905
- ✍ Chin, S.T., Nazimah, S.A.H., Quek, S.Y., Man, Y.B.C., Rahman, R.A. and Hashim, D.M. (2007), Analysis of volatile compounds from Malaysian durians using headspace SPME coupled to fast GC-MS. *Journal of Food Compost Anal*, 20:31-44.
- ✍ Conde, J., Doria, G. and Baptista, P. (2012), Noble metal nanoparticles applications in cancer, *Journal of drug delivery*, 751075.
- ✍ Costa C., Tsatsakis A., Mamoulakis C., Teodoro M., Briguglio G., Caruso E., Tsoukalas D., Margina D., Efthimious D., Kouretas D., et al. Current evidence on the effect of dietary polyphenols intake on chronic diseases. *Food Chem. Toxicol.* 2017;110:286-299.

- ✍ Din, F., Aman, W., Ollah, I., Qureshi, O.S., Mustapha, O., Shafique, S. and Zeb, A. (2017), Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors, *International journal of Nanomedicines*, 12:7291-7309.
- ✍ Ediriweera, M.K., Tennekoon, K.H., Samarakoon, S.R., Thabrew, I and de Silva, E.D. (2016), Cytotoxic and Apoptotic Effects of the Bark of Two Common Mango (*Mangifera indica*) Varieties from Sri Lanka on Breast and Ovarian Cancer Cells, *Journal of Pharmacological Research*, 10, 1-7.
- ✍ El-Nour, K. M. A., Eftaiha, A. A., Al-Warthan, A. and Ammar, R. A. (2010), Synthesis and applications of silver nanoparticles, *Arabian Journal of Chemistry*, 3(3):135-140.
- ✍ Emeka, E.E., Uzoh, I., Babalola, O.O. and Onwudiwe, D. (2019), The role of nanotechnology in the fortification of plant nutrients and improvement of crop production, *Applied sciences*, 9:499.
- ✍ Evans, W. C. (1996), *Grease and Evans pharmacognosy*, WB sunders, 119-159.
- ✍ Evans, W. C. (1996), *Grease and Evans pharmacognosy*, WB sunders, 119-159.
- ✍ Ganapathy, S. and Karpagam, S. (2016) *In vitro* antibacterial and phytochemical potential of *Aegle marmelos* against multiple drug resistant (MDR) *Escherichiacoli*, *Journal of Pharmacognosy and Phytochemistry*, 5, 253-255.
- ✍ Genskowsky, E., Puente, L.A., Pérez-Álvarez, J.A., Fernández-López, J., AMuñozc, L. and Viuda-Martos, M. (2016), Determination of polyphenolic profile, antioxidant activity and antibacterial properties of maqui [*Aristotelia chilensis* (Molina) Stuntz] a Chilean blackberry, *Journal of Science and Food Agriculture*, 96, 4235-4242.
- ✍ Gul, H., Ahmad, M., Sarwar, N., Abbasi, S.K., Minhas, N.M., Jabeen, N., Ahmad, M.S. and Gulfraz, M. (2014) Phytochemical analysis and biological activities of *Diospyros lotus* L. fruit extracts, *International Journal of Pharmacy and Chemistry*, 4, 155-159.
- ✍ Harbone, J. B. (1998), *Phytochemicals methods, A guide to modern techniques of plant analysis*, Chapman and Hall.
- ✍ Harder, A. and Kang, I. (2015) Preparation of silver nanoparticles and their industrial and biomedical applications: A comprehensive review, *Advances in materials science and engineering*, 3:1-16.

- ✍ Harika, P., Kannamba, B., Ramana, G.V. and Haribabu, B. (2017) Effect of solvent composition on total phenol and flavonoids content of *Withaniasomnifera*, Journal of Chemical and Pharmaceutical Science, 10, 601-603.
