Green synthesis of silver nanoparticle from Durian fruit (*Durio zebenthinus*) and its antimicrobial activity and antioxidant properties

By

MRUDULA, C.

16PBC008

A thesis submitted to

Avinashilingam Institute for Home Science and Higher Education for Women

Coimbatore- 641043.

In partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE IN BIOCHEMISTRY

April, 2018

10/4/18

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1.0. INTRODUCTION

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. These medicinal plants are consider as a rich resources o ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole World. Moreover, some plants are considered as important source of nutrition and as a result of that these plants recommended for their therapeutic values. These plants include ginger, green tea, walnuts and some others plants. Other plants their derivatives consider as important source for active ingredients which are used in aspirin and toothpaste. Medicinal plants have a promising future because there are about half million plants around the world, and for most of them medical activities have not investigate yet and their medical activities could be decisive in the treatment of present or future studies. (Rasool Hassan., *et al* 2012).

Medicinal plants have been used in virtually all cultures as a source of medicine. Assurance of the safety, quality and efficacy of medicinal plants and herbal products has now become a key issue in industrialized and in developing countries. The widespread use of herbal remedies and healthcare preparations is described in the Vedas and the Bible .Medicinal Plants have been used for thousands of years as flavor and conserve food, to treat health disorders and to prevent diseases including epidemics. The knowledge of their healing properties has been transmitted over the centuries within and among human communities. Active compounds produced during secondary metabolism are usually responsible for the biological properties of plant species used throughout the globe for various purposes, including treatment of infectious diseases. Currently, data on the antimicrobial activity of numerous plants, so far considered empirical, have been scientifically confirmed, with the increasing number of reports on pathogenic microorganisms resistant to antimicrobials. Products derived from plants may potentially control microbial growth in diverse situations and in the specific case of disease treatment, numerous studies have been aimed to describe the chemical composition of these plant antimicrobials and the mechanisms involved in microbial growth inhibition, either separately or associated with conventional antimicrobials.(Singh, *et al* ., 2015).

Nanotechnology is the study of extremely small structures, having size of 0.1 to 100 nm. Nano medicine is a relatively new field of science and technology. The development in the field of nanotechnology started in 1958. Advancement in the field of nanotechnology and its applications to

the field of medicines and pharmaceuticals has revolutionized the twentieth century. Nanotechnology is the study of extremely small structures. The prefix “nano” is a Greek word which means “dwarf”. The word “nano” means very small or miniature size. Nanotechnology is the treatment of individual atoms, molecules, or compounds into structures to produce materials and devices with special properties. (Anna Pratima Nikalje, *et al*., 2015).

With the increasing demand for advances in diagnosis and treatment modalities, nanotechnology is being considered as a groundbreaking and viable research subject. This technology, which deals with matter in nanodimensions, has widened our views of poorly understood health issues and provided novel means of diagnosis and treatment. Researchers in the field of dentistry have explored the potential of nanoparticles in existing therapeutic modalities with moderate success.

The key implementations in the field of dentistry include local drug delivery agents, restorative materials, bone graft materials, and implant surface modifications. This review provides detailed insights about current developments in the field of dentistry, and discusses potential future uses of nanotechnology. Current nanotechnological research falls under two approaches. The bottoms-up approach deals with the creation and development of new ‘intelligent’ materials or devices, wherein various processes are utilized to induce nanostructures to self assemble at a desired scale and then organize into higher macroscale structures. (Bhavikatti *et al*., 2013).

Nanotechnology is sometimes referred to as a general purpose technology because in its advanced version it will have significant impact on almost all industries and all areas of society (Bhattacharyya, *et al*., 2009).

The combination of green chemistry techniques with nanotechnology applications has thus become a key component of the nanotechnology future. The use of natural ingredients to synthesize nanomaterials and design environmentally benign synthetic processes has been extensively explored. While many of these so-called ‘‘green nanotechnologies’’ are now finding their way from the laboratory to commercial application, green nanotechnology still faces significant challenges.

Nature provides us with numerous chemical substances that serve as suitable reducing agents for the synthesis of nanoparticles, including plant extracts, biopolymers, vitamins, proteins, peptides (e.g., glutathione), and sugars (e.g., glucose, fructose). Plant extracts are the most studied category to date. Given their abundance, plant extracts are regarded as one of the most promising natural reducing agents . One area of particular success is the synthesis of metal nanoparticles, useful in electronics and medical applications, using plant extracts as reducing agents (Soydan Ozcan, *et al*., 2015).

Nanomaterials have been extensively investigated during the last decade due to their Wide variety of applications. It is observed that field of nanomaterial synthesis is very dynamic. Many process such as gas condensation, chemical vapor synthesis, mechanical attrition, chemical precipitation, Sol-Gel technique, electrodeposition, some other methods widely used are molecular beam epitaxy, ionised cluster beam, liquid metal ion source, consolidation, sputtering and gas aggregation of monomers chemical precipitation in presence of capping agents, reaction in microemulsions and autocombustion are commonly used techniques for synthesis of nanophosphors. Nanomaterials prepared using the processes include a wide variety.(Namita Rajput *et al*., 2015).

The nanoparticles can also be prepared using a variety of chemicals and physical methods such as chemical reduction, photochemical reduction, electrochemical reduction and heat vaporization. Inorganic compounds are used as reagents that have the capacity of being oxidized. Because noble metals nanoparticles are now widely used in areas of human contact, there is growing need to develop ecofriendly processes it do not use toxic chemicals in their synthesis.plants play a major role in the synthesis of nanoparticle to overcome the problem of toxicity in synthesis (Arunachalam *et al*., 2013).

Ecofriendly or Green synthesis of metal nanoparticles has become an important branch of nanotechnology and there is an increasing commercial demand for nanoparticles due to their wide applications. Biological synthesis process provides a wide range of environmentally acceptable methodology, low cost production and minimum time required. At the same time the biologically synthesized silver nanoparticles has many applications such as catalysts in chemical reactions. silver nanoparticles have been successfully synthesized by Escherichia coli, in which exposure of supernatant to silver ions resulted in the extracellular reduction of the metal ions and formation of silver nanoparticles. (Hinal Gandhi *et al*., 2016)**.** The synthesized nanoparticle are carried out by characterization technique like UV-visible, FTIR, SEM, TEM, EDX, XRD, ZETA and DLS and so on.

In developing countries, the frequency of life-threatening infections were caused by pathogenic microorganisms has led to increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients1. The historical point, plants have been used as an important source of natural products for human health. All over the world, the antimicrobial properties of plants have been investigated by a number of studies and many of them have been used as therapeutic alternatives because of their antimicrobial properties and they contains secondary metabolites such as alkaloids, phenolic compounds, etc.(Vimalaand *et al*., 2014).

Disease-causing microbes that have become resistant to drug therapy are an increasing public health problem. Many researchers are now engaged in developing new effective antimicrobial reagents with the emergence and increase of microbial organisms resistant to multiple antibiotics, which will increase the cost of health care. Therefore, there is an urgent need to develop new bactericides. Silver has been used for years in the medical field for antimicrobial applications such as burn treatment. The mechanism of the bacterial effect of AgNP as proposed is due to the attachment of AgNP to the surface of the cell membrane, thus disrupting permeability and respiration functions of the cell. It is also proposed that AgNP not only interact with the surface of a membrane but can also penetrate inside the bacteria. The antibacterial activity of AgNP is significantly enhanced when it is modified with sodium dodecyl sulfate (SDS) (Maiti *et al*., 2014).

The antimicrobial activity of Ag nanoparticles was investigated against yeast, Escherichia coli, and Staphylococcus aureus. In these tests, Muller Hinton agar plates were used and Ag nanoparticles of various concentrations were supplemented in liquid systems. The antibacterial effects of Ag salts have been noticed since antiquity, and Ag is currently used to control bacterial growth in a variety of applications, including dental work, catheters, and burn wounds. In fact, it is well known that Ag ions and Ag-based compounds are highly toxic to microorganisms, showing strong biocidal effects on as many as 12 species of bacteria including E. coli (Kim *et al*., 2007).

The complex biochemical reactions of the body and increased exposure to environmental toxicants and dietary xenobiotics result in the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), leading to oxidative stress under different pathophysiological conditions. Antioxidants prevent oxidative damage through one-electron reactions with free radicals [superoxide radicals (O_2-), hydroxyl radicals (OH·), singlet oxygen (O·), and hydrogen peroxide (H_2O_2)] that adversely alter cellular lipids, protein, DNA and polysaccharides. Therefore, a balance between free radical and antioxidant concentrations is necessary to maintain proper physiological functions. Many people consume antioxidants as a defense against oxidative stress. Antioxidants in the form of commercial food additives have been manufactured synthetically and may contain high amounts of preservatives (Tanvir *et al*., 2017).

Durio zibethinus, is a high medicinal value fruit, a number of health protective effects of phenolic compounds have been reported due to their antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory, antimicrobial, and other biological possessions (Ashraf *et al*., 2011).

The work has been done on "Green synthesis of silver nanoparticle and Antimicrobial and Antioxidant activity of *Durio zibethinus*". The present study are indicated by the following objectives.

1. Green synthesis of silver nanoparticle from Durian fruit extract by exposureto sunlight.
2. Antimicrobial activity of selected phyto pathogen using different extract green synthesized silver nanoparticle from Durian fruit.
3. Antioxidant activity of Durian fruit.

2.0.REVIEW OF LITERATURE

The review of literature pertaining to the study entitled Green synthesis of silver nanoparticle from Durian fruit (Durio zebenthinus) and its antimicrobial and antioxidant properties discussed under the following headings.

2.1. Medicinal plants

Plants are the basis of life on earth and are central to people's livelihoods. Tribal people are the ecosystem people who live in harmony with the nature and maintain a close link between man and environment (Sajem *et al*., 2006).

Medicinal plant is an important element of indigenous medical systems in all over the World. The ethno botany provides a rich resource for natural drug research and development. “Traditional” use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as “traditional herbal medicines”. In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in

order to meet health care needs. Although modern medicine may exist side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons. Natural products have played an important role throughout the world in treating and preventing human diseases. Natural product medicines have come from various source materials including terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates and its importance in modern medicine has been discussed in different reviews and reports (Hosseinzadeh *et al*.,2015).

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants consider as important source of nutrition and as a result of that these plants recommended for their therapeutic values. These plants include ginger, green tea, walnuts and some others plants (Hassan *et al.*, 2012).

Medicinal plants have been used in traditional health care systems since prehistoric times and are still the most important health care source for the vast majority of the population around the world e.g. It is estimated that 70-80% of people worldwide rely on traditional herbal medicine to meet their primary health care needs. Globally, millions of people rely on medicinal plants not only for primary health care, but also for income generation and livelihood improvement. Annual sales of herbal-based medicines range between 7.5 billion US$ and 108 billion US$ worldwide, the latter number representing sales of processed medicines. In Canada annual market sales of medicinal plants reached 400 million US$ in 2001, and are growing at a pace of 15% annually.

2.2 *Durio zibethinus*

Durian is a very popular tropical fruits and often referred as the King of tropical fruits in South East Asia. Durian has a special shape and nutrient content. It possesses strong aroma and unique taste. In Indonesia, durian is not only eaten as a fresh fruit but also used as ingredient for ice cream, pudding, juices and in various food products. Durian cultivars are very diverse in taste, smell, texture and flesh color, as well as variations in the shape and size of the fruits (Belgis *et al.*, 2015).

Durian (Durio zibenthinus Linn) belongs to the family Bombacaceae and features large seeds surrounded by fleshy arils. Durian has a characteristic large size, unique odour and formidable thorn-covered husk. It is otherwise known as “king of tropical fruit” owing to its highly nutritious superlative pulp and outer thorny appearance, resembling the thrones of ancient Asian era kings. Durian fruit can reach up to 30 cm in length, 15 cm in diameter and can weigh between 1 and 3 kg. Durian grows well in warm tropical areas with 75%–80% humidity and is abundantly available from mid-May until July. Though there are 200 different varieties grown, Mon Thong, Chani and Kan Yao varieties are most well-known. Durian is used extensively by naturopaths who use it as a traditional treatment of fevers, jaundice and infertility. Villagers of southern India use durian for fertility enhancing and consume the fruits available from Nilgris, southern India(Ansari *et al*., 2016).

Durian is rich in carbohydrate, protein, fat, phosphorus, iron and vitamin A. Durian is usually used for fresh consumption. The edible portion (aril) of durian has a very strong odor. Most of the photochemicals are an integral part of the durian fruit and also being used in medicinal formulations. A number of health protective effects of phenolic compounds have been reported due to their antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory, antimicrobial, and other biological possessions. Currently durian fruit is popular in daily utilization because of their first-class flavor and health-promoting compounds, such as flavonoids, phenolics and carotenoides contents. Durian flesh, is said to serve as a medication to eliminate parasitic worms. Moreover, in Malaya, decoction of durian leaves and fruits are applied to swellings and skin diseases while the ash of the burned rind is taken after childbirth (Sivananthan *et al*., 2013).

2.3 Nanotechnology

Nanotechnology is the study of extremely small structures. The prefix “nano” is a Greek word which means “dwarf”. The word “nano” means very small or miniature size. Nanotechnology is the treatment of individual atoms, molecules, or compounds into structures to produce materials and devices with special properties. Nanotechnology involve work from top down i.e. reducing the size of large structures to smallest structure e.g. photonics applications in nano electronics and nano engineering, top-down or the bottom up, which involves changing individual atoms and molecules into nanostructures and more closely resembles chemistry biology. Nanotechnology deals with materials in the size of 0.1 to 100 nm; however it is also inherent that these materials should display different properties such as electrical conductance chemical reactivity, magnetism, optical effects

and physical strength, from bulk materials as a result of their small size. Nanotechnology works on matter at dimensions in the nanometer scale length (1-100 nm), and thus can be used for a broad range of applications and the creation of various types of nano materials and nano devices (Nikalje *et al*., 2015).

Nanotechnology has gained increased popularity largely due to the design, creation" utilization of materials whose constituent structures exist at Nanoscale i.e. physical dimensions that are in the range of 'one-billionth (1O¯9) of a meter.(Bhasin *et al*., 2007).

2.4.The Structure of Nanotechnology

Nanotechnology is distinguished by its interdisciplinary nature. For one thing, investigations at the nanolevel are occurring in a variety of academic fields. More important, the most advanced research and product development increasingly requires knowledge of disciplines that, until now, operated largely independently. These areasinclude:

• Physics — The construction of specific molecules is governed by the physical forces between the individual atoms composing them. Nanotechnology will involve the continued design of novel molecules for specific purposes. However, the laws of physics will continue to govern which atoms will interact with each other and in what way. In addition, researchers need to understand how quantum physics affects the behavior of matter below a certain scale.

• Chemistry — The interaction of different molecules is governed by chemical forces. Nanotechnology will involve the controlled interaction of different molecules, often in solution. Understanding how different materials interact with each other is a crucial part of designing new nanomaterials to achieve a given purpose.

• Biology — A major focus of nanotechnology is the creation of small devices capable of processing information and performing tasks on the nanoscale. The process by which information encoded in DNA is used to build proteins, which then go on to perform complex tasks including the building of more complex structures, offers one possible template. A

better understanding of how biological systems work at the lowest level may allow future scientists to use similar processes to accomplish new purposes. It is also a vital part of all research into medical applications.(Jim Saxton *et al*., 2007).

2.5. Nanoparticle

Nanotechnology employs knowledge from the fields of physics, chemistry, biology, materials science, health sciences, and engineering. It has immense applications in almost all the fields of science and human life. Nanoparticles can be defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed(Nikam et al ., 2014).

Nanoparticles are of great interest because of their technological and fundamental scientific importance. These materialsoften exhibit fascinating properties which cannot be achieved by their bulk counterparts. Their applications, or potential applications, are in many fields [and references therein]. Nanoparticles have advantages in application in life science and the environment. Their particle size is comparable with the dimension of small molecules (about 1–10 nm) or of viruses (about 10–100 nm). This allows nanoparticles to attach to biological entities without changing their functions. Large surface area of nanoparticles permits strong bonds with surfactant molecules. In the environment, the small size of nanoparticles, together with their large surface area can lead to very sensitive detection of a specific contaminant from the presence of which pollution often arises. Nanoparticles can also be engineered to actively interact with a pollutant and treat them.

2.6. Classification of nanoparticle

Nanoparticles are broadly classifi ed in to three classifi cations

• One dimension nanoparticles

One dimensional system (thin fi lm or manufactured surfaces) has been used for decades. Thin fi lms (sizes 1–100 nm) or monolayer is now common place in the field of solar cells offering, different technological applications, such as chemical and biological sensors, information storage systems, magneto-optic and optical device, fiber-optic systems.

