International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Green Synthesis of Silver Nanoparticles and Its Antioxidant and Antimicrobial Activity

Miji T V[1], Aruna S Harry[2], G Goshitha[3], Liyana A[4], Sunaina S[5], Nabeesathul Shabna K[6], Ranjini B[7]

[1]Assistant Professor, Department of Biotechnology Mercy College, Palakkad-678006 Kerala, India
[2,3,4,56,7]Student, Department of Biotechnology, Mercy College, Palakkad-678006 Kerala, India

ABSTRACT

Nanotechnology is a standout amongst the most dynamic region of examine in the present day material science. The metallic nanoparticles have great attention of chemist, physicist, biologist, engineers who wish to use them for development of new generation nanodevices. Plant mediated synthesis of nanoparticles is a green chemistry approach that connects nanotechnology with plants. Silver nanoparticles are broadly utilised as a part of different research because of their unique physiochemical properties. These unique characteristics make them useful in various applications like antioxidant and antimicrobial activities. It has been realised that nanoparticles and its compounds have solid inhibitory and antimicrobial activities for microorganisms. By this present study, we will be able to understand that how silver nanoparticles of *Ocimum* leaf extract is important for antioxidant activity and its effectiveness against microorganisms.

INTRODUCTION

Over the past few years, the synthesis of metal nanoparticles is an important topic of research in modern material science due to their distinctive potential applications in the field of electronic, magnetic, optoelectronic, information storage and drug delivery. Nanocrystalline silver particles have been found tremendous applications in the field of high sensitivity biomolecular detection, diagnostics, antimicrobials, therapeutics, catalysis, micro-electronics. However, there is still need for economic commercially viable as well as environmentally clean synthesis route to synthesize the silver nanoparticles.

The broad spectrum of silver nanoparticles is produced by a variety of methods. In environmental substenance, there is a need to develop the environmental friendly procedures to avoid the toxic chemicals in the synthesis protocols to avoid adverse effect in medical applications. From recent results, researchers inspired on biological systems to develop benign nanoparticles using microorganisms, yeast and plant or plant extracts terme as green chemistry approaches. Group of researchers develop nanoparticles being extensively synthesized using various plant leaf extracts such as *Camellia sinensis, Magnolia kobus and Diopyros kaki leaf, Geranium leaf, Acalypha indica leaf, Coriandrum sativum, Sorbus aucuparia leaf, Gliricidia sepium, Rose leaf, Cinnamomum camphora, Aloe vera* and *Neem*.

Ocimum sanctum (local name Tulasi) is a traditional medicinal plant of India, a source of bio-reductant and stabilizers. It has been reported to contain alkaloids, glycosides, tannins, saponins and aromatic compounds. It is used in the treatment of headaches, coughs, diarrhea, constipation, worts,

worms and kidney malfunctions. Recent interest on Ocimum has resulted from its inhibitory activity against HIV-1 reverse transcriptase and platelets aggregation induced by collagen and ADP[22] (adenosine 5-diphosphate). Hence, the present study was carried out to synthesize and characterize the silver nanoparticles using Ocimum leaf extract and to check their antioxidant and antimicrobial activity.

OCIMUM SPECIES

Tulsi is considered to be a ubiquitous plant in India. Ocimum tenuiflorum (tulsi) is an aromatic plant in the family Lamiaceae. It is an erect, much branched sub shrub 30-60 cm tall with hairy stems and simple opposite green leaves that are strongly scented. tulsi plays a vital role in our everyday life and is said to be the queen of herbal plants. It is the most common household plant in india and it is sacred in hindu tradition. Many Hindu epics explain the importance, properties and uses of tulsi.

Tulsi is an erect sweet scented shrub which grows upto a height of 3 -5 feet commonly grown in gardens and in the periphery of temples. It has got a pungent taste and fragrant smell. Tulsi is the only plant that can absorb carbon dioxide through-out its life. It releases the oxygen in the early morning which is beneficial for the people in breathing dis-orders. Tulsi plant has a lot of significance for mankind, due to the manifold medicinal benefits it provides. Tulsi leaves are widely used in the preparation of Ayurvedic medicines. It is known to promote the longevity of life.

Traditional medicine claims tulsi a cure to a number of ailments, being supposedly anti-oxidant, anti-inflammatory, anti-fungal, antibacterial, insecticide and anti-diabetic. Although it has been used for thousands of years in Ayurveda, sound scientific research on tulsi properties is scarce.

Some trustworthy studies did however confirm the medicinal properties of tulsi. One article published in Photomedecine in 2000 described a neat study of the compounds found in fresh Ocimum sanctum leaf and stem extracts: cirsilineol, cirsimaritin, isothymusin, isothymonin, apigenin, rosmarinic acid, and eugenol (Kelm et al., 2000). It was the first time that cirsimaritin (a flavonoid) and isothymusin (a phenolic active exhibiting antioxidant and antiinflammatory properties) have been identified as compounds in tulsi. As expected, anti-inflammatory activity was observed.