- ✍ Hayakawa, S., Ohishi, T., Miyoshi, N., Oishi, Y., Nakamura, Y. and Isemura, M. (2020), Anticancer effects of green tea Epigallocatechin - 3- gallate and Coffee chlorogenic acid, Molecules, 25, 4553
- ✍ He, D., Dorantes-Aranda, J. J., and Waite, T. D. (2012). Silver nanoparticle-algae interactions: oxidative dissolution, reactive oxygen species generation and synergistic toxic effects. *Environ. Sci. Technol.* 46, 8731-8738.
- ✍ He, X., Deng, H. and Hwang, H. (2019), The current application of nanotechnology in food and agriculture, Journal of food and drug analysis, 27: 1-21.
- ✍ Ho, L. and Bhat, R. (2015), Exploring the potential nutraceutical values of durian (*Duriozibethinus* L.)—An exotic tropical fruit. Food Chemistry, 168:80-89.
- ✍ Hodoroaba, V. D., Rades, S., Salge, T., Mielke, J., Ortel, E. and Schmidt, R. (2016). Characterisation of nanoparticles by means of high-resolution SEM/EDS in transmission mode, In IOP Conference Series: Materials Science and Engineering, 109(1): 012006.
- ✍ Hoshyar, R., Mahboob, Z. and Zarban, A. (2015), The antioxidant and chemical properties of *Berberis vulgaris* and its cytotoxic effect on human breast carcinoma cells, Cytotechnology.
- ✍ Hossen, S., Hussain, M. k., Basher, M. K., Mia, M. N. H., Rahman, M. T. and Uddin, M. J. (2019), Smart nanocarrier - based drug delivery systems for cancer therapy and loxicity studies: A review, Journal of advanced research, 15: 1-18.
- ✍ Husin, N.A., Rahman, S., Karunakaran, R. and Bhore, S. J. (2018), A review on the nutritional, medicinal, molecular and genome attributes of Durian (*Duriozibenthinus*), King of Fruits in Malaysia, Bioinformation, 14(6).
- ✍ Igarashi, M. And Miyazawa, T. (2001) The growth inhibitory effect of conjugated linoleic acid on a hepatoma cell line Hep G2, is induced by a change in fatty acid in cells, Biochem. Biophys. Acta/ Molecular and Cell biol. lipids, 1530, 162-171.
- ✍ Jyoti, K., Baunthiyal, M. and Singh, A. (2016), Characterization of silver nanoparticles synthesized using *Urtica dioica* Linn. leaves and their synergistic

- effects with antibiotics, *Journal of Radiation Research and Applied Sciences*, 9(3): 217-227.
- ✍ Karatoprak, G.S, K peli Akkol, E., Gen, Y., Bardakc , H., Y cel, . and Sobarzo-S nchez, E. (2020), Combretastatins: An Overview of Structure, Probable Mechanisms of Action and Potential Applications, *Molecules*, 25, 2560.
 - ✍ Kaul, A., Chaturvedi, S., Attri, A., Kalri, M. and Mishra, A. K. (2016) Targeted theranostic liposomes: rifampicin and ofloxacin loaded pegylated liposomes for theranostic application in mycobacterial infections, *RSC advances*, 34.
 - ✍ Kchaou, W., Abb s, F., Attia, H. and Besbes, S. (2014), *In Vitro* Antioxidant Activities of Three Selected Dates from Tunisia (*Phoenix dactylifera* L.), *Journal of Chemistry*.
 - ✍ Keshari, A. K., Srivastava, R., Singh, P., Yadav, V. B. and Nath, G. (2020), Antioxidant and antibacterial activity of silver nanoparticles synthesized by *Cestrum nocturnum*, *Journal of Ayurveda and integrative medicine*.1-8.