• Two dimension nanoparticles

Carbon nanotubes

• Three dimension nanoparticles

Dendrimers, Quantum Dots, Fullerenes (Carbon 60), (QDs) (Bhatia *et al*., 2016).

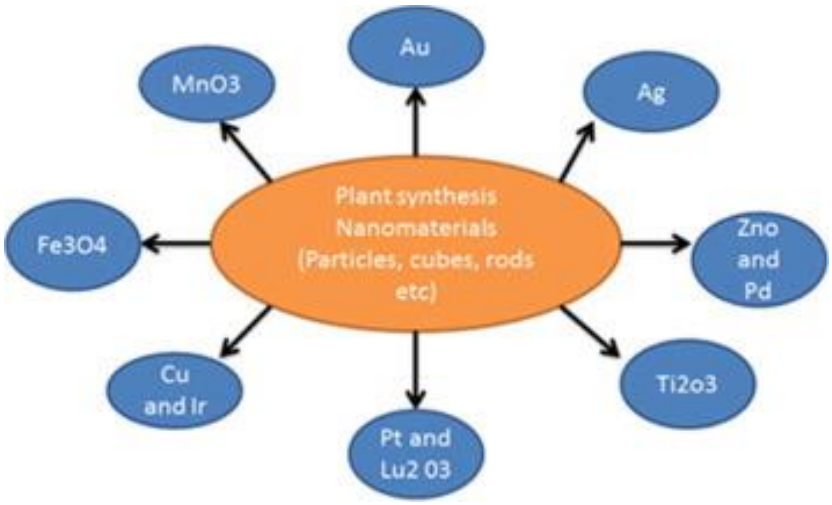
2.7. Types of nanoparticle

Silver**:** Silver nanoparticles have proved to be most effective because of it's good antimicrobial efficacy against bacteria, viruses and other eukaryotic micro-organisms16,17. They are undoubtedly the most widely used nanomaterials among all, thereby being used as antimicrobial agents, in textile industries, for water treatment, sunscreen lotions etc18,19. Studies have already reported about the successful biosynthesis of silver nanoparticles by plants such as *Azadirachta indica*, *Capsicum annuum* and *Carica papaya*22.

Gold**:** Gold nanoparticles (AuNPs) are used in immunochemical studies for identification of protein interactions. They are used as lab tracer in DNA fingerprinting to detect presence of DNA in a sample. They are also used for detection of aminoglycoside antibiotics like streptomycin, gentamycin and neomycin. Gold nanorods are being used to detect cancer stem cells, beneficial for cancer diagnosis and for identification of different classes of bacteria.

Figure 1

Types of plant synthesized Nanoparticle



Alloy**:** Alloy nanoparticles exhibit structural properties that are different from their bulk samples25. Since Ag has the highest electrical conductivity among metal fillers and, unlike many other metals, their oxides have relatively better conductivity 26, Ag flakes are most widely used . Bimetallic alloy nanoparticles properties are influenced by both metals and show more advantages over ordinary metallic NPs27

Magnetic**:** Magnetic nanoparticles like Fe3O4 (magnetite) and Fe2O3 (maghemite) are known to be biocompatible. They have been actively investigated for targeted cancer treatment (magnetic hyperthermia), stem cell sorting and manipulation, guided drug delivery, gene therapy, DNA analysis, and magnetic resonance imaging (MRI) (Hasan *et al*., 2015).

2.8. Application of nanoparticle

Applications in drugs and medications

Nano-sized inorganic particles of either simple or complex nature, display unique, physical and chemical properties and represent an increasingly important material in the development of novel nanodevices which can be used in numerous physical, biological, biomedical and pharmaceutical applications. NPs have drawn increasing interest from every branch of medicine for

their ability to deliver drugs in the optimum dosage range often resulting in increased therapeutic efficiency of the drugs, weakened side effects and improved patient compliance

Applications in manufacturing and materials

Nanocrystalline materials provide very interesting substances for material science since their properties deviate from respective bulk material in a size dependent manner. Manufacture NPs display physicochemical characteristics that induce unique electrical, mechanical, optical and imaging properties that are extremely looked-for in certain applications within the medical, commercial, and ecological sectors. NPs focus on the characterization, designing and engineering of biological as well as non-biological structures < than 100 nm, which show unique and novel functional properties.

Applications in the environment

The increasing area of engineered NPs in industrial and household applications leads to the release of such materials into the environment. Assessing the risk of these NPs in the environment requires on understanding of their mobility, reactivity, Eco toxicity and persistency. The engineering material applications can increase the concentration of NPs in groundwater and soil which presents the most significant exposure avenues for assessing environmental risks.

Due to high surface to mass ratio natural NPs play an important role in the solid/water partitioning of contaminants can be absorbed to the surface of NPs, co-precipitated during the formation of natural NPs or trapped by aggregation of NPs which had contaminants adsorbed to their surface. The interaction of contaminants with NPs is dependent on the NPs characteristics, such as size, composition, morphology, porosity, aggregation/disaggregation and aggregate structure. The luminophores are not safe in the environment and are protected from the environmental oxygen when they are doped inside the silica network (Khan *et al*., 2017).

2.9. Different methods of silver nanoparticle synthesis

Physical Approach

In physical processes, metal nanoparticles are generally synthesized by evaporation condensation, which could be carried out using a tube furnace at atmospheric pressure. The source material within a boat centered at the furnace is vaporized into a carrier gas. Nanoparticles of various materials, such as Ag, Au, PbS and fullerene, have previously been produced using the evaporation/condensation technique. However, the generation of AgNPs using a tube furnace has several drawbacks, because a tube furnace occupies a large space, consumes a great deal of energy while raising the environmental temperature around the source material, and requires a lot of time to achieve thermal stability. A typical tube furnace requires power consumption of more than several kilowatts and a preheating time of several tens of minutes to attain a stable operating temperature. Furthermore, silver nanoparticles have been synthesized with laser ablation of metallic bulk materials solution.

Biological Approach

Recently, biosynthetic methods using naturally reducing agents such as polysaccharides, biological microorganism such as bacteria and fungus or plants extract, i.e. green chemistry, have emerged as a simple and viable alternative to more complex chemical synthetic procedures to obtain AgNPs. Bacteria are known to produce inorganic materials either intra- or extracellularly. This makes them potential biofactories for the synthesis of nanoparticles like gold and silver. Particularly, silver is well known for its biotical properties.

Chemical approach

Chemical reduction is the most common method because of its convenience and simple equipment. Control over the growth of metal nanoparticles is required to obtain nanoparticles of small size with a spherical shape and narrow distribution in diameter. It is well known that silver nanoparticles can be produced by chemical reaction at low cost and in high yield. Generally, the chemical synthesis process of AgNPs in solution usually employs the following three main

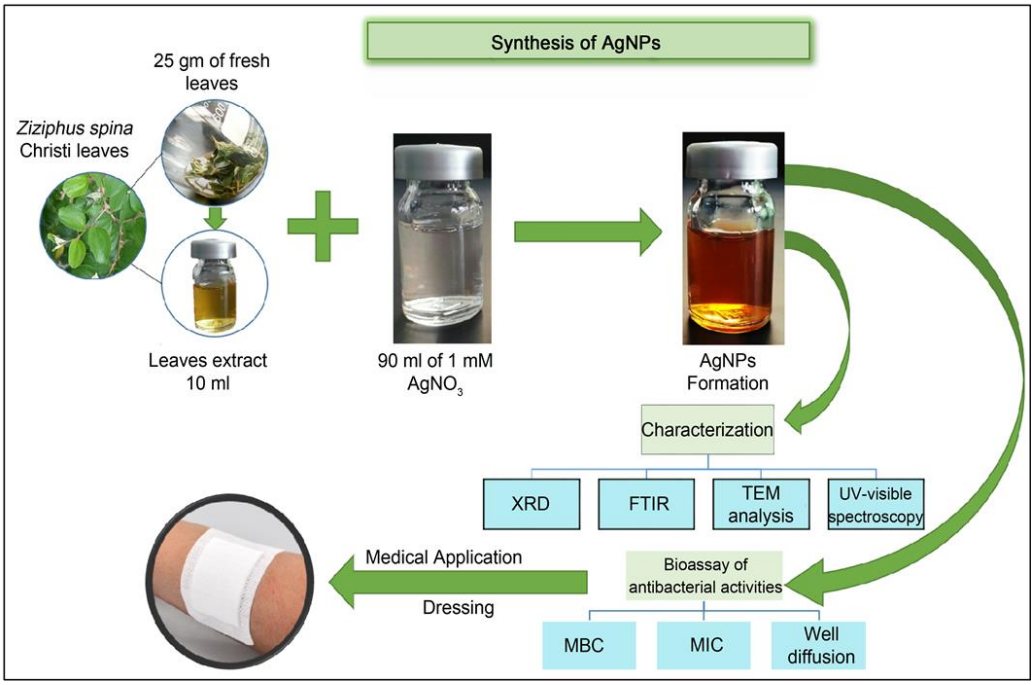
components: (1) metal precursors, (2) reducing agents and (3) stabilizing /capping agents (Natsuki *et al*., 2015).

2.10. Green synthesis of Nanoparticle

The synthesis of silver nanomaterials or nanoparticles extensively studied by using chemical and physical methods, but the development of reliable technology to produce nanoparticles is an important aspect of nanotechnology. Biological synthesis process provides a wide range of environmentally acceptable methodology, low cost production and minimum time required. At the same time the biologically synthesized silver nanoparticles has many applications includes catalysts in chemical reactions. Microbial source to produce the silver nanoparticles shows the great interest towards the precipitation of nanoparticles due to its metabolic activity. Of course the precipitation of nanoparticles in external environment of a cell, it shows the extracellular activity of organism. Extracellular synthesis of nanoparticles using cell filtrate could be beneficial over intracellular synthesis, the fungi being extremely good candidates for extracellular process and also environmental friendly. There are few reports published in literature on the biosynthesis of silver nanoparticles using fungal as source

Figure 2

Green synthesis of silver nanoparticle



Over the past several years, plants, algae, fungi, bacteria and viruses have been used for production of inexpensive, energy-efficient and eco-friendly metallic nanoparticles. Many bacterial cultures were used for different kind of nanoparticles some are gold nanoparticles using *Shewanella algae*; it's a kind of marine bacterium, silver nanoparticles by *Cynobacteria Plectonema boryanum*, cadmium nanoparticles biosynthesis was done by *Clostridium thermoaceticum*, magnetite nanoparticles by *Actinobactor* sp., *Shewanella oneidensis* used for uranium nanoparticles (Mathur *et al*., 2014).

The use of plant and plant extract in nanoparticle synthesis is considered advantageous over microbial based system because it reduces the elaborate process of maintaining cell cultures. The particle size growth can also be controlled by altering synthesis conditions like pH, reductant concentration, temperature, mixing ratio of the reactants etc. The plant based synthesis can be carried out either extracellularly or intracellularly. Intracellular synthesis takes place inside the plant whereas the extracellular synthesis occurs *in vitro*. The studies reveals that extracellular synthesis using plant extracts has been considered better as compared to intracellular synthesis because it eliminates the extraction and purification procedures. Biosynthesis of AgNPs by plant extracts such as neem, *Chenopodium album, Allium cepa, Eucalyptus hybrid*, Cycas, *Tribulus terrestris* etc.have been reported. Recent examples of plants reported in literature for AgNPs synthesis. Till date, lot of papers has been published in this area which describes the mechanism and role of active biomolecules in synthesis (Khatoon *et al*., 2017).

Biosynthesis of silver nanoparticles has already been reported as clean, cost effective and non-toxic to environmental routes. Green synthesis offers improvement over synthetic, chemical or micro-organisms methods as it is cost effective, environmentally friendly and can easily be scaled up for large scale synthesis.9 The methods used for the synthesis of silver nanoparticles and toxic chemicals are used for the reduction process of substances such as citrates, NaBH4, or ascorbates (Paramasivam *et al*., 2017).

Apart frombeing effective,AgNPs still remain a popular choice due to their nontoxicity towards human in comparison to other metals or materials. However, scarcity makes them expensive and limits their application. To overcome the problem, numerous synthesis methods have been developed. Most of the conventional methods for producing AgNPs require numerous chemicals, which not only is expensive but also could produce hazardous residue. Therefore, a green synthesis

of AgNPs is desirable to provide an economic, eco-friendly, and cleaner synthesis route (Ali *et al*., 2015).

2.11. Characterization of Silver nanoparticle

2.1.1.1Scanning Electron Microscopy (SEM)

The Scanning Electron Microscope is a electron microscope that images the sample surface by scanning it with a high energy beam of electron. Conventional light microscope use a series of glass lenses to bend light waves and create a magnified image while the scanning electron microscope creates the magnified image by using electrons instead of light waves.

2.1.1.2Energy Dispersive X- ray Analysis (EDX)

EDX is technique to analyze near surface elements and estimate their proportion at different position, thus giving an overall mapping of the sample(Joshi *et al*., 2008).

2.1.1.3. FTIR

Infrared (IR) or Fourier transform infrared (FTIR) spectroscopy has a large application range, from the analysis of small molecules or molecular complexes to the analysis of cells or tissues. The imaging of tissues is one of the recent developments of infrared spectroscopy, taking advantage of infrared microscopy and of the use of synchrotron IR radiation. It is used for the mapping of cellular components (carbohydrates, lipids, proteins) to identify abnormal cells. FTIR spectroscopy has also been increasingly applied to the study of proteins. This concerns the analysis of protein conformation, protein folding, and of molecular details from protein active sites during enzyme reactions using reaction-induced FTIR difference spectroscopy. (Berthomieu et *al*., 2009).

2.1.1.4. X-RAY DIFFRACTION (XRD)

X-ray Powder Diffraction (XRD) is an efficient analytical technique used to identify and characterize the unknown crystalline material. (Berthomieu et *al*., 2009).

2.1.1.5. Zeta potential

The zeta-potential is used in colloid chemistry for observing the behaviour of dispersive systems in liquids. Besides, the zeta-potential characterizes the electrical double layer on the solidmiquid interface, a fact very important in flotation and flocculation processes (Salopek *et al*., 1992).

2.1.1.6. UV-Vis Absorption Spectroscopy

UV-Vis Absorption Spectroscopy gives UV absorption of the amorphous gels and crystalline ceramic samples heated at different temperatures. Many molecules absorb visible or ultraviolet light. The absorbance of a solution is directly proportional to attenuation of the beam, i.e., it increases as attenuation of the beam increases. Absorbance is also directly proportional to the path length "b" and the concentration "c" of the absorbing species. Beer's Law states that $A = \varepsilon bc$, where ε is a constant of proportionality, called the absorbtivity. Different molecules absorb radiation of different wavelengths. An absorption spectrum will show a number of absorption bands corresponding to structural groups within the molecule (Srivastava *et al*., 2012).

2.12. HPTLC

The HPTLC is very useful qualitive analysis method.its combine arts of chromatography with quickness at moderate cost.its major advance to TLC principle shorten time duration & better resolution. HPTLC is playing an important role in today analytical world, not in competition to HPLC but as complementary method. One of the most obvious orthogonal features of the two techniques is the primary use of reversed phases in HPLC versus unmodified silica gel in HPTLC, resulting in partition chromatography and adsorption chromatography respectively. Unlike other methods,

HPTLC produces visible chromatograms complex information about the entire sample is available at a glance. Multiple samples are seen simultaneously, So that reference and test samples can be compared for identification. Similarities and differences are immediately apparent and with the help of the image comparison. Several chromato-grams can be compared directly, even from different plates. In addition to the visible chromatograms, analog peak data are also available from the chromatogram. They can be evaluated either by the image based software Videoscan or by

scanning densitometry with TLC Scanner, measuring the absorption and/or fluorescence of the substances on the plate. TLC is an offline technique: the subsequent steps are relatively independent, allowing parallel treatment of multiple samples during chromatography (Vikramkumar *et al*., 2014).

2.13. Antimicrobial avtivity

Structural modification of antimicrobial drugs to which resistance has developed has proven to be an effective means of extending the life-span of antifungal agents such as the azoles, antiviral agents such as the non-nucleoside reverse transcriptase inhibitors, and various antibacterial agents including β-lactams and quinolones. This problem encourages the researchers to study the new agents which can effectively inhibit microbial growth.

An alternative approach to overcome these issues might be using natural antimicrobial or antiviral products and phytochemicals. The Middle East has unique niches for medicinal plants, which have been used for treating diseases and infections for thousands of years in traditional medicine (Hee Lee *et al*., 2015).

With the emergence and increase of microbial organisms resistant to multiple antibiotics, and the continuing emphasis on health-care costs, many researchers have tried to develop new, effective antimicrobial reagents free of resistance and cost. Such problems and needs have led to the resurgence in the use of Ag-based antiseptics that may be linked to broad-spectrum activity and far lower propensity to induce microbial resistance than antibiotics. The antibacterial effects of Ag salts have been noticed since antiquity, and Ag is currently used to control bacterial growth in a variety of applications, including dental work, catheters, and burn wounds. In fact, it is well known that Ag ions and Ag-based compounds are highly toxic to microorganisms, showing strong biocidal effects on as many as 12 species of bacteria including E. coli (Kim *et al*., 2006).