 - ✍ Khalil, M. M. H., Ismail, E.H., El-Baghdady, K.Z. and Mohamed, D. (2014), Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity. *Arabian Journal of Chemistry* 7(6):1131-1139
 - ✍ Khan,I.,Saeed,K. and Khan,I.(2019),Nanoparticles:Properties,applications and toxicities,*Arabian journal of chemistry*,12:908-931
 - ✍ Khidir, A. M. and Saeed, A. A. (2020), Ligand targeted liposomes, *Health and Primary care*, 2515-107X.
 - ✍ Klein S.M., Cohen G., Cederbaum A.I. Production of formaldehyde during metabolism of dimethyl sulphoxide by hydroxyl radical generating system. *Biochemistry*. 1991;20:6006-6012.
 - ✍ Krithiga, N., Rajalakshmi, A. and Jayachitra, A. (2015), Green synthesis of silver nanoparticles using leaf extracts of *Clitoria ternatea* and *Solanum nigrum* and study of its antibacterial effect against common nosocomial pathogens, *Journal of Nanoscience*, 1-8.
 - ✍ Kulkarni, N. and Muddapur, U. M. (2014), Biosynthesis of metal nanoparticles: A review, *Journal of Nanotechnology*, 416-430.
 - ✍ Lee, S.H, Cho, J.Y., Jeong, H.Y., Jeong, D.E., Kim, D., Cho, S.Y., Kim, W.S. and Moon JH. (2015) Comparison of bioactive compound contents and *in vitro* and *ex*

- vivo* antioxidative activities between peel and flesh of pear (*Pyrus pyrifolia* Nakai), Food Science and Biotechnology, 24(1): 207-216.
- ✍ Li, S., Chen, G., Zhang, C., Wu, M., Wu, S. and Liu, Q. (2014), Research progress of natural antioxidants in foods for the treatment of diseases, Food Science and Human Wellness, 3, 110-116.
 - ✍ Lin, P. C., Lin, S., Wang, P. C. and Sridhar, R. (2014), Techniques for physicochemical characterization of nanomaterials, Biotechnology Advances, 32(4): 711-726.
 - ✍ Logeswari, P., Silambarasan, S. and Abraham, J. (2015), Synthesis of silver nanoparticles using plant extract and analysis of their antimicrobial property, Journal of Saudi chemical society, 19: 311-317.
 - ✍ Logothetidis, S. (2012), Nanotechnology: Principles and applications, Nanoscience and technology, 59:1.
 - ✍ Luo, Q., Zhang, J.R., Li, H.B., Wu, D.T., Geng, F., Corke, H., Wei, X.L. and Gan, R.Y. (2020), Green extraction of antioxidant polyphenols from green tea (*Camellia sinensis*).
 - ✍ Lyubchyk, S., Shapovalova, O., Oliveira, M.C., Appazov, N., Lyubchyk, A., Charmier, A. J., Lyubchik, S. and Pombeiro, A.J.L. (2019), Integrated green chemical approach to the medicinal plant *Carpobrotus edulis* processing, Scientific reports, 9:1-12.
 - ✍ Malaikozhundan, B. (2018), Pharmaceutical Applications of Zinc Oxide Nanoparticles-A Review, Acta Scientific Pharmaceutical Sciences, 2(1): 11-12.
 - ✍ Malik, P., Shankar, R., Malik, V., Sharma, N. and Mukherjee, T. K. (2014), Green synthesis, Journal of nanoparticles, 302429.
 - ✍ Manchanda, S., Das, N., Chandra, A., Bandyopadhyay, S. and Chaurasia, S. (2020), Chapter 2 - Fabrication of advanced parenteral drug-delivery systems, Advances in Pharmaceutical Product Development and Research, 47-84
 - ✍ Mankad, M., Patil, G., Patel, D., Patel, P. and Patel, A. (2018), Comparative studies of sunlight mediated green synthesis of silver nanoparticles from *Azadirachta indica* leaf extract and its antibacterial effect on *Xanthomonas oryzae* pv. *Oryzae*, Arabian Journal of Chemistry, 1-8.

- ✍ Mehenna, M., El-Kader, N. and Smaha, M. W. (2017), Liposomes as potential carriers for ketorolac ophthalmic delivery: Formulations and stability issues, *Brazilian Journal of Pharmaceutical Science*, 53(2).
- ✍ Mensor, L.L., Mennezes, F.S., Leitao, G.G., Reis, A.S., Santos, T.C., Coube, C.S. and Leitao, S.G. (2001), Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method, *Phytotherapy Res.*, 15, 127-130.
- ✍ Mhatre, S., Srivastava, T., Naik, S. and Patravale, V.(2020), Antiviral activity of green tea and black tea polyphenols in prophylaxis and treatment of COVID-19: *Phytomedicine*, 212: 1-9.
- ✍ Mishra, P. K., Mishra, H., Ekielski, A., Talegaonkar, S. and Vaidya, B. (2017), Zinc oxide nanoparticles: a promising nanomaterial for Biomedical applications, *Drug Discovery Today*, 22(12): 1825-1834. Srirangam, G. M. and Rao, K. P. (2017), Synthesis and characterization of silver nanoparticles from the leaf extract of *Malachra capitata (L.)*, *Rasayan Journal Chemistry* 10: 46-53.
- ✍ Mittal, J., Sharma, M. and Batra, A. (2014), *Tinosporacordifolia* a multipurpose medicinal plant -A review, *Journal of medicinal plants studies*, 2:32-47.
- ✍ Mohamed , M., Lisa, A. S. A., Shimizu, T., Alaaeldin, E., Hussein, A., Sarhan, H. A., Szabein, J. and Ishida, T. (2019), PEGylated liposomes: Immunological responses, *Science and Technology of Advanced Materials*, 20(1): 710-724.
- ✍ Mostofsky, E., Johansen, M. B., Tjønneland, M. A., Chahal, H. S. and Mittleman, M. A. (2017), Chocolate intake and risk of clinically apparent atrial fibrillation: The Danish Diet, Cancer, and Health Study, 103, 1163-1167
- ✍ Mourdikoudis, S., Pallares, R. M. and Thanh, N. T. (2018), Characterization techniques for nanoparticles: Comparison and complementarity upon studying nanoparticle properties, *Nanoscale*, 10(27): 12871-12934.
- ✍ Nabikhan ,A., Kandasamy,K., Raj,A. and Alikunhi, N. (2010) synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, *Sesuviumportulacastrum*, *colloids and surfaces B: Biointerfaces*, 79:488-493.
- ✍ Netala, V.R., Kotakadi, V.s., Domdi, L., Gaddam, S.A., Bobbu, P., Venkata , S.k., Ghosh, S.b. and Tarte, V. (2016), Biogenic silver nanoparticles : efficient and effective antifungal agents, *applied nanoscience*, 6:475-484.

- ✍ Njoya, E.M., Munvera, A.M., Pierre Mkounga, P., Nkengfack, A.E. and McGaw, L.J. (2017), Phytochemical analysis with free radical scavenging, nitric oxide inhibition and antiproliferative activity of *Sarcocephaluspobeguunii* extracts, *BMC Complement. Alternative Medicine*, 17, 199.
- ✍ Nurani, S. J., Saha, C. K., Khan, M. R. and Sunny, S. M. H, (2015) Silver Nanoparticles Synthesis, Properties, Applications and Future Perspectives: A Short Review, *Journal of Electrical and Electronics Engineering*, 10(6): 117-126.
- ✍ Park Y., Cvikrova M., Martincova O., Ham K., Kang S., Park Y., Namiesnik J., Rambola A.D., Jastrzebski Z., Gorinstein S. In vitro antioxidative and binding properties of phenolics in traditional, citrus and exotic fruits. *Food Res. Int.* 2015;74:37-47.
- ✍ Parveen, A. and Rao, S. (2015), Mechanistic Approach of Functionalized Noble Metal Nanoparticles Synthesis from *Cassia auriculata* L, *Journal of Cluster Science*, 26(4), 1295-1303.