The mechanism of the bacterial effect of AgNP as proposed is due to the attachment of AgNP to the surface of the cell membrane, thus disrupting permeability and respiration functions of the cell. It is also proposed that AgNP not only interact with the surface of a membrane but can also penetrate inside the bacteria. The antibacterial activity of AgNP is significantly enhanced when it is modified with sodium dodecyl sulfate (SDS) (Maiti *et al*., 2014).

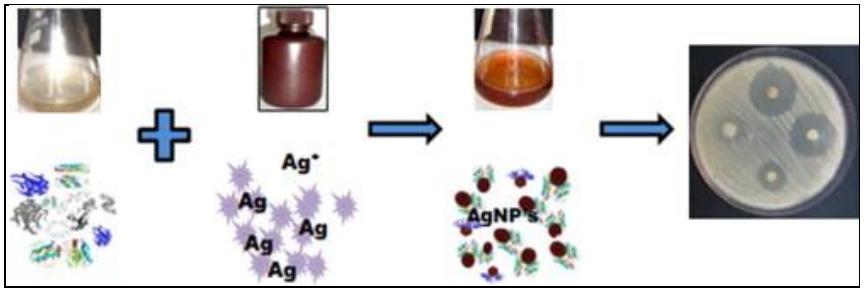
Several chemical methods have been developed for the synthesis of silver nanoparticles including chemical reduction, aqueous solution chemical reduction, nonaqueous chemical reduction, the template method, electrochemical reduction, ultrasonic-assisted reduction, photo-induced or photo-catalytic reduction, microwave assisted synthesis, irradiation reduction, the microemulsion method, biochemical method etc., but these chemical methods have been reported along with various

drawbacks, including the use of toxic solvents, generation of hazardous by-products, and high energy consumption, which pose potential risks to human health and to the environment. Currently, there is a growing need to develop an environment friendly nanoparticle synthesis that does not use toxic chemicals in the process of its synthesis. The microbial-mediated biological synthesis of metallic nanoparticles has recently been recognized as a promising source for mining nanomaterials. The microbial recovery of precious metals with the formation of their nanoparticles is a green alternative to the conventional method. Biosynthesis of silver nanoparticles using bacteria, fungi, and plants are already well-documented. However, the exploration of actinomycetes has recently gained interest for the efficient biological synthesis of metallic nanoparticles. The bonding reaction between the antibiotic and nanoparticles enhances the inhibition effect against the test organisms. The antibiotic molecules contain many active groups such as hydroxyl and amide groups, which react easily with nanosilver by chelation, and helps in effective inhibition (Gandhi *et al*., 2016).

Xanthones are naturally-occurring compounds with a distinct chemical structure, known as tricyclic aromatic system, with known antibacterial properties. Natural compounds with antibacterial properties may be applied to treat local infections, wounds and lesions difficult to heal, circumventing antibiotic resistant pathogens with multidrug resistance (MDR) genes, or may be combined with antibiotics to increase their effect. Therefore, studies on bacteria inhibition in vitro or in vivo have been performed on a wide array of natural compounds and peptides. In microbiology, the minimum inhibitory concentration (MIC) is the lowest concentration of a chemical that prevents visible growth of bacterium, whereas the minimum bactericidal concentration is the concentration that results in the microbial death (Narasimhan *et al*., 2017).

Figure 3

Antimicrobial activity of silver nanoparticle



2.14. Antioxidant activity

Reactive oxygen species (ROS) in the forms of superoxide anions, hydroxyl radicals and hydrogen peroxide are generated from the auto-oxidation of lipids, as well as reactive nitrogen species (RNS). Formations of these excess ROS and RNS by Ultraviolet (UV) irradiation, smoking and drug metabolisms are likely to damage several cellular components such as lipids, proteins, nucleic acids, and DNAs through the oxidation or nitration processes. In addition, these reactive oxygen species cause inflammation or lesions on various organs and are associated with various degenerative diseases, including cancer, ageing, arteriosclerosis, and rheumatism.

All aerobic organisms, including human beings, have antioxidant defences that protect against oxidative damages, numerous damage removal and repair enzymes to remove or repair damaged molecules (Mohadjerani *et al*., 2012).

The health protective effect of natural products such as fruits and vegetables is mostly related to their antioxidants, phenolic compounds, and to a lesser extent, dietary fiber (Avila *et al*., 2008).

Durian fruit contain a considerable amount of phenolics, flavonoids and carotenoids and their quantitative fingerprint shows differences in the proportions because of various locations of origins, agro climatic conditions and dreadful diversity in species. The importance of this fruit is mostly connected with its composition and antioxidant properties (Ashraf *et al*., 2010).

Phenolic acids are antioxidant molecules that are in the limelight of clinical and epidemiological research because their demonstrated value as the antioxidant components of fruits and vegetables. These foods also contain a wide variety of antioxidant bioactive compounds (carotenoids, vitamins, among others) that provide health benefits to consumers. Mango (*Mangifera indica* L.) fruit is an excellent source of dietary antioxidants, such as ascorbic acid, carotenoids, and especially phenolic compounds (Carlos *et al*., 2012).

3.0. MATERIALS AND METHODS

Traditional medicine has remained as the most affordable and easily accessible source of treatment in the primary health care system of resource poor communities. The local people have a long history of traditional plant usage for medicinal purposes. The medicinal use of plants is very old. The writings indicate that therapeutic use of plants is as old as 4000 - 5000 B.C. and Chinese used first the natural herbal preparations as medicines. In India, however, earliest references of use of plants as medicine appear in Rig-Veda, which is said to be written between 1600 - 3500 B.C. Later the properties and therapeutic uses of medicinal plants were studied in detail and recorded empirically by the ancient physicians (an indigenous system of medicine) which are a basic foundation of ancient medical science in India.(Hosseinzadeh *et al*.,2015) .

Medicinal plants have a promising future because there are about half million plants around the World, and for most of their medical activities have not investigate yet and their medical activities could be decisive in the treatment of present or future studies (Hassan *et al*., 2012).

Durian is a very popular tropical fruits and often referred as the king of tropical fruits in South East Asia. Durian has a special shape and nutrient content. It possesses strong aroma and unique taste (Belgis *et al* ., 2016).

3.1. COLLECTION OF SAMPLE

The Durian fruit was collected from Oot

FIGURE 4

3.1.1. Preparation of methanolic extract

10g of Durian fruit pulp was weighed and cut into small pieces and ground with 100 ml of methanol. And the mixture was allowed to stand over night in a shaker at room temperature. Then the methanol extract was filtered using a Whatmans No.1 filter paper. Stored at 4˚C for further use.

3.1.2 Preparation of ethanol extract

10g of Durian fruit pulp were weighed and cut into small pieces and ground with 100ml of ethanol. And the mixture was allowed to stand over night in a shaker at room temperature. The ethanol filtered using a What man's No.1 filter paper. Stored at 4˚C for further use.

3.1.3 Preparation aqueous extract

10g of Durian fruit pulp was weighed and cut into small pieces and ground with 100ml of aqueous. And the mixture was allowed to stand over night in a shaker at room temperature. The aqueous filtered using a Whatman's No.1 filter paper. Stored at 4˚C for further use.

3.2.ANTIOXIDANTS

3.2.1. Enzymatic antioxidants

Enzymatic antioxidants such as superoxide dismutase, catalase, peroxidase, glutathione S-transferase of Durian fruit extract was estimated.

3.2.1.1Superoxide dismutase

Estimation of superoxide dismutase in pulp of the Durian fruit was done by the method Misra and Fridovich, (1972)

3.2.1.2Catalase

Estimation of catalase present in the pulp of the durian fruit was done by the method of Luck (1974)

3.2.1.3.Peroxidase

Estimation of peroxidase of pulp of the durian fruit was done by the method of Reddy *et al.,* (1995)

3.2.1.4. Glutathione S-transferase

Glutathione S-transferase activity of Durian fruit pulp was followed by the method Habig *et al.,* (1974) involving 2, 4 dichloronitrobenzene as substrate by spectrophotometrically.

3.3.1. Non enzymatic antioxidants

Non enzymatic antioxidants are ascorbic acid, α-tocopherol, reduced glutathione, polyphenols, flavanoids present in durian fruit extract were assayed.

3.3.1.1Ascorbic acid

Activity of ascorbic acid was determined by using a method by Rou and Kuether (1943).

3.3.1.2. Alpha -tocopherol

Activity of the α-tocopherol by Durian fruit pulp was described by Rosenberg (1992)

3.3.1.3. Reduced glutathione

Reduced glutathione activity of pulp of Durian fruit was determined by the method by Moron *et al.,* (1979).

3.4. SYNTHESIS OF SILVER NANO PARTICLE

Aqueous solution of silver nitrate (1mm) was prepared and used for the synthesis of silver nano particle. 10 ml of the extract was added into 90ml of aqueous solution of silver nitrate for reduction into Ag+ ions

3.4.1. Heating in water bath

The silver nitrate solution and sample were incubated at room temperature for about 72 hours (Paulkumar *et al.,* 2014).

The methanolic extract of Durian fruit indicate the presence of silver nitrate which heated at different time period (5,10,15 and 20 minutes) in a water bath and the temperature was maintained at 60°C(Gulcin *et al.,*2011; Mubarakali *et al.,*2011).

3.4.2. Heating in microwave

The methanolic extract of Durian fruit with silver nitrate solution were heated in a microwave at different time limit namely 10, 20, 30, and 40 seconds (Nooroozi *et al.,* 2012).

3.4.3. Exposure to sunlight

The methanolic extract of durian fruit with silver nitrate solution were exposed to sunlight at different time period such as 5, 10,15 and 20 minutes(Sulaiman *et al.,*2013).

3.5. CHARACTERIZATION

Characterization of silver nano particle is important in order to evaluate the functional aspects of synthesized particle. Characterization is performed using a variety of analytical technique including UV vis spectroscopy, X- ray diffractometry (XRD), FTIR,, SEM- EDX, Zeta potential

3.5.1.SCANNING ELECTRON MICROSOPE (SEM)

Nanoparticle synthesis was characterized using high resolution scanning electron microscopy. Scanning electron microscopy is discussed in light of its principle. The morphology of the nanoparticles was identified by scanning electron microscope

3.5.2. Zeta potential

Zeta potential is a measure of the magnitude of the repulsion or attraction between particles. Its measurement brings detailed insight into the dispersion mechanism and is the key to electrostatic dispersion control. The measurement of zeta potential is an extremely important parameter across a wide range of industries including brewing, ceramics, pharmaceuticals, medicine, mineral processing and water treatment (Khoshnevisan *et al* ., 2015).

3.5.3. UV visible spectroscopy

Uv spectroscopy is routinely used in analytical chemistry for quantitative determination of different analytes. The light nanoparticle exhibits unique and optical properties on account of their surface Plasmon resonance (SPR).

3.5.4. X -ray diffraction (XRD)

A primary use of the technique is the identification and characterization of compounds based on their diffraction pattern and also used for the phase identification of a crystalline material and can provide information on unit cell dimensions.

3.5.5. Fourier transform infra red (FTIR) spectroscopy

FTIR is an analytical technique used to identify organic (and in some cases inorganic) materials. This technique measures the absorption of infrared radiation by the sample material versus wavelength. The infrared absorption bands identify molecular components and structure. FTIR analysis is done to obtain the infrared spectra of absorption, emission and to ensure the formation of nanoparticle.

3.5.6. ENERGY DISPERSION X-REAY SPECTROSCOPY (EDAX)

It is an analytical technique used for elemental analysis or chemical characterization of a sample. The reduced silver solution of aqueous extract of Durio zebenthinus was dried and drop coated on to the carbon grid which was used for the EDAX analysis.

3.6. TEST MICROORGANISM

The bacterial and fungal strains were got from Microlab Coimbatore. The bacterial strains are *staphylococcus aureus, pseudomonas aeruginosa, staphylococcus haemolyticus , Eschericia coli* The fungal strains are *candida glabarata* and *candida tropicalis.*

3.7.ANTI BACTERIAL ACTIVITY

3.7.1. Agar well diffusion method

The silver nano particle synthesized from the pulp of the Durian fruit was dissolved in a known amount of dimethyl sulphoxide. 20ml of Muller Hinton agar was poured in to the petriplates then it is allowed to solidify and the well was cut using gel puncture in the sizes of 6 mm diameter. Then 20 µl of sample and chloramphenicol was impregnated in to the well. The plates were kept in 37˚c for 24 hours. The obtained zone of inhibition around the well was measured in millimeters (NCCLS, 2000).

3.8. ANTIFUNGAL ACTIVITY

3.8.1Agar plug method

Potato dextrose agar medium was prepared and poured into the petriplates and allowed to solidify. A fungal plug was placed in the centre of the plate. Sterile disc immersed in the fruit extract were also placed in the agar plate. Nystatin was used as the antifungal control. The antifungal effect was seen as crescent shaped zones of inhibition (Schlumbaum *et al*., 1986).

4.0. RESULTS AND DISCUSSION

Medicinal plants and herbs have been proved to be of great importance to the health of the individuals and communities. In recent years, many scientific investigations of traditional herbal remedies for several diseases have been carried out and this has lead in the development of alternative drug and therapeutic strategies. Since the consumption of medicinal plants is increasing, it is interesting to use these plants as a supplement in food taking into account that these plants can present a significant amount of trace elements and other nutrients (Prabhavathi *et al*., 2016).

Plant extracts, with their reducing properties are responsible for the reduction of metal compounds into their respective nanoparticles in cheaper ways for nanoparticles synthesis. These approaches have many advantages over chemical, physical, and microbial synthesis because there is no need of the elaborated process of culturing and maintaining the cell, hazardous chemicals, high-energy requirements, and wasteful purifications. Several researchers developed and reported metallic nanoparticles using various plants and their extracts such as *Jatropha curcas* (Latex), *Clove, Brassica, Morganella, Cinnamon zeylanicum* (Bark extract), *Jatropha cruces* (seed) etc. Biosynthesis using leaves extracts of *Murraya koenigii Eucalyptus hybrida* , *Artocarpus heterophyllus*, *Camellia Sinensis*, *Mollugo nudicaulis* , *Panicum virgatum* and fruit extracts of *Capsicum annum* L, *Carica papaya*. L *Citrullus colocynthis* and *Lantana camara* have been reported so far. But information on silver nanoparticle synthesis using papaya leaves at room temperature is scanty. Hence the present work focused on to study the synthesis of silver nanoparticles using papaya leaves and to evaluate its antibacterial activity against pathogenic bacterial strains (Sridevi *et al*., 2015).

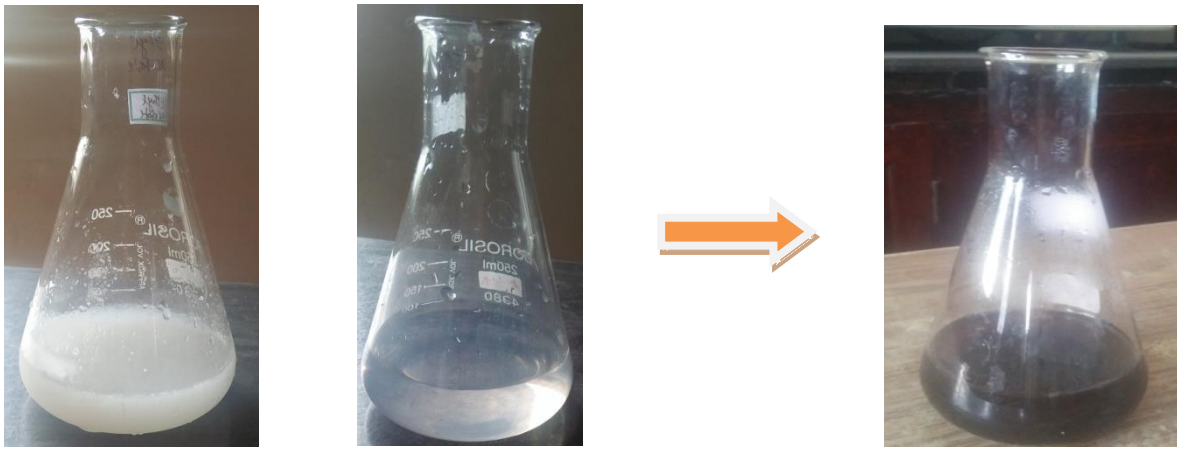
Biological synthesized nanoparticles are new generation antibiotics in the field of medicine because of their good conductivity, chemical stability, catalytic and antibacterial activity. The procedures using for the synthesis of nanoparticles should be clean, non-toxic and ecofriendly. Nanoparticles are relatively new in modern medicine. Silver has a long and intriguing history as an antibiotic in human health care. The antimicrobial properties of silver have been known to cultures all around the world for many centuries. The silver ion is biologically active and readily interacts with proteins, amino acid residues, free anions and receptors on mammalian and eukaryotic cell membranes (Swathi *et al*., 2014).