- ✍ Patra, J.K., Das, G., Fraceto, L.F., Campos, E.V.R., Rodriguez, M.D.P., Acosta, L.S., Diaz, L.A., Swamy, M.K., Sharma, S., Habtemariam, S. and Shin, H.S., (2018), Nano based drug delivery system: recent developments and future prospects, 71:1-33.
- ✍ Pavithra, K. and Vadivukkarasi, S. (2015) Evaluation of free radical scavenging activity of various extracts of leaves from *Kedrostisfoetidissima*(Jacq.), *Food Science and Human Wellness*, 4, 42-46.
- ✍ Pieme, C.A., Santosh, G.K., Tekwu, E.M., Askun, T., Aydeniz, H., Ngogang, J.Y., Bhushan, S. and Saxena, A.K. (2014), Fruits and barks extracts of *Zanthozyllum heitzii* a spice from Cameroon induce mitochondrial dependent apoptosis and Go/G1 phase arrest in human leukemia HL-60 cells, *Biological Research*, 47, 2-13.
- ✍ Pourmand, A., Pourmand, M. R., Wang, J. and Shesser, R. (2012), Application of nanomedicine in emergency medicine; point-of-care testing and drug delivery in twenty-first century, *DARU Journal of Pharmaceutical Sciences*, 1-3.
- ✍ Prabhu, S., Vinodhini, S., Elanchezhian, C. and Rajeswari, D. (2018), Evaluation of antidiabetic activity of biologically synthesized silver nanoparticles using

Pouteria sapota in streptozotocin-induced diabetic rats, Journal of Diabetes 10: 28-42

- ✍ Prathna, T. C., Raichur, A. M., Chandrasekaran, N. and Mukherjee, A. (2014), Sunlight irradiation induced green synthesis of stable silver nanoparticles using *Citrus limon* extract. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 84(1): 65-70.
- ✍ Preeti, N. (2016), Green Chemistry for Nanotechnology: Opportunities and Future Challenges. Research and Reviews: Journal of Chemistry, 5(1).
- ✍ Premasudha, P., Venkataramana, M., Abirami, M., Vanathi, P., Krishna, K. and Rajendran, R. (2015), Biological synthesis and characterization of silver nanoparticles using *Eclipta alba* leaf extract and evaluation of its cytotoxic and antimicrobial potential. Bulletin of Materials Science, 38(4): 965-973.
- ✍ Premasudha, P., VenkataRamana, M., Mariappan, A., Vanathi, P., Kadirvelu, K. and Rajendran, R. (2015) Biological synthesis and characterization of silver nanoparticles using *Eclipta alba* leaf extract and evaluation of its cytotoxic and antimicrobial potential, Bulletin of materials science, 38(4).
- ✍ Priyaragini, S., Sathishkumar, S.R. and Bhaskararao, K.V. (2013), Biosynthesis of silver nanoparticles using actinobacteria and evaluating its antimicrobial and cytotoxicity activity, International Journal of Pharmacy and Pharmaceutical Sciences, 5(2): 709-712.
- ✍ Raj, V.K., Kumar, J.R., Balasubramanian, S., Swamy, S.N., Keerthini, D., Shambhavi, N., Kanthesh, B.M. and Avinash, K.O. (2016) Comparative evaluation of hepatoprotective effects of exotic fruits and common vegetables extracts on CCl₄ induced hepatotoxicity: an *in vitro* study, International Journal of Pharmacological Science, 7, 3388-3393.
- ✍ Ramesh, M., Anbuvaran, M. and Viruthagiri, G. (2015), Green synthesis of ZnO nanoparticles using *Solanum nigrum* leaf extract and their antibacterial activity, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 136: 864-870.