In this present study the aqueous extract of Durio zebenthinus was used to synthesize silver nanoparticle

4.0. SYNTHESIS OF SILVER NANOPARTICLE FROM ETHANOLIC EXTRACT OF *Durio zebenthinus*

FIGURE 5

Durian fruit extract $AgNo_3$ solution synthesized Ag Np



The extract of Durian fruit with silver nitrate solution was subjected to more than 25-30 minutes in the sunlight, that gives the maximum yield of silver nanoparticle was observed. In this method the hydrated electrons (e- aq) behaves as strong reducing agent and can reduce Ag ions (Ag+) into zero valent Ag atoms (Ag°). The result is same which is observed by Bhumi *et al*., (2015) when the leaf extract of *Adhatoda vasica nees.* was added to the silver nitrate solution, The leaf extract was added to 1 mM $Ag(NO_3)_2$ solution. The color change of the solution from yellow to brown indicated that the AgNPs were synthesized from the leaf. The formation of AgNPs is Green method. It involves the formation of the atom by the nucleation process; which is followed by the formation of nanoparticles by the aggregation. In the nucleation process, the hydrated electrons (e-aq) behaves as strong reducing agent and can reduce Ag ions (Ag+) into zero-valent Ag atoms (Ag°).

4.2.CHARACTERIZATION OF SILVER NANOPARTICLE OF *Durio zebenthinus*

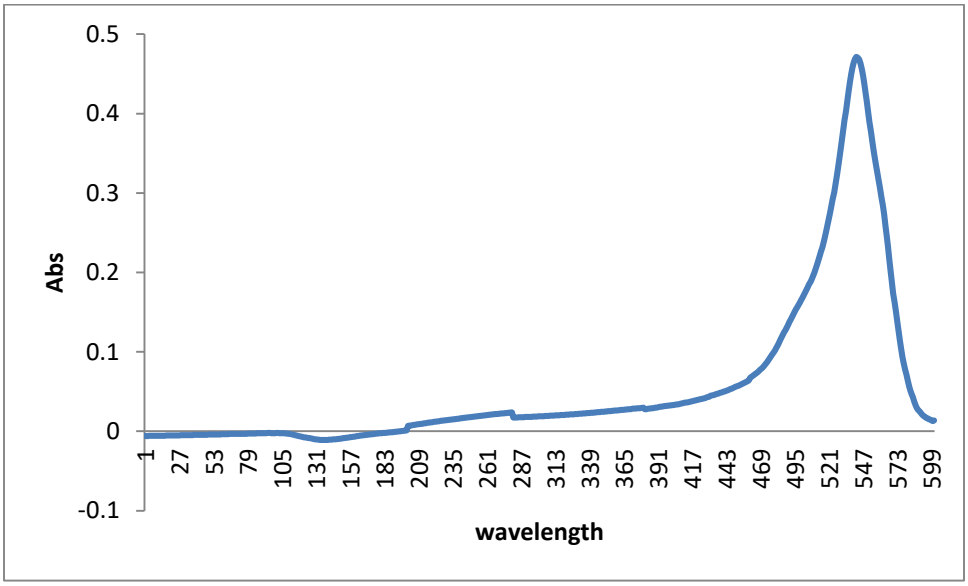
Silver nanoparticle synthesized from durian fruit were characterized for UV-visible, Fourier transform infrared (FTIR), SEM, EDAX, Zeta potential, X-ray diffraction.

4.2.1. UV-VISIBLE SECTROSCOPY

Ultraviolet – visible spectrometry was used to examine the size and shape of the nanopartile in aqueous suspension.

UV-VISIBLE SPECTRA OF SYNTHESIZED SILVER NANOPARTICLE OF AQUEOUS EXTRACT OF Durio zebenthinus

FIGURE6



The reduction of the silver nanoparticle from pure silver ions was visualized by the help of UV visible spectra.The absorption spectrum of the synthesized silver nanoparticle from Durio zebenthinus shown in the figure 6. The absorbance was measured in the range of 400-600 nm.

Anadalakshmi *et al*., (2016) reported that the formation of the AgNPs during the reduction process is indicated by the change in the colour of the reaction solution from colorless to dark brown which can be visually observed by UV visible spectroscopy. Metal nanoparticles have free electrons, which yield a surface Plasmon resonance (SPR) absorption band, due to the mutual vibration of electrons of metal nanoparticles in resonance with light wave. The appearances of the peaks show the characteristics of surface plasmon resonance of silver nanoparticles.

Pak *et al*., (2016) Due to surface plasmon resonance (SPR) phenomena, resonant peak occurs at a variety of wavelengths for various NPs mixture and according to the theory of resonance

maximum wavelength, is absorbed at resonant wavelengths. The previous studies suggest that a usual AgNP shows SPR patterns at wavelengths in the range of 400–480 nm. In his study the surface Plasmon resonance pattern of silver nanoparticle produced from *Dracocephalum moldavica* Seed extract was obtained at the wavelength of 443 confirming the presence of AgNPs.

Showmya *et al*., (2012) suggested that the reaction mixtures developed an array of colour after 3 hours of incubation under dark condition (to minimize photoactivation of silver nitrate), at 37°C temperature In UV-VIS spectroscopy the peak was observed in the range of 475 nm. The visual observation and UV-VIS spectra were thus indicative of nanoparticles synthesis. Silver nanoparticles are known to display vivid colour due to the phenomenon of Surface Plasmon Resonance

Similar results were expressed by Kumar *et al*., (2013) The solution turned to brown color indicating the for-mation of AgNPs. UV–Vis spectra of silver nano particles giving a plasmon resonance band at 410 nm

According to Ojha et al., (2017) The color change of the silver nitrate solution from transparent to golden yellow color was observed after addition of leaf extract at an optimal ratio. This color change is due to the excitation of surface plasmonic vibrations of silver nanoparticles. Absorbance spectra of all the prepared solutions containing varying ratios of leaf extract (10 μg ml−1) and silver nitrate (1 mM). A peak at 442 nm wavelength indicated the SPR of the synthesized silver nanoparticles in the solution containing leaf extract and silver nitrate at 1:3 ratio. The SPR peak reached its maxima at 430 nm after 24 h of addition of all the components which articulates maximum reduction of silver ions after 24 h. UV–Vis absorption spectrum of the reaction mixture was measured for 24 h at 2 h interval. From the absorbance spectra it has been observed that the absorbance of nanoparticles intensified with time at 430 nm.

4.2.2. FOURIER TRANSFORM INFRARED SPECTROSCOPY

Fourier transform infrared spectroscopy is an analytical technique used to identify organic materials. The technique measures the absorption of infrared radiation by the sample material versus wavelength.

FTIR SPECTRUM OF SYNTHESIZED SILVER NANOPARTICLE OF AQUEOUS EXTRACT OF *Durio zebenthinus*

FIGURE 7

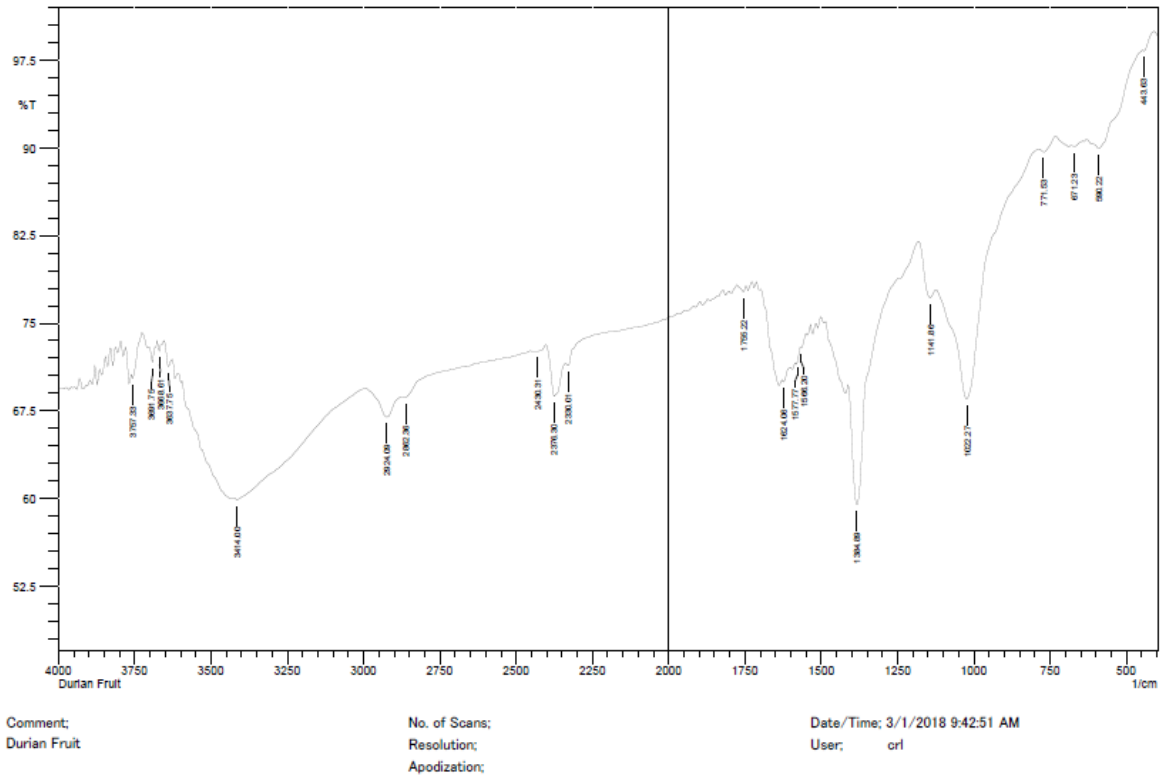


Figure shows that the sharp FTIR spectrum of silver nanoparticle synthesized from aqueous extract of Durian fruit located at 443.63, 590.22, 771.53, 1022.27, 1384.89, 1624.06, 1755.22, 2330.01, 2430.31, 2862.36, 3414.00, 3757.33 cm-1

It shows a broad strong band from 3132cm-1 to 3414 cm-1indicating the prescence of OH group and 678.55 cm-1 – 960 cm-1 indicate the presence of =C-H functional groups. peak at 721.4 cm-1 indicate methylene functional group. The band region of 1377.23 – 1465.03cm-1 can be ascribed to the bending vibration of C-H methyl groups. peak at wavenumber 1745.89 cm-1 which is strongest in the spectrum is attributed to C=O groups with the stretching mode of vibration. The peaks at 2850.41 cm-1 and 2924.32 cm-1 indicate symmetric and asymmetric stretching vibrations of C-H alkane groups respectively.

Bagyalakshmi *et al*., (2017) Reported that**,** In the *Pterocarpus marsupium* bark and wood FTIR spectrum strong absorption peaks at 3693.01 and 3413 indicates OH stretching due to the

presence of alcohol and phenol. Peaks at 1619.91 and 1530.24 indicates C=C stretching of α, β, unsaturated ketone and N-O stretching of nitro compounds absorption peaks at 1384.64, 821.527 and 786.815 and 727.996 indicates the presence of alkanes, alkenes, and aromatic rings. In the FTIR spectra of silver nanoparticles band between 3700-3584 corresponds to OH stretching of free alcohol, 3550- 3200 corresponds to hydrogen bonded alcohols and phenols, 2830-2695 corresponds to CH stretching in aldehyde, 1720-1706 C=O stretching in carboxylic acid, 1550-1500 corresponds to nitro compounds. From the FTIR spectral analysis it is concluded that hydroxyl and carboxyl groups present may act as reducing and stabilizing agent and phenolic group present may act as capping agent.

Similar results were reported by Khan et al., (2018) FT-IR spectrum of synthesized AgNPs located at about 663.52 cm-1, 1401.31 cm-1, 1642.41 cm-1 and 3361.02cm-1. Absorption peaks at 663.52 cm-1 assigned to C-Cl stretching for halogen compounds, 1401.31 cm-assigned to C-O stretching for alcohol and phenols,1642.41 cm-1 assigned to C=O stretching for tertiary amides 3361.02 cm-1 assigned to O-H stretching for alcohols and phenols. IR spectroscopic study confirmed that carbonyl group form amino acid residues and proteins has the stronger ability to bind metal, could possibly form a layer covering the metal nanoparticles (i.e., capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium. These results suggest that the biological molecules perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium.

Anandalakshmi1 *et al*., (2016) repoted that FTIR measurements were carried out to identify the possible biomolecules in the Pedalium murex extract. FTIR spectra of dried aqueous extract and synthesised AgNPs. The phytochemical analysis of Pedalium murex reveals the presence of flavonoids, alkaloids, steroids, rosins, saponins and proteins. In leaf extract, the peaks are observed at 445, 617, 1075, 1287, 1421, 1602, 3157 and 3785 cm-1,respectively. After reaction with AgNO3, the peaks are shifted to a higher wave number side, such as 456, 614, 1074, 1382, 1592, 3158 and 3881 cm-1. The peak at 445 cm-1 of the extract is shifted toward a higher wave number side at 456 cm-1 due to the O–Si–O network and ring opening vibration. The band observed at 617 cm-1 is shifted to the lower side at 614 cm-1, which corresponds to C–Cl stretching in the alkyl group.

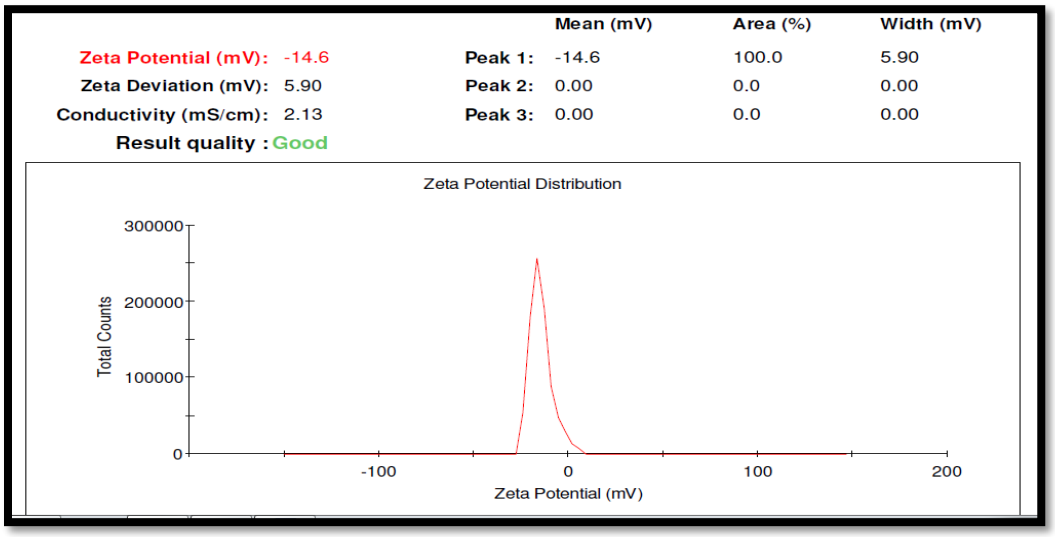
4.2.3. ZETA POTENTIAL

Zeta potential is an essential parameter for the characterization of stability in aqueous nanosuspensions. A minimum of ±30 mV zeta potential values is required for indication of stable nanosuspension. At 48 h of stirring time, the zeta potential was equal to 35.5 ± 3.7 mV. So, this result clearly indicated that the particles are fairly stable due to the electrostatic repulsion. Shameli et al., (2012).

The zeta potential of the synthesized silver nanoparticles of Durian fruit is shown in the figure

ZETA POTENTIAL GRAPH OF SYNTHESIZED SILVER NANOPARTICLE OF AQUEOUS EXTRACT OF *Durio zebenthinus*

FIGURE8



Zeta potential activity against the Durian fruit has more stability of size distribution. The zeta potential value of synthesized silver nanoparticle from aqueous extract of Durian fruit was found to be -14.6mV shows that size distribution has very strong aggregation.

Dispersions of both nanoparticle variations (NP1 and NP2) were sonicated for 20 min and diluted to final concentration of 75 _g/mL in phosphate buffer saline (PBS) pH = 7.4. The zeta potential of either pure nanoparticle variations NP1 or NP2 and *E. coli* cells were measured using a Zetasizer Nano ZS instrument.