- ✍ Rautela, A., Rani, J. and Das, M. D. (2019), Green synthesis of silver nanoparticles from *Tectona grandis* seeds extract: characterization and mechanism

- of antimicrobial action on different microorganisms, *Journal of Analytical Science and Technology*, 10(1): 5.
- ✍ Rehana, D., Mahendiran, D., Kumar, R. S. and Rahiman, A. K. (2017), *In vitro* antioxidant and antidiabetic activities of zinc oxide nanoparticles synthesized using different plant extracts, *Bioprocess and biosystems engineering*, 40(6): 943-957.
 - ✍ Reverberi, A. P., Vocciante, M., Lunghi, E. and Pietrelli, L. (2017), New trends in the synthesis of nanoparticles by green methods, *Chemical engineering transactions*, 61, 667-672.
 - ✍ Roa , J.P. and Geckeler , K.E.(2011),*Polymer Nanoparticles: Production techniques and size _ control parameters*,*progress in polymer science*,7.
 - ✍ Roy, A., Bulut, O., Some, S., Mandal, A. K. and Yilmaz, M. D. (2019), Green synthesis of silver nanoparticles: Biomolecule - nanoparticle organizations targeting antimicrobial activity, *RSC advances*, 9: 2673-2702.
 - ✍ Ruch, R.J., Cheng, S.J. and Klaunig, J.E. (1989) Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea, *Carcinogenesis*, 10, 1003-1008.
 - ✍ Saha, M.R., Dey, P., Chaudhuri, T.K., Goyal, A.K., Sarker, D.D. and Sen, A. (2016), Assessment of haemolytic, cytotoxic and free radical scavenging activities of an underutilized fruit, *Baccaurearamiflora*Lour. (Roxb.) Muell. Arg, *Indian Journal of Expression Biology*, 54: 115-125.
 - ✍ Saji, V. S., Choe, H. C. and Yeung, K. W. (2010). Nanotechnology in biomedical applications: a review, *International Journal of Nano and Biomaterials*, 3(2), 119-139.
 - ✍ Salata, O. V. (2004), Applications of nanoparticles in biology and medicine. *Journal of nanobiotechnology*, 2(1): 1-6.
 - ✍ Saminathan, V. and Doraiswamy, R. (2020), Phytochemical analysis, antioxidant and anticancer activities of durian (*Duriozibenthiummurr*) fruit extract, *Journal of research in pharmacy* 24(6): 882-892.
 - ✍ Santhoshkumar, J., Kumar, S. V. and Rajeshkumar, S. (2017), Synthesis of zinc oxide nanoparticles using plant leaf extract against urinary tract infection pathogen, *Resource Efficient Technologies*, 3(4): 459-465.

- ✍ Sarma, R., Das, M., Mudoi, T., Sharma, K.K., Kotoky, J. and Devi, R. (2016), Evaluation of Antioxidant and Antifungal Activities of Polyphenol-rich Extracts of Dried Pulp of *Garcinia pedunculata* Roxb. and *Garcinia morella* Gaertn.(Clusiaceae), *Tropical Journal of Pharmacology*, 15: 133-140.
- ✍ Sau, T. P. and Rogach, A. L. (2010), Nanospherical noble metal nanoparticles: Colloid - chemical synthesis and morphology control, *Advanced materials*, 22: 1781-1804.
- ✍ Scimeca, M., Bischetti, S., Lamsira, H. K., Bonfiglio, R. and Bonanno, E. (2018), Energy Dispersive X-ray (EDX) microanalysis: A powerful tool in biomedical research and diagnosis, *European Journal of Histochemistry*, 62(1).
- ✍ Seifipour, R., Nozari, M. and Pishkar, L.(2020), Green Synthesis of silver nanoparticles using *Tragopogon collinus* leaf extract and study of their antibacterial effects, *Journal of Inorganic and organometallic polymers and materials*,30: 2926-2936.
- ✍ Sekhar, S. M., Vyas, N., Unnikrishnan, M. K., Rodrigues, G. S., and Mukhopadhyay, C. (2014). Antimicrobial susceptibility pattern in diabetic foot ulcer: A pilot study, *Annals of Medical and Health Sciences Research*, 4(5), 742-745.
- ✍ Semsri, S., Anuchapreeda, S. and Janwitayanuchit, W. (2015), Inhibitory effects of crude ethanolic leave extract from *Moringa oleifera* Lam. on Wilms' tumor 1 protein expression in K562 leukemic cell line, *Medical science*, 49, 53-64.
- ✍ Senthilraja, P. and Kathiresan, K. (2015), *In vitro* cytotoxicity MTT assay in Vero, HepG2 and MCF -7 cell lines study of Marine Yeast, *Journal of Applied Pharmacological Science*, 5,080-084.
- ✍ Senthilraja, P. and Kathiresan, K. (2015), *In vitro* cytotoxicity MTT assay in Vero, HepG2 and MCF -7 cell lines study of Marine Yeast, *Journal of applied pharmacological sciences*, 5,080-084.
- ✍ Sercombe, L., Veerati, T., Moheimani, F., Wu, S. Y., Sood, A. K. and Hua , S. (2015), Advances and challenges of liposome assisted drug delivery, *Frontiers in Pharmacology*, 6: 286.

- ✍ Shah, M., Fawcett, D., Sharma, S., Tripathy, S. and Poinern, G. (2015), Green synthesis of metallic nanoparticles via biological entities, *Materials*, 8(11): 7278-7308.
- ✍ Shameli, K., Ahmad, M. B., Zamanian, A., Sangpour, P., Shabanzadeh, P., Abdollahi, Y. and Zargar, M. (2012), Green biosynthesis of silver nanoparticles using *Curcuma longa* tuber powder. *International Journal of Nanomedicine*, 7: 5603.
- ✍ Shanmugam, S., Baby, J.P., Chandran, R., Thankarajan, S. and Thangara, P. (2016) *Maesa indica*: a nutritional wild berry rich in polyphenols with special attention to radical scavenging and inhibition of key enzymes, α -amylase and aglucosidase, *Journal of Food Science and Technology*.
- ✍ Sharma, R., Bisen, D. P., Shukla, U. and Sharma, B. G. (2012), X-ray diffraction: a powerful method of characterizing nanomaterials, *Recent Research in Science and Technology*, 4(8).
- ✍ Sharma, S., Sing, S., Ganguli, A.K. and Shanmugam, V. (2017), Anti-drift nano-strickersmade of graphene oxide for targeted pesticide delivery and crop pest control, *Research gate*, 212:1-15.
- ✍ Shirwaiker, A., Rajendran, K. and Kumar, C.D. (2006) *In vitro* antioxidant studies of *Annona squamosa Linn* leaves, *Int. J. Exp. Biol.*, 42, 803-807.
- ✍ Silva, F.S.G., Starostina, I.G., Ivanova, V.V., Rizvanov, A.A., Oliveira, P.J., and Pereira, S.P. (2016), Determination of metabolic viability and cell mass using a tandem resazurin/sulforhodamine B assay, *Current Toxicology*, 68, 2.24.1-2.24.15.
- ✍ Singh, R., Vora, J., Nadhe, S. B., Wadhvani, S. A., Shedbalkar, U. U. and Chopade, B. A. (2018), Antibacterial activities of bacteriogenic silver nanoparticles against nosocomial *Acinetobacter*, *Nanoscience and Nanotechnology*, 18(6):3806-3815.
- ✍ Skehan,P.A.,Storeng, R., Scudiero,D.A. and Monks,A. (1990),New colorimetric cytotoxicity assay for anticancer drug screening, *Journal of the National Cancer Institute*,82: 1107-12.
- ✍ Sreejayan.andRao,M.N. (1997), Nitric oxide scavenging by curcuminoids, *Journal of pharmacy and pharmacology*, 49(1): 105-7.