Anandalakshmi *et al*., (2017) reported that the Ag NPs obtained have a negative zeta potential value. Zeta potential is a basic parameter for classification of stability in aqueous Ag NPs

suspensions. The Zeta potential measurements of the biosynthesised Ag NPs show a sharp peak at -13.5 mV indicative of that the surface of the nanoparticles is negatively charged. Generally, the zeta potential of the nanoparticles should be either highest than +30 mV on lower than -30 mV. But, the synthesized Ag NPs by *Vitex negundo* shows incipient instability.

4.2.4. X-RAY DIFFRACTION

The synthesized silver nanoparticle of Durian fruit was dried with the help of hot air oven so that a small amount of dry silver nanoparticle obtained for XRD. The crystalline nature of silver nanoparticle of *Durio zebenthinus* was confirmed by the analysis of XRD pattern and the results presented in figure 9.

XRD PATTERN OF SYNTHESIZED SILVER NANOPARTICLE OF AQUEOUS EXTRACT OF Durio zebenthinus

FIGURE 9

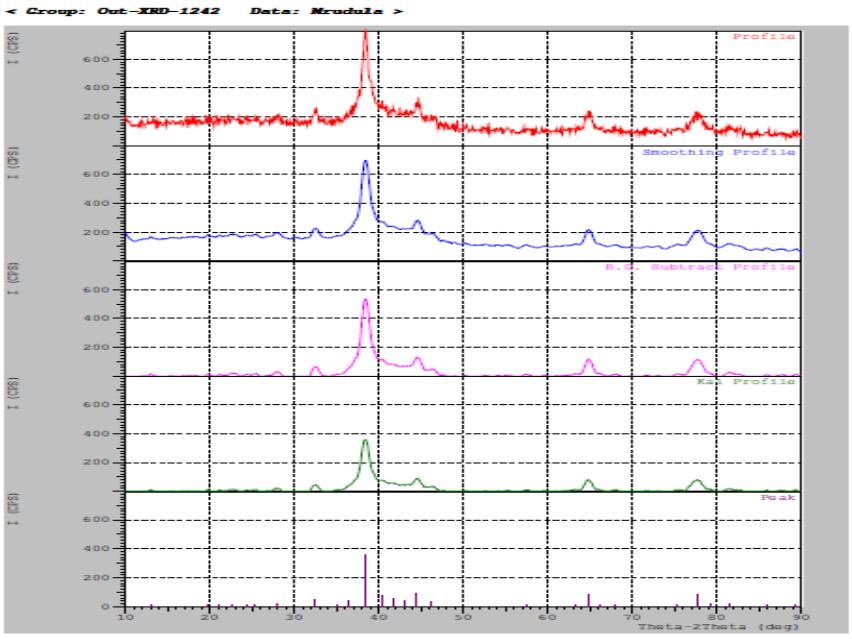


Figure shows the XRD pattern of synthesized silver nanoparticle. Diffraction peaks at (222), (250), (300), (311) were observed in the range of 30-80. The XRD pattern confirms that structure of nanoparticle is Face centered cubic (FCC) which is confirmed with jcpds card No-087-0720.

Kalainila et al., (2014) reported that the dried silver nanoparticles synthesized using *Erythrina indica* leaf extract at room temperature the XRD patterns of AgNPs indicated that the structure of silver

nanoparticles is face cubic center (fcc). In addition, the XRD peaks could be attributed to the crystallographic planes [38]. All the prominent peaks at 2 theta values of about 28º, 32º and 46ºrepresenting the (2 2 0), (3 1 1) and (4 2 0) Bragg's reflections of 'fcc' structure of silver. Hence, from the XRD result, it is clear that AgNPs formed using *Erythrina indica* leaf were essentially crystalline. The average Nano crystalline size has been estimated by using well known Debye–Scherrer formula, $D = K\lambda / \beta \cos\theta$ Where, D is particle diameter size, k is a constant (k=1), λ is wavelength of X-ray source (1.5405 nm), and βis the full width at half maximum (FWHM). The average crystalline size according to Debye–Scherrer equation calculated is found to be 8nm.

Similar results were observed in Umoren et al., (2014) The XRD spectrum showed four distinct diffraction peaks at 38.28 °, 44.33 °, 64.33 °, and 77.53 ° corresponding to lattice plane value indexed at (111), (200), (220) and (311) planes of face centered cubic (FCC) silver with a lattice parameter of a = 4.08 Å which were in good agreement with reference of FCC structure from joint committee of powder diffraction standard (JCPDS) Card No-087-0720.

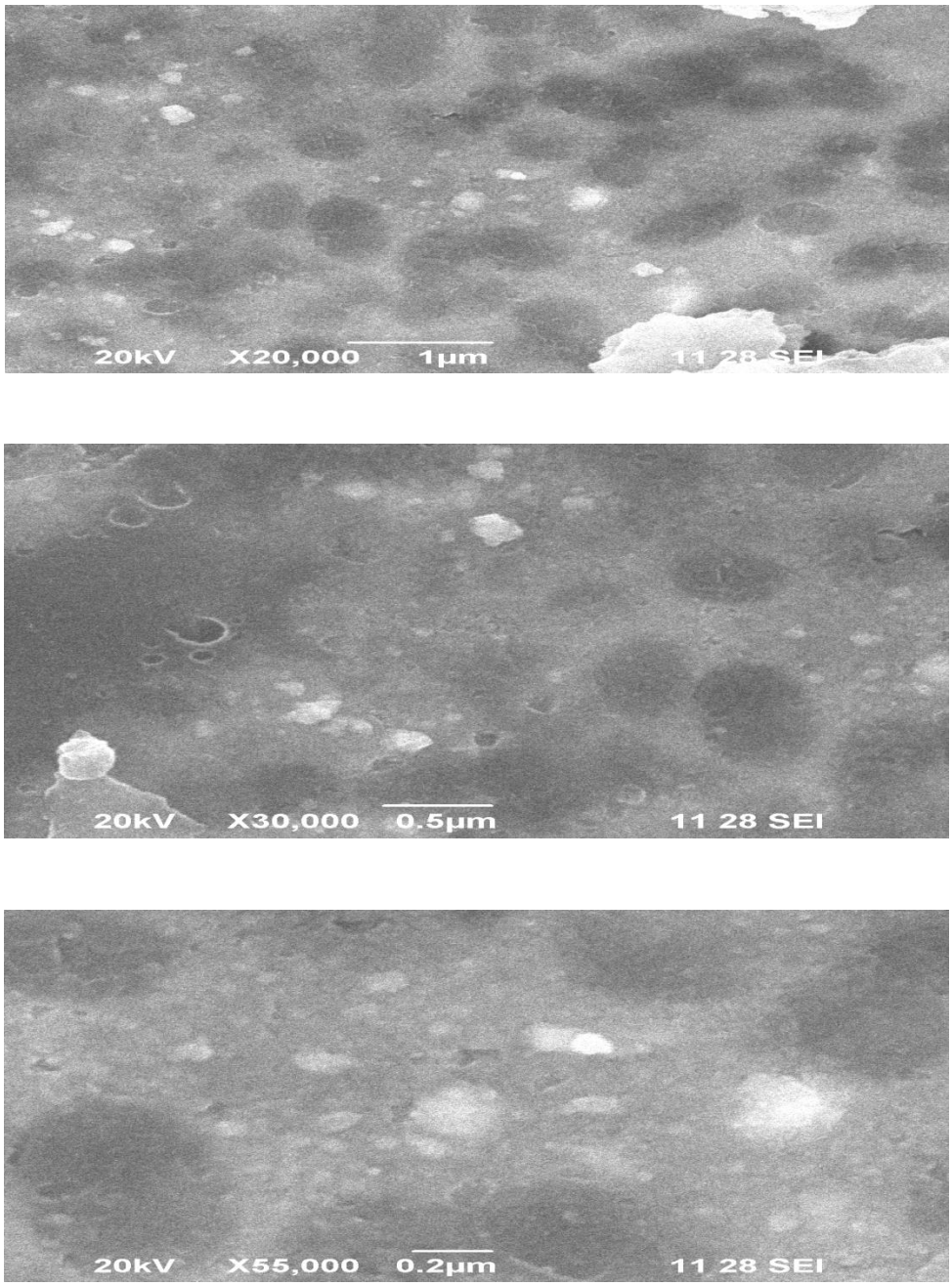
4.2.5. SCANNING ELETRON MICROSCOPY

Scanning electron microscopy is used to study the topography and composition of synthesized silver nanoparticle that were formed.

The synthesized silver nanoparticles were further characterized by SEM analysis where it was noted that the particles were predominantly spherical in shape, although other than the spherical shaped particles were also present. The particle size around 100nm (figure). The different sizes of particles may be correlated with the variable shapes. Our results are similar to those reported by Alkammash *et al*., 2017

SEM IMAGES OF SYNTHESIZED SILVER NANOPARTICLE

FIGURE 10



In the present study, the biosynthesis of silver nanoparticles was successfully obtained using a green method of preparation. This method used showed that selected plant can be used as an effective stabilizing reducing agent for the synthesis of AgNPs. The methodology employed here is very simple, easy to perform inexpensive, eco-friendly and provide an improved alternative to chemical synthesis. The formed AgNPs are highly stable spherical shaped particles, (when observed

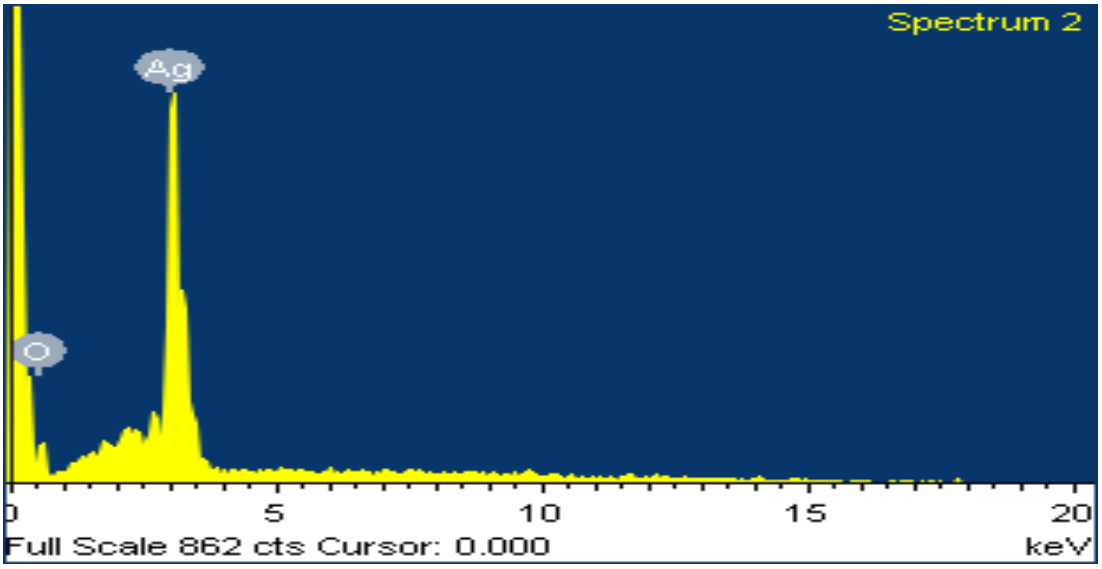
under the SEM). It is hoped that novel and improved silver nanoparticles will be produced in further studies.

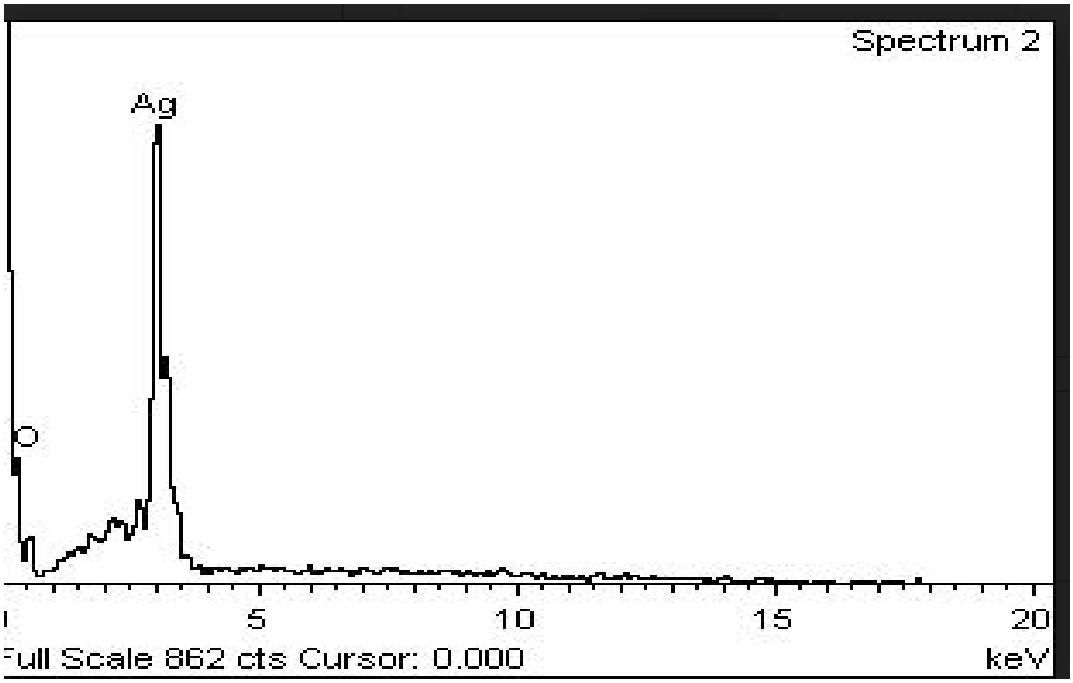
With the help of the SEM the morphology of the silver nanoparticle were analyzed , the SEM image were shown in the figure. The silver nanoparticle were roughly spherical in shape. The average size of the synthesized silver nanoparticle from the aqueous extract of *Durian* fruit is less than around 100 nm. Under different magnification the synthesized nanoparticle were examined such as 20000, 30000, 55000 X. the particle aggregation was clearly viewed in the 55000X.

4.2.6. ENERGY DISPERSIVE X-RAY ANALYSIS (EDAX)

EDAX IMAGE OF SYNTHESIZED SILVER NANOPARTICLE OF AQUEOUS EXTRACT OF Durio zebenthinus

FIGURE 11





From the graph obtained by the EDAX analysis, It is well known that silver nanoparticles show typical optical absorption peak approximately at 3 KeV due to Surface Plasmon Resonance . showed the absorption peak at 3 KeV regions which revealed that nanoparticles were formed exclusively highest proportion of silver with crystalline nature. The graph which confirms the nanostructure of the silver nanoparticle of the extract of *Durio zebenthinus*. It contain nearly 86.04wt.% Ag, 13.96 wt.% oxygen (O).

Kumara *et al*., (2017) reported that EDX spectrum reveals strong signal in the silver region and confirms the formation of silver nanoparticles. It is well known that silver nanoparticles show typical optical absorption peak approximately at 3 KeV due to Surface Plasmon Resonance , showed the absorption peak at 3 KeV regions which revealed that nanoparticles were formed exclusively highest proportion of silver with crystalline nature.

Manjula *et al*., (2016) reported that EDAX provides the supporting confirmation for the formation of silver nanoparticle. EDAX Spectrum showed the signal for silver which confirmed the presence of silver nanoparticles. The signal was observed at 3Kev, which is typically for silver nanoparticles due to the surface Plasmon resonance. The other spectral signals such as O, K, Ca, Ag, Si, Cl, and Al were the noticed in the EDAX spectrum. And also rereported that EDAX spectrum of silver nanoparticles synthesized from the leaves Jasminum sambac(Mogra).Showed signal for Ca, K, Cl, O, Mg, and Si both these observation were confirmed the presence of common spectral signals found in both Amorphophallus campanulatus and *Jasminum sambac*.

4.3. ANTIBACTERIAL ACTIVITY

Medicinal plants are abundant source of antimicrobial molecules. A wide range of medicinal plants extracts are used to treat several infections as they have potential antimicrobial activity. Some of these bioactive molecules are screened and traded in market as raw material for many herbal industries. Experts turned their concentration back towards obtaining advantages from medicinal plants after observing more side effects of synthetic drugs compared to their benefits (Javid *et al*., 2015).

Higher plants as a natural source which give rise to a sorce of antimicrobial agent. The drugs are resistance to human pathogen against particular type of antibiotics. New antimicrobial organisms are derived from other sources. Medicinal plants are screened for antimicrobial activity to find a new substances for therapeutic purposes (Krithiga *et al*., 2014).

Synthesized silver nanoparticle from Durian fruit extract is dissolved in DMSO. The organism namely *staphylococcus aureus, pseudomonas, E.coli, staphylococcus haemolyticus*. The antimicrobial activity of silvernanoparticle synthesized from the extract of Durian fruit with gram positive and gram negative bacteria using the control as chloramphenicol. The zone of inhibition is measured in mm.

The assessment of antimicrobial activity of *Elettaria Cardamomom* from synthesized silver nanoparticle shows similar result against *B. subtilis* and *K. planticola* has minimum zone of inhibition (GnanaJobitha *et al*., 2012).the control chloramphenicol have the maximum zone of inhibition compared with *Elettaria Cardamomom* .

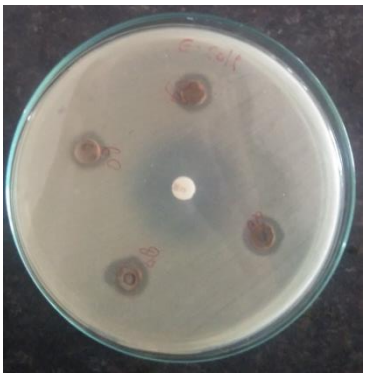
ZONE OF INHIBITION OF SYNTHESIZED SILVER NANOPARTICLE OF ETHANOL METHANOL AND AQUEOUS EXTRACT FROM Durio zebenthinus AGAINST MICROORGANISM

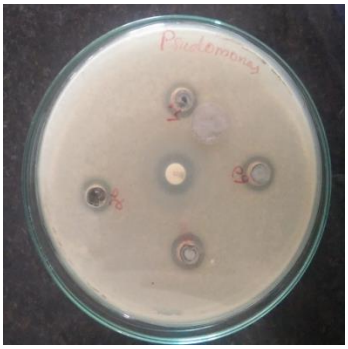
Table 1

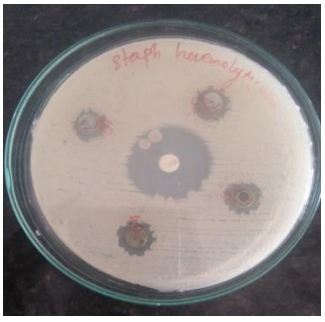
Sl.no	Microorganism	Extracts used	Zone of inhibition (in mm)				Control chloramphenicol
			20µl	40µl	60µl	80µl	
1	Escherichia coli	Aqueous	4	6	9	12	18
		Methanol	7	9	11	15	19
		Ethanol	4	6	8	11	17
2	Pseudomonas	Aqueous	8	10	14	16	21
		Methanol	3	8	13	17	20
		Ethanol	3	7	9	13	19
3	Staphylococcus auerus	Aqueous	6	11	16	19	25
		Methanol	5	9	14	21	24
		Ethanol	3	7	9	12	16
4	Staphylococcus haemolyticus	Aqueous	4	7	8	10	15
		Methanol	6	9	11	14	19
		Ethanol	3	5	7	12	17

PLATES 1

METHANOL ETHANOL AQUEOUS







Different concentration of silver nanoparticle synthesized from different extract of durian fruit have the effective antibacterial activity against gram positive and gram negative bacteria that confirms by the zone of inhibition in the plate 1. However the highest zone of inhibition was found

against S.aureus (21mm) in methanol extract while comparing with other microorganism in other extracts.

The silver nanoparticle undergo an interaction with the cell wall of the bacteria results in the bacterial death. The synthesized silver nanoparticle from cappris spinosa leaf extract exhibits an excellent antibacterial activity against Staphylococcus aureus, E.coli etc (Benakashani *et al*., 2016). Biosynthesis of silver nanoparticle from Durio zebenthinus have highest zone of inhibition with minimum concentration, so itcan be potentially eliminates the problem of disease causing pathogens. So which may have the greatest application to make them more biocompatible.

4.4. ANTIFUNGAL ACTIVITY

Among fungus, *Candida* species is the most common pathogen which is responsible for majority of fungal infections. Candida is a dimorphic fungus as it can exist in two forms, blastoconidial and hyphal form. biosynthesized silver nanoparticles using plant leaf extract and explored them for antifungal activity against three opportunistic human fungal pathogenic Candida species (Khatoon *et al*., 2015).

Anti fungal activity of silver nanoparticle produced from Aspeergillus niger shows maximum zone inhibition for Candida glaberata and candida species (Khan *et al*., 2016).

The silver nanoparticle synthesized from aqueous extract of durian fruit shows the maximum zone of inhibition. Minimum zone of inhibition shows the ethanol extract of durian fruit for both *candida tropicalis* and *candida glbarata*.

TABLE 2

ANTIFUNGAL ACTIVITY OF SILVER NANOPARTICLE SYNTHESIZED FROM Durio zebenthinus USING NYSTATIN AS CONTROL

FUNGI	EXTRACTS			CONTROL Nystatin
	Methanol	Ethanol	Aqueous	
Candida glaberata	2	*5*	*7*	*5*
|Candida tropicalis	6	*4*	*9*	*5*

The zone of inhibition is highest in the aqueous extract in the candida glaberata and candida tropicalis, 7mm and 9mm respectively. And the minimum inhibition is in the methanol extract in candida glaberata and ethanol extract in candida tropicalis, so the durian fruit can be used to identify the new antibiotic to control the candida species.

PLATE 2

Candida glabarata *candida tropicaliS*

4.5. ANTIOXIDANT ACTIVITY OF *Durio zebenhinus* FRUIT

It is generally accepted that reactive oxygen species (ROS) and/or free radicals play an important role in the development of tissue damage and pathological events in living organisms. This is often attributed to the antioxidants in the fruits and vegetables such as vitamin C, E, carotenoids, lycopenes and flavonoids that prevent free radical damages. attention has been focused on the investigation of antioxidants that can scavenge ROS, especially natural antioxidants, phenolic

and flavonoids from plants which are mostly used as protective agents against free radical-mediated diseases (Semiz et al., 2007).

Free radicals play an important role in development of tissue role and pathological events in living organisms. There are evidences that explain that increased uptake of fruits and vegetables reduce the risk of cancer. This is attributed by antioxidants present in fruits and vegetables (Leelaprakash *et al*., 2011).

Natural products from dietary components such as Indian species and medicinal plants are known to possess antioxidant activity. Increasing intake of dietary antioxidants may help to maintain an adequate antioxidant status and, therefore, the normal physiological function of a living system. To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system (Chirag *et al*., 2013).

4.5.1. ENZYMATIC ANTIOXIDANT ACTIVITY OF Durio zebenthinus FRUIT

The endogenous antioxidants are categorized into two group namely enzymic and non enzymic antioxidants. The activity of the enzymatic antioxidant such as catalase, peroxidase and superoxide dismutase were determined and given in the table below

TABLE 3

Enzymic antioxidant activity of *Durio zibenthinus*

S.No	Enzymatic antioxidant	Fresh fruit sample
1	Catalase	2.037±0.91
2	Peroxidase	0.495±0.63
3	Superoxide dismutase	4.037±0.15

From the above table it is clear that the Durio zebenthinus fruit was found to exhibit significant activities of catalase 2.037±0.91 U/g in the fresh sample. The present study indicates that *durio zebenthinus* has considerable catalase activity in the fruit.

The present study also indicates that the fruit of *Durio zebenthinus* was found to be exhibits significant activities of superoxide dismutase 4.037±0.15in the fresh sample and also exhibits the significant activities of peroxidase 0.495±0.63 in the fresh sample.

Similar results were observed in Suresh *et al*. (2014) the leaves of *Artemisia niligirica* exhibited the maximum activities of Glutathione reductase and Superoxide dismutase when compared to other enzymic antioxidants.

According to Abrahim *et al*., (2012) The ethyl acetate extracts of P. betle (64 µg/ml) was incubated with the MCF-7 cells for 24 and 48 h for determination of SOD activities. MCF-7 cells treated with the plant extract showed a time-dependent increase in SOD activities, almost doubling at the 48 h incubation point compared to untreated cells.

4.5.2. Non-enzymic antioxidant activity of Durio zebenthinus fruit.

The non enzymic antioxidants are present mostly on natural products and they have significant role in the cellular system against reactive oxygen species. Ascorbic acid, alpha tocopherol , phenols these antioxidants are rich in fruits and other nutritional suppliments. The activity of non-enzymic antioxidants was determined and given in the table.

Non-enzymic antioxidant activity of *Durio zebenthinus*

TABLE 4

S.No	Non-enzymicAntioxidant	Fresh fruit sample
1	Ascorbic acid	9.01±0.206
2	Alpha tocopherol	10.01±0.83
3	Phenol	1.99±0.69
4	Reduced glutathione	20.15±0.15

Table 6 shows the level of Ascorbic acid found in Durio zebenthinus and it was found to be 9.01±0.20 U/g in the fresh sample. The alpha tocopherol content of the Durio zebenthinus was found to be 10.01±0.83 U/gin the fresh sample. The reduced glutathione content of the Durio zebenthinus

was found to be 20.15±0.15U/g in the fresh sample . and the phenol content of the *Durio zebenthinus* is1.99±0.69U/g respectively.

Racchi *et al*., (2013) reported that Tocopherols are lipid-soluble antioxidant synthesized by all plants. They protect lipids and other membrane components by scavenging and quenching various ROS and lipid by-products of oxidative stress. Out of four isomers of tocopherols present in plants, α-tocopherol has the highest antioxidant activity and represents the major vitamin E compound; it is the only tocopherol absorbed efficiently by humans. α-Tocopherol is located in the chloroplast envelope and thylakoid membranes; high levels have been found in leaves of many plant species.

Poovadorom *et al*., (2010) reported that it was found a similarity 0.08, ± in acetone extracts between durian and mango in the contents of polyphenols (1.66 0.05, mg GAE g ± 1.48^{-1} DW, respectively), Durian and avocado were similar in the contents of polyphenols, and ABTS and DPPH values in water and in methanol extracts, respectively. Based on the obtained results the nutritional and bioactive properties of durian are comparable with those indices in mango and avocado. In conclusion, durian can be recommended as a part of disease prevented diets.

According to Asharaf *et al*., (2011) The amount of vitamin C found in the durian extract samples varied widely among varieties. The concentration of vitamin C was found maximum in D11 variety (25.13mg/L) and that of least in Ang Jin (18.87mg/L). Based on these findings, the order of vitamin C contents in various varieties of durian was as follows; D11 > Chaer Phoy > Yah Kang > Ang Jin. Statistical analysis showed the significant ($p < 0.05$) variation in vitamin C with respect to durian varieties.

4.6. GAS CHROMATOGRPHY-MASS SPECTROMETRY (GC-MS)

Gas chromatography- mass spectrometry is an analytical method that combines the features of gas chromatography and mass spectrometry to identify substances within a test sample.

GRAPHIC REPRESENTATION OF GCMS RESULT OF METHANOLIC EXTRACT OF Durian FRUIT

FIGURE 12

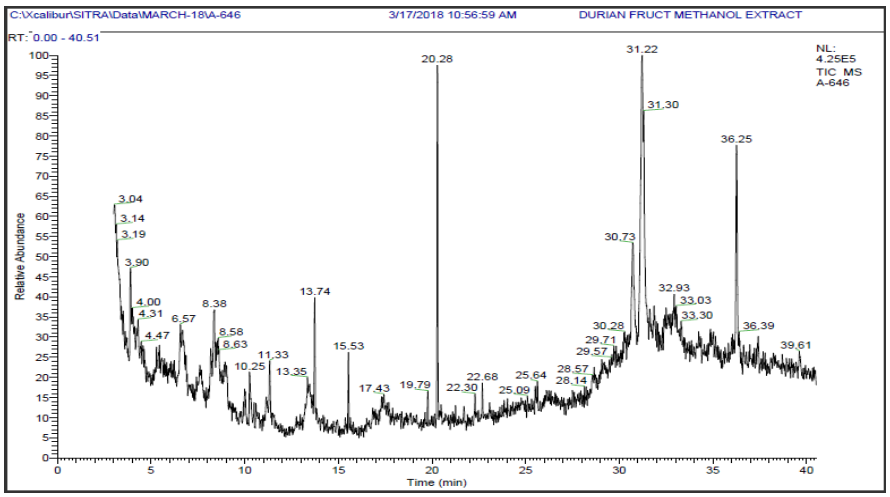


TABLE 5

GCMS ANALYSIS FOR DIFFERENT COMPOUNDS PRESENT IN METHAOL EXTRACT OF *Durio zebenthinnus*

Peak	Retention time	Name of the compounds	Probability	Area %
1.	8.36	Methyl 13c hexadecatrienoate	21.14	9.29
2.	11.33	Ethenol, 2-ethoxy-, acetate	11.14	2.95
3.	13.74	Cyclohexyl(2-methylenecyclohexyl)methanol	53.06	3.03
4.	17.43	5-Isoxazolecarboxylic acid, 4,5-dihydro-3,5-dimethyl-, methyl ester, (s)	15.81	2.44
5.	20.24	Isopropyl myristate	71.58	8.87
6.	25.62	Pregn-4-ene-3,20-dione, 17,21-dihydroxy-, bis(O-methyloxime)	24.69	1.04
7.	31.22	3-Oxo-20-methyl-11-à-hydroxy-N-demethylconanine-1,4,20-triene	73.03	20.66
8.	36.25	13-Docosenamide	41.56	8.18

From the results in the table above, it can't be exactly determined how much amount of the concentration of these compounds. But the results of the curve can be calculated the area of each peaks. Comparison between the area of each peak area to the total area of the graph as a whole generate data % area as shown in the table 5. The percentage of this area shows how much the content of these compounds in the samples tested. The data above shows that the 3-Oxo-20-methyl-11-à-hydroxy-N-demethylconanine-1,4,20-triene compound is 73.03% majority of the total compounds contained in the sample. And the second major compound is Isopropyl myristate have 71.58%.

The result of methanol extract of durian fruit was analyzed using Gas Chromatography-Mass Spectroscopy (GC-MS). From the library was obtained 8 peaks, the first peak has a higher area, ie peak to 8. Compound at the peaks 8 appeared at a retention time of 36. 25 minutes by area% 41.56.

GRAPHIC REPRESENTATION OF GCMS RESULT FOR ETHANOLIC EXTRACT OF DURIAN FRUIT

FIGURE 13

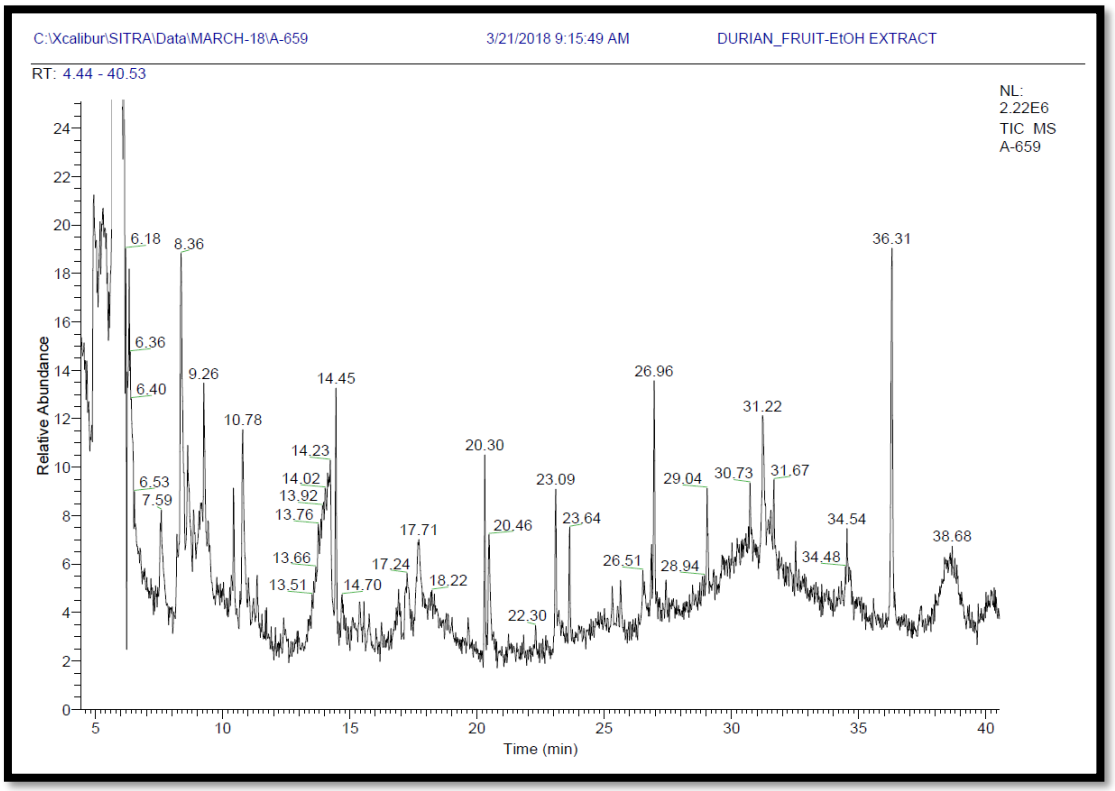


TABLE 6

GCMS ANALYSIS FOR DIFFERENT COMPOUNDS PRESENT IN ETHANOL EXTRACT OF Durio zebenthinus

Peak	Retention time	Name of the compounds	Probability	Area %
1.	5.71	á-Terpinyl acetate	8.09	25.55
2.	8.38	Dihydro-5-(1-hydroxyethyl)-2(3H)-furanone	19.93	4.37
3.	14.17	à-D-Glucopyranoside, O-à-D-glucopyranosyl-(1.fwdarw.3)-á-D-fructofuranosyl	23.85	7.15
4.	17.71	5-Nitro-3-methyl-2-cyanomethylpyridine	16.77	1.57
5.	25.09	Hexadecanoic acid, ethyl ester	66.67	0.90
6.	26.96	Heptadecene-(8)-carbonic acid-(1)	4.61	2.30
7.	31.24	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	36.59	1.93
8.	36.29	13-Docosenamide	63.12	4.67

From the results in the table above, it can't be exactly determined how much amount of the concentration of these compounds. But the results of the curve can be calculated the area of each peaks. Comparison between the area of each peak area to the total area of the graph as a whole generate data % area as shown in the table 5. The percentage of this area shows how much the content of these compounds in the samples tested. The data above shows that the 13-Docosenamide compound is 63.12% majority of the total compounds contained in the sample.