- ✍ Sulaiman, G., Mohammed,W., Marzoog,T., Al-Amiery , A.A.A., Kashim, A.A.H., Mohammad ,A.B. and Bagmati,R. (2013), Green synthesis, antimicrobial and cytotoxic effects of silver nanoparticles using *Eucalyptus chapmanianaleaves* extract,Asian Pacific journal of tropical biomedicine,3(1):58-63.
- ✍ Sumitha, S., Vasanthi, S., Shalini, S., Chinni, S. V., Gopinath, S. C., Anbu, P. and Ravichandran, V. (2018), Phyto-Mediated Photo Catalysed Green Synthesis of Silver Nanoparticles Using *Duriozibethinus*Seed Extract: Antimicrobial and Cytotoxic Activity and Photocatalytic Applications, Molecules, 23(12): 3311.
- ✍ Surendiran, A., Sandhiya, S., Pradhan, S. C. and Adithan, C. (2009), Novel applications of nanotechnology in medicine, Indian Journal of Medical Research, 130(6).
- ✍ Suresh, J., Pradheesh, G., Alexramani, V., Sundrarajan, M. and Hong, S. I. (2018), Green synthesis and characterization of zinc oxide nanoparticle using insulin plant (*Costus pictus D. Don*) and investigation of its antimicrobial as well as anticancer activities, Advances in Natural Sciences: Nanoscience and Nanotechnology, 9(1), 015008.
- ✍ Syafiuddin, A., Salmiati, Salim, M.R., Kueh, A.B.H., Hadibrata, T. and Nur, H.(2017), A revieoof silver nanoparticles: Research trends, global consumption, synthesis, properties and future challenges, Journal of the Chinese chemical society, 64:732-756.
- ✍ Thrikawala, V. S., Deraniyagala, S. A., Dilanka Fernando, C. and Udukala, D. N. (2018), *In Vitro* α -Amylase and Protein Glycation Inhibitory Activity of the Aqueous Extract of *FlueggealeucopyrusWilld*, Journal of Chemistry, 1-7.
- ✍ Tundis, R., Loizzo, M. R. and Menichini, F. (2010), Natural products as α -amylase and α -glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update, Mini reviews in medicinal chemistry, 10(4): 315-331.
- ✍ Umar, H., Kavaz, D. and Rizaner, N. (2019), Biosynthesis of zinc oxide nanoparticles using *Albizia lebbeck*stem bark and evaluation of its antimicrobial, antioxidant, and cytotoxic activities on human breast cancer cell lines, International journal of nanomedicine, 14:87-100.

- ✍ Vanaja, M., Paulkumar, K., Gnanajobitha, G., Rajeshkumar, S., Malarkodi, C. and Annadurai, G. (2014). Herbal plant synthesis of antibacterial silver nanoparticles by *Solanum trilobatum* and its characterization. *International Journal of Metals*.
- ✍ Vasundhara, C. C. S. and Devi, G.S. (2014), In vitro antidiabetic activity of leaves and seeds of *Boerhaviadiffusa*, *Journal of Pharmaceutical and Biological Research*, 2: 184-9.
- ✍ Yasir, M., Singh, J., Tripathi, M. K., Singh, P. and Shrivastava, R. (2017). Green synthesis of silver nanoparticles using leaf extract of common arrow head houseplant and its anticandidal activity, *Pharmacognosy magazine*, 13(4), 840-844.
- ✍ Yildirim, A., Mavi, A. and Kara, A.A. (2001), Determination of antioxidant and antimicrobial activities of *Rumexcrispus* L. extracts, *Journal of Agriculture and Food Chemistry*, 49:4083-4089.
- ✍ Zhang, X. F., Liu, Z. G., Shen, W. and Gurunathan, S. (2016), Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches, *International Journal of Molecular Sciences*, 17(9): 1534.