The result of ethanol extract of durian fruit was analyzed using Gas Chromatography-Mass Spectroscopy (GC-MS). From the library was obtained 8 peaks, the first peak has a higher area, ie peak to 8. Compound at the peaks 8 appeared at a retention time of 36. 29 minutes by area% 63.12. Illing *et al*., (2017) reported that From the results in the table above, it can't be exactly determined how much amount of the concentration of these compounds. But the results of the curve can be

calculated the area of each peaks. Comparison between the area of each peak area to the total area of the graph as a whole generate data % area . The percentage of this area shows how much the content of these compounds in the samples tested. The data above shows that the beta-sitosterol compound is 61.95% majority of the total compounds contained in the sample.

4.7. HIGH PERFORMANCE THIN LAYER LIQUID CHROMATOGRAPHY

Thin Layer Chromatography (HPTLC) is a sophisticated, reliable, efficient and automated form of TLC having the latest technical developments for quality assessment and evaluation of botanical materials. A chromatographic fingerprint of extract represents a chromatographic pattern of pharmacologically active or chemically characteristic constituents present in the extract.

Different composition of the mobile phase for HPTLC were tested and the desired resolution of compounds, together with symmetrical and reproducible peaks, was achieved using ethyl acetate >formic acid >acetic acid > water in the ratio of 10:1.1:1.1:2.6 as the mobile phase.

HPTLC chromatogram pattern of standard flavanoids and extracts of Durian fruit

FIGURE 14

Before derivatization

254nm 366nm white remission

After derivatization

254nm

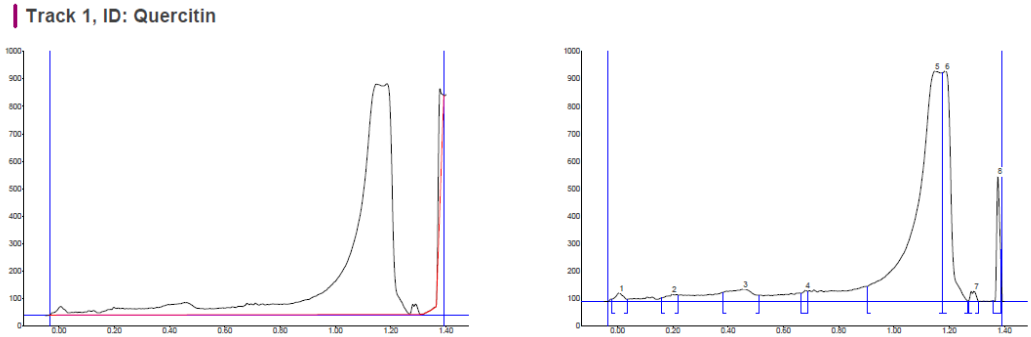
366nm

Lane A: Quercetin, B:Ngn std, C:EAE, D:ME, E:AE, F:EE

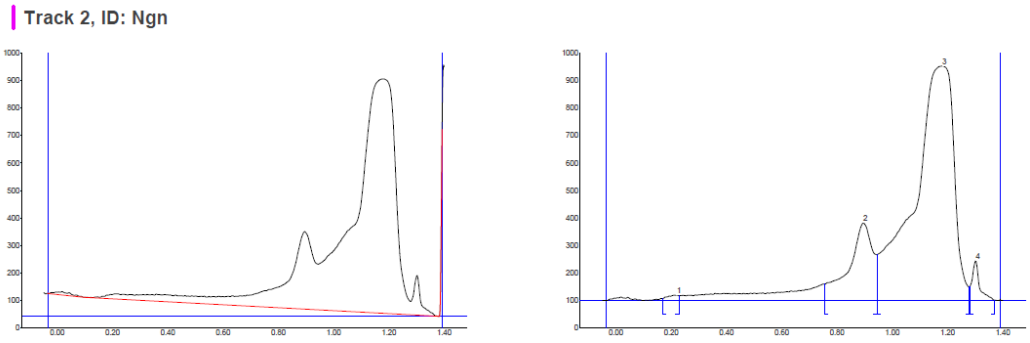
FIGURE 15

PEAK DESTINOGRAM OF HPTLC ANALYSIS

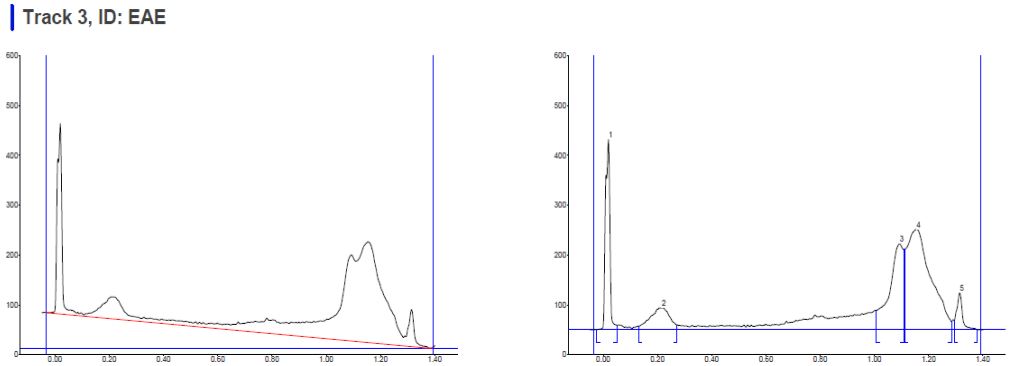
Peak densitogram of standard flavonoid mixture (Quercetin)



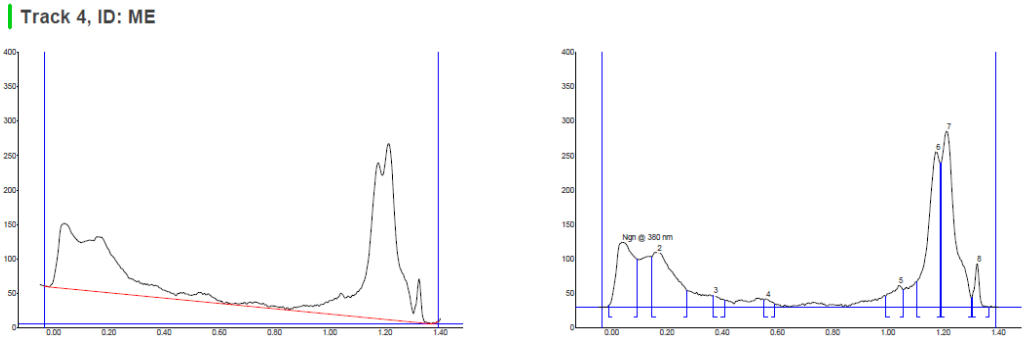
Peak densitogram of standard flavonoid mixture (Ngn)



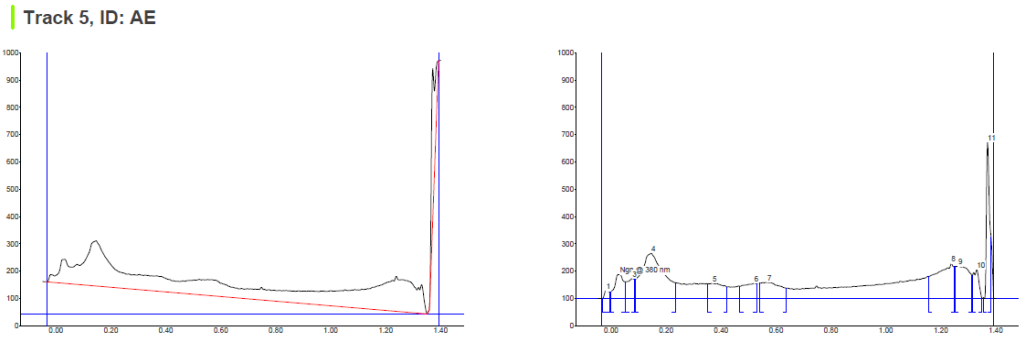
Peak densitogram of standard flavonoid profile of EAE extract



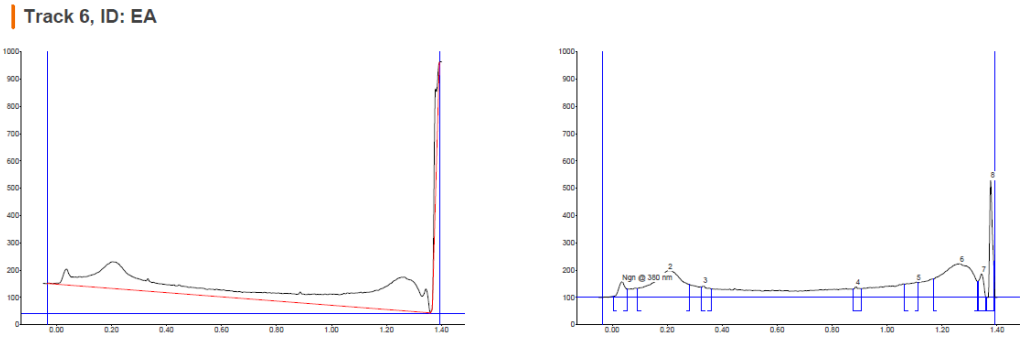
Peak densitogram of standard flavonoid profile of ME extract



Peak densitogram of standard flavonoid profile of AE extract



Peak densitogram of standard flavonoid profile of EE extract

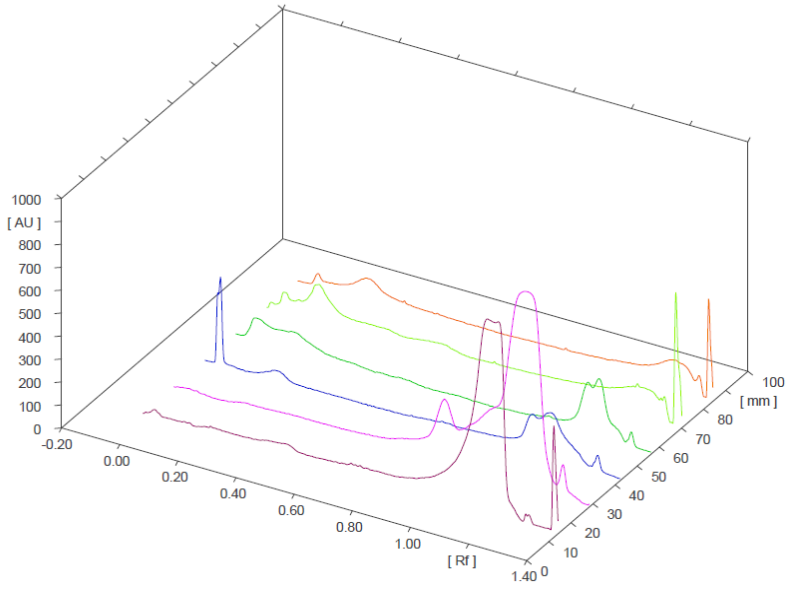


The figure shows the flavanoids profile of ethyl acetate, Methanol, Aqueous, Ethanol extracts against the flavanoid standards. The Rf value of standard of flavanoid Ngn was found to be maximum on 1.18 out of 4 peaks. The standard flavanoid Rf value was found to be 0.04 out 8 peaks in the methanol extract. The flavanoid standard was found in methanol extract at 200 nm.

The standard flavanoid Rf value of aqueous extract was found to be 0.03 out of 11 peaks stamdard flavanoid found at 200 nm. Then the standard Rf value of Ethanol extract was found to be 0.04 out of 8 peaks at 322 nm

HPTLC chromatogram of *Durio zebenthinus* extracts: All tracks at wavelengths.

FIGURE 16



The above figure shows the HPTLC fingerprint also revealed the the presence of flavonoids in methanol, aqueous, and ethanol extracts.

Annapandin *et al*., (2017) HPTLC studies showed blue, greenish blue and yellowish green coloured fluorescent zones at UV 366 nm after derivatization. These fluorescent zones confirmed the presence of flavonoids in the L. aspera leaf extracts. The order of presence of flavonoids (fluorescent zone) in L. aspera extract was ethanol > isopropyl alcohol > ethyl acetate > chloroform > petroleum ether. The Rf value of the extracts were found to be 0.61, 0.66, 0.67, 0.70, 0.76 and 0.77 of the peaks. Out of 10 peaks 6 were identified as flavonoid compounds. The complete details of Rf values and area of the peaks were presented in Table 3. Rutin was used as the standard compound for flavonoid identification.

Nile *et al*., (2014) The densitometric scan of each extract was done using rutin as the reference compound. The range 200–400 nm (with intervals of 20 nm) was monitored, with the cut-off filter set at 370, 420, 450 and 550 nm. Because the plant extract fingerprint is highly complex, and due to the difficulty of meeting commercial standards, detection was done at 300 nm and the

cut-off filter was at 550 nm which corresponds to the maximum intensity of fluorescence and a condition selective for the fluorescence yellow bands corresponding to rutin derivatives.

5.0. SUMMARY AND CONCLUSION

Medicinal plants are capable of preventing, relieving or curing diseases. Herbal medicines are the end product of technological transformation operations such as division, classification, extraction, purification, concentration, and drying that is performed on the plant materials to obtain medicinal drugs. The Medicinal plants are now universally recognized as the basis for a number of critical human health, social, and economic support systems and benefits. India is the one of the largest producers of medicinal plants globally. Various forms of alternative medicinal practices like Siddha, Ayurveda and Unani for the treatment of several diseases.

The current study has been focused on the "Green synthesis of silver nanoparticle from Durian fruit (*Durio zebenthinus*) and its antimicrobial and antioxidant properties".

With the increasing demand for advances in diagnosis and treatment modalities, nanotechnology is being considered as a groundbreaking and viable research subject. Nanotechnology plays an important role in the area of medicine and health care system. For example dendrimers are a type of nanostructure that can be precisely designed and manufactured for a wide variety of applications, including the treatment of cancer and other diseases. The nanotechnology also plays an important role in the field of pharmaceutical industries.

The silver nanoparticles was synthesized using by exposure to sunlight. The silver nanoparticle were synthesized and which is confirmed by the color change from clear solution to dark brown color and also by increase in the absorbance. There are other methods also present for the synthesis of the nanoparticle , but in the present study only one method, exposure to sunlight is followed to the synthesis of the silver nanoparticle. Got maximum yield of nanoparticle synthesis obtained when the aqueous extract of *Durio zebenthinus* was subjected to sunlight for more than 30 minutes.

The synthesized silver nanoparticle were subjected to absorption spectrum in UV visible range and it showed the characteristic peak between 400-600 nm. The peak indicates the synthesis of silver nanoparticle increase with increase in the time of exposure.

The SEM and EDAX results shows the morphology and where the nanoparticle were roughly spherical shape and found to be aggregates at nanoscale. The XRD analysis confirms the nature of the silver nanoparticle synthesized from the *Durio zebenthinus* fruit extract. The FTIR analysis also confirms the formation or synthesis of the silver nanoparticle. Zeta potential for check the stability in aqueous nanosuspension, and the *Durio zebenthinus* fruit shows the good source of stability. The stability of the durian fruit is -14.6mV.

The antioxidant activity of *Durio zebenthinus* was assayed for both enzymatic and non-enzymattic antioxidants. The enzymatic antioxidants such as catalase, peroxidase, superoxide dismutase was moderately present in the *Durio zebenthinus*. The non – enzymatic antioxidants such as ascorbic acid, alpha tocopherol, phenol reduced glutathione which were found to be moderately.

The silver nanoparticle synthesized from the aqueous, methanol and ethanol extract of *Durio zebenthinu* is carried out for the antibacterial activity against the bacterial strains such as *pseudomonas, staphylococcus aureus, Escherichia coli* and *staphylococcus haemolyticus*. Showed the minimum zone of inhibition.

The synthesized silver nanoparticle from aqueous, methanol and ethanol extract of durian fruit shows the minimum zone of inhibition against the fungal strains such as *Candida glaberata, Candida tropicalis.*

The HPTLC analysis were carried out to confirm the presence of flava The result showed the presence of flavonoids against reference standard flavonoid (Rf = 0.04, 0.03, 0.04) and 8 unknown flavonoid, present in the fruit of *Durio zibenthinus* . methanol, aqueous and ethanol extract respectively.

The GCMS results shows the presence of various compounds present in the methanol and ethanol extracts of *Durio zebenthinus*.

Based on the result, can be concluded that fruit extract of *Durio zebenthinus* have great potential of antimicrobial capacity against *staphylococcus aureus, pseudomonas, staohylococcus*

haemolyticus, Escherichia coli. . Due to their antioxidant and antimicrobial activities of *Durio zibenthinus* has been potential as a source of natural antioxidant and antimicrobial agents.

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APPENIX - I

ANTIBACTERIAL ASSAY

Preparation of medium

Muller Hinton Agar medium

The medium was prepared by dissolving 13.56g of the Muller Hinton Agar in 800ml of distilled water. Various type of extracts in the several bacterial strains was assayed by agar well diffusion method. The medium was autoclaved at 15 lbs pressure at 121°C for 1 hour. The autocalved medium poured on to the petriplates (20-30 ml/plate).

Preparation of the test culture

Inoculums of the microorganism were prepared from overnight culture growth in nutrient broth and the suspension was adjusted with a turbidity equivalent to that of 0.5 MacFarland standards.

Agar well diffusion method

Principle

The antimicrobials are allowed to diffuse out into the medium and interact in a plate freshly fruit extract with the test microoraganism. The resulting zone of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

Procedure

Petriplates containing 20ml of Muller Hinton Agar medium of extracts were inoculated by preparing form a nutrient broth that has been incubated for 6 hours, when the growth in the logarithmic phase, 100µl were spread in plates. Wells were cut in the agar and different µl of extracts were added. The plates were incubated at 37°C for 24 hours. The antibacterial activities were assayed by the diameter of zone of inhibition formed around the well (NCCLS, 1993). Chloramphenicol was used as standard antibacterial control agent.

ANTIFUNGAL ASSAY

Agar plug method

Principle

The fungicidal effect of plant extract of the plant extract can be assessed by the inhibition of the mycelia growth of the fungus and is observed as a zone of inhibition near the disc or the well.

Reagents

1. Potato dextrose Agar medium
   The commercially available (HiMedia) potato dextrose agar medium (39g) was suspended in 1000ml of distilled water. The medium was dissolved completely by boiling and then autoclaved at 15 lbs pressure (121°C) for 15 minutes.
2. Nystatin (standard antifungal agent)

Procedure

Potato dextrose agar medium was prepared and poured on to the petriplates. A fungal plug was placed in the centre of the plate. Sterile disc immersed in the fruit extract were also placed in the plates. Nystatin was used as a antifungal control. The antifungal effect was seen as crescent shaped zone of inhibition (schlumbaum et al., 1986).

APPENDIX - II

ESTIMATION OF CATALASE ACTIVITY

(Luck, 1974)

Principle

The UV light absorption of H_2O_2 can be easily measured between 230-250nm. On decomposition of hydrogen peroxide by catalase, the absorption decreases with time. The enzyme activity can be estimated by this decrease in absorption.

Reagents

1. Phosphate buffer: 0.067M (pH 7.0)

Dissolved 3.522g of KH_2PO_4 and 7.268g of $KHPO_4.2H_2O$ in distilled water and made up the volume to one liter.

2. Hydrogen peroxide in phosphate buffer (2mM)

Dissolved 0.16ml of hydrogen peroxide (10%W/V) to 100ml phosphate buffer, prepared fresh. The absorbance of the solution should be about 0.5 at 240nm with 1cm light path.

Procedure

A 20% homogenate of the plant sample were prepared in phosphate buffer (0.006M, pH 7.0) and the homogenate was employed for the assay. The sample were read against a control without homogenate, but containing the H_2O_2- phosphate buffer. To the experimental cuvette, 3ml of H_2O_2 -phosphate buffer was added, followed by the rapid addition of 40µl enzyme extract and mixed thoroughly. The time interval required for a decrease in absorbance by 0.05 units was recorded at 240nm. The enzyme solution containing H_2O_2 free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240nm by 0.05 units.

Calculation

Calculated the concentration of the H_2O_2 using the extinction coefficient 0.036µ mole/ml.

APPENDIX - III

ESTIMATION OF PEROXIDASE ACTIVITY

(Reddy *et al.*, 1995)

Principle

Peroxidase catalyses the conversion of H_2O_2 to H_2O and O_2, in the presence of the hydrogen donor pyrogallol. The oxidation of coloured product called purpurogalli can be measured spectrophotometrically at 430nm with the specified time interval. The intensity of the product is proportional to the activity of the enzyme.

Reagents

1. Pyrogallol (0.05M in 0.1M phosphate buffer, pH 6.5)

   630 mg of pyrogallol in100 ml of 0.1 M phosphate buffer.

2. H_2O_2 (1% in 0.1M phosphate buffer)

Procedure

The plant samples were prepared as 20% homogenate in 0.1M phosphate buffer (pH 6.5) and used for the assay. Pyrogallol solution (3.0 ml) and enzyme extract (0.1 ml) were pipetted out into a cuvette. The spectophotometer was adjusted to read zero at 430nm followed by the addition of 0.5 ml of 1% H_2O_2 and mixed. The change in absorbance was recorded every 30 seconds upto 3 minutes.

Calculation

Change in absorbance / min	=	x
Weight of the plant material taken	=	300 mg
Volume of the extract taken for the assay	=	0.02 ml
Change in absorbance for 1.5 ml extract	=	(X / 0.02 ml) x 1.5 - Y
(i.e) peroxidase activity in 300 mg plant tissue	=	Y
Peroxidase activity / g plant tissue	=	Y x (1000 / 300) units

APPENDIX - IV

ESTIMATION OF POLYPHENOL OXIDASE (PPO)

(Esterbauer *et al.*, 1977)

Principle

Phenol oxidases are copper proteins of wide occurrence in nature, which catalyes the aerobic oxidation of phenolic substrates to quinines, which are auto oxidized to dark brown

pigments generally known as melanins, which can be estimated spectro-photometrically at 495nm.

Reagents

1. Tris HCl (50mM, pH 7.2)
2. Sorbitol (0.1M)
3. NaCl (10mM)
4. Catechol (0.01M) in phosphate buffer (0.1M. pH 6.5).

Procedure

The leaves were homogenized in about 20 ml medium containing 50 mM Tris HCl, pH 7.2, 0.4M sorbitol and 10mM NaCl. The homogenate was centrifuged at 2000rpm for 10 minutes and the supernatant was used for the assay. The assay mixture contained 2.5ml of 0.1M phosphate buffer and 0.3 ml of catechol solution (0.01M). The spectrophotometer was set at 495nm. The enzyme extract (0.2ml) was added to the same cuvette and the change in absorbance was recorded every 30 seconds up to 5minutes.

Enzyme unit = K x (A/ min)

Where,

K for catechol oxidase = 0.272

K for laccase = 0.242

APPENDIX - V

ESTIMATION OF SUPEROXIDE DISMUTASE ACTIVITY

(Misra and Fridovich, 1972)

Principle

Superoxide dismutase uses the photochemical reduction of riboflavin as oxygen generating system and catalyses the inhibition of nitro blue tetrazolium (NBT) reduction, the extent of which can be assayed spectrophotometrically at 600nm.

Reagents

1. Potassium phosphate buffer (500 mM, pH 7.8)
2. Methionine (450 µM)
3. Riboflavin (50 mM)
4. Nitro blue tetrazolium NBT(840µM)
5. Potassium cyanide (200 µM)

Procedure

The incubation medium contained a final volume of 3.0 ml, 50 mM potassium phosphate buffer (pH 7.8), 45µM methionine, 5.3mM riboflavin, 84µM NBT and 20µM potassium cyanide. The amount of homogenate added to this medium was kept below one unit of enzyme to ensure

sufficient accuracy.

The tubes were placed in an aluminium foil-lined box maintained at 25ºC and equipped with 15W fluorescent lamps. After exposure to light for 10 minutes, the reduced NBT was measured spectrophotometrically at 600 nm. The maximum reduction was observed in the absence of the enzyme giving a 50% inhibition of the reduction of NBT.

APPENDIX - VI

ESTIMATION OF GLUTATHIONE S-TRANSFERASE ACTIVITY

(Habig *et al.*, 1974)

Principle

Glutathione S- transferase conjugates GSH with CDNB and the extent of conjugation is used as a measure of enzyme activity from the proportional change in the absorption at 340 nm.

Reagents

1. Tris HCl
2. K_2HPO_4 buffer (0.5 ml, 0.5 M, pH 6.5)
3. CDNB (25 mM)
4. 20 mM glutathione

Procedure

Ground about 5.0g of the sample in a medium and made upto 20ml with the medium

containing 50mM Tris HCl (pH 7.2, 0.4M sorbitol and 10mM NaCl). Centrifuged the homogenate at 2000 rpm for 10 min and used the supernatant for the assay. K_2HPO_4 buffer (0.5ml, 0.5M, pH 6.5) was taken in the tube and 0.1ml of CDNB (25mM) was added. Added

. ml of distilled water. Incubated the tubes at 3 ˚C for 0minutes. Then 0. ml of 0mM glutathione was added to the reaction mixture. Added 0.2ml of enzyme extract to the reaction mixture. Run a blank like test without the addition of enzyme. Measured the absorbance at 340nm. Glutathione S- transferase activity in the extract is expressed as µmoles of CDNB-GSH conjugate / min /mg protein.

APPENDIX -VII

ESTIMATION OF ASCORBIC ACID

(Roe and Kuether, 1943)

Principle

Ascorbate is converted to dehydroascorbate by treatment with activated charcoal or bromine. Dehydroascorbic acid then reacts with 2, 4 – dinitrophenyl hydrazine to form osazones, which dissolves in sulphuric acid to give an orange coloured solution. The coloured product can be measured spectrophotometrically at 540 nm.

Reagents

1. Trichloroacetic acid (4%)
2. Sulphuric acid (9N)
3. 2, 4 dinitrophenyl hydrazine reagent (2% in 9N sulpuric acid)
4. Thiourea solution (10%)
5. Sulphuric acid (85%)
6. Standard ascorbate solution: 10mg ascorbate in 100ml of 4% TCA.
7. Working standard solution: Diluted 10 ml of the stock solution to100ml with 4% TCA.

Procedure

The plant sample of 1g was taken and homogenized with 4% TCA to extract the ascorbate and the final volume was made up to 10ml with 4% TCA. The supernatant obtained after centrifugation at 2000 rpm for 10minutes was treated with pinch of activitated charcoal, shaken well and kept for 10 minutes. Centrifugation was repeated once again to remove the charcoal residue. The volume of the clear supernatants obtained were noted. Two different aliquots of the supernatant were taken for the assay (0.5ml and 1.0ml). The assay volumes were made up to 2.0ml with 4% TCA. A range of 0.2 to 1.0ml of the working standard solution containing

20 – 100g of ascorbate respectively were pipetted into clean dry test tubes, the volumes of which

were also made up to 2.0ml with 4% TCA, DNPH reagent (0.5ml) was added to all tubes. Followed by two drops of 0% thiourea solution. The osazones formed after incubation at 3 ˚C for 3 hours, were dissolved in 2.5ml of 85% H_2SO_4, in cold condition, to avoid an appreciable rise in temperature. To the blank alone, DNPH reagent and thiourea were added after the addition of H_2SO_4. After incubation for 30 minutes at room temperature, the sample were read at 540nm and calculate the content of ascorbic acid in the sample using standard graph.

APPENDIX – VIII

ESTIMATION OF α - TOCOPHEROL

(Emmerie- Engel 1938 method, as described by Rosenberg, 1992)

Principle

The estimation of tocopherol can be done using Emmerie- Engel reaction, based on the reduction of ferric to ferrous ions by tocopherols, which forms a red colour with , ‘ – dipyridyl. Tocopherols and carotenes are first extracted with xylene and read at 460 nm to measure carotenes. A correlation is made for this after adding ferric chloride and read at 520 nm.

Reagents

1. Absolute alcohol
2. Xylene
3. , ‘- dipyridyl (1.2 g in 1 litre of n- propanol)
4. Ferric chloride (1.2g in 1 litre of ethanol stored in brown bottle)
5. Standard solution

Dissolved 0mg 0mg of α-tocopherol, 10 mg/1L in absolute alcohol. 91mg of

α- tocopherol is equivalent to 100 mg of tocopherol acetate.

Procedure

The plant sample (2.5g) were homogenized in a small volume of 0.1N sulphuric acid and the volume was finally made upto 50ml by adding 0.1N sulphuric acid slowly, without shaking and the contents were allowed to stand overnight. The contents of the flask were shaken vigourously on the next day and filtered through Whatman No.1 filter paper. Aliquots of the filtrate were used for the estimation of tocopherol. The plant extract, standard and water of

1.5ml were pipetted out into three centrifuge tubes namely test, standard and blank respectively. To all the tubes, 1.5ml each of ethanol and xylene were added, stoppered, mixed well and centrifuged. After centrifugation, the xylene layer was transferred into another tube, taking care not to include any ethanol or protein. To .0 ml of xylene layer, .0 ml of , ‘ dipyridyl reagent was added, stoppered and mixed. This reaction mixture was taken in the spectrophotometric cuvettes and the extinction of the test and the standard were read against the blank at 460 nm. Then, in turn, beginning with the blank, 0.33 ml of ferric chloride solution was added, mixed well and exactly after 15 minutes, the test and the standard were read against the blank at 520nm. The levels of tocopherol can be calculated by using the formula,

$$\text{Amount of tocopherols in } \mu g = \frac{\text{Reading at 520nm} - \text{Reading at 460nm}}{\text{Reading of standard at 520nm}} \times 0.24 \times 15$$

APPENDIX - IX

ESTIMATION OF POLYPHENOLS

(Malick and Singh, 1980)

Principle

Phenols react with phosphomolybdic acid in Foli -Ciocalteau reagent in alkaline medium and produce blue coloured complex (molybdenum blue), which is read in a spectrometer at 650nm.

Reagents

1. 80% ethanol

2. Diluted Folin-Ciocalteau reagent

3. 20% Sodium carbonate

4. Stock solution:

100mg of catechol was made up with 100ml distilled water 5.
Working standard:

0ml of stock standard was diluted to 00ml. .0ml of this contains 00µg of catechol.

Procedure

1g of sample was homogenized using 20ml of 80% ethanol. The homogenate was centrifuged at 10,000rpm for 20 minutes. The supernatant was saved. The residue was reextracted with 10ml of 80% ethanol, centrifuged and collected the supernatant and evaporated to dryness. The residue was dissolved in a known volume of distilled water (50ml) and 2.0ml was taken for the experiment. A working standard of 0.5 – 2.5ml catechol solution corresponding to 50 - 0µg of catechol were pipetted out into a series of test tubes. The volume was made upto 2.5ml with water. To all the tubes added 0.5ml of diluted Folin–Ciocalteau reagent. After 3 minutes, added 2.0ml of 20% Na2CO3 solution to each tube and mixed thoroughly.

The tubes were placed in a boiling water bath for exactly one minute. Cooled and measured at 650nm against a reagent blank. Constructed a standard graph by plotting theconcentration of catechol on X-axis and absorbance on Y-axis.

From the graph, the amount of polyphenols present in the sample was estimated and expressed as mg of polyphenols per g of the sample.

APPENDIX - X

ESTIMATION OF REDUCED GLUTATHIONE

(Moron *et al.*, 1979)

Principle

Reduced glutathione (GSH) is measured by its reaction with 5, 5-dithio- 2 -nitrobenzoic acid (DTNB) Ellman‘s reaction to give a compound that absorbs at nm.

Reagents

1. DTNB

2. 5%TCA

3. 0.2M Sodium phosphate buffer

Procedure

1g of the sample was homogenized in 5%TCA to give a 20% homogenate. The precipitated protein was centrifuged at 1000rpm for 10 minutes. The homogenate was cooled on ice and 0.1ml of supernatant was taken for the estimation. The volume of the aliquot was made up to 1.0ml with 0.2M sodium phosphate buffer (pH 8.0), 2ml of freshly prepared DTNB solution (0.6mM) in 0.2M phosphate buffer (pH 8.0), was added to the tubes and intensity of the yellow colour formed was read at 412nm in a spectrophotometer after 10 minutes.

A standard curve of GSH was prepared using concentration ranging from 2 to 10 moles of GSH in 5%TCA.