Other studies have shown that constituents of Tulsi leaf extracts have stimulatory effects on physiological pathways of insulin secretion which may underlie its reported antidiabetic action (Hannan et al., 2006); that it is an antioxidant (Geetha et al., 2004), antiparasitic (Asha et al., 2001) and anticancer (Karthikeyan et al., 1999).

The extracts obtained from the plant are extensively brought to use for curing various diseases such as the common cold, inflammation, malaria, heart disease, headaches, stomach disorders, kidney stones, heart disorders, and many more. The Indian basil Tulsi also aids in the purification of atmosphere. Tulsi plant serves as a fabulous repellant in fighting against flies, mosquitoes and insects. It is especially valuable in combating malarial fever. It is said that at the time of establishment of Victoria gardens in Bombay (now Mumbai), the workers became victims of mosquito bites and suffered from chronic malaria. Seeing the pitiable situation of the workers, some of the Hindu managers recommended the plantation of Tulsi plant in the garden. On following their advice, fruitful results were obtained. Thus, holy basil Tulsi helped to abate the growth of mosquitoes and control malaria. There are numerous uses of Tulsi plant. The plant is increasingly finding its way in the Ayurvedic treatment of diseases. Tulsi leaves are widely used due to their

healing power. It is a tonic for the nervous system and thus, helps a great deal in sharpening the memory. This aromatic plant supports the removal of phlegm and catarrhal matter from the bronchial tube. It also works wonders in preventing stomach disorders. The herb Tulsi is known to cure the respiratory disorders. The decoction prepared by mixing honey, ginger and Tulsi leaves is quite helpful in combating bronchitis, influenza and asthma.

PLANT PROFILE

Kingdom : Plantae

Order : Lamiales

Family : Lamiaceae

Genus : Ocimum

Species : O. tenuiflorum

BOTANICAL IDENTITY OF TULSI HERB

The Tulsi herb is found quite commonly all over the Indian sub continent. The plant can grow in the wild in the tropical warm regions. The plant's height varies from 2 to 4 feet. The flowering season is winter (December to February). The Tulsi leaves have a marked strong aroma and an astringent taste. There are some biologically active compounds like urosolic acid, luteolin and apigenin that can be extracted from the Tulsi leaves. Though it is the leaves that are usually used the flowers, seeds and roots also find good usage.

There are three vareieties of Tulsi considered here in our experiment – Krishna Tulsi, Rama Tulsi and Vana Tulsi.

It is said that Krishna Tulsi got its name because of the purple leaves as Lord Krishna's skin colour is dark according to the Vedas. Krishna Tulsi is also famous for its crispy and peppery taste. It will not be right to say that Krishna Tulsi grows less, but it is hard to find in comparison to the other types of tulsi. Purple leaf tulsi is also used to treat throat infections, respiratory system, nasal lesions, earache and skin diseases.

Rama tulsi is also known as Sri or Lakshmi Tulasi, Ocimum tenuiflorum, Ocimum sanctum, and green leaf tulsi (Basil). The Rama tulsi emits a strong aroma from its every part. It is also found in Eastern Nepal, Brazil, China, as well as in Bengal, Bihar, Chatgaon. Rama Tulsi is widely famous for its cooling taste.

The third one from the different types of tulsi is Vana Tulsi. It is native to India, Sri Lanka, Java and the northern and eastern parts of Africa. The scientific name of Vana tulsi is Ocimum gratissimum. It can grow up to 2m high with highly aromatic and slightly hairy green leaves. The strong antioxidant activity of Vana Tulsi slows down the ageing process.

CHEMICAL COMPOSION AND ITS USES

The chemical composition of Tulsi is very complicated. It is eugenol, or 1-hydroxy-2-methoxy-4-allylbenzene. This chemical formula contains many phyto-chemicals referred as compounds. These

numerous compounds present in entire plant consist of antioxidant, adaptogenic, anti-inflammatory, antibacterial and immune-enhancing properties. With these properties when anyone consumes Tulsi in any form their body gets prepared to fight against the diseases and other health problems.

ANTIOXIDANT - Polyphenol Rosmarinic acid present in the Tulsi chemical composition acts as the powerful antioxidant. It protects the cells in the body from smash up due to the presence of free radicals. Excess of oxidation in the body also causes the cell damage. This acid prevents the formation of excess oxidation.

ANTIBACTERIAL – Carvacrol and terpene are the antibacterial agents present in this remarkable plant. Sesquiterpene B-caryophyllene also severs the same purpose. This constituent is FDA approved food additive which is naturally present in Tulsi. It helps keeping the body safe from bacterium that causes illness.

Green chemistry is also known as environment friendly chemistry, or sustainable chemistry. Perhaps the most widely accepted definition of green chemistry is the one offered by chemists **Paul Anastas and John Warner**, who defined green chemistry as the ***design of chemical products and processes that reduce or eliminate the use and generation of hazardous substances***. Green synthesis of nanoparticles had got valuable attention in past few years. A number of approaches are available for the synthesis of nanoparticles, for example, reduction in solution, photochemical and chemical reaction in reverse micelles thermal decomposition of nanoparticles (Akl Awwas and Nida Salem, 2012), radiation assisted, electrochemical, microwave assisted process and recently via green chemistry route (Ravindra *et al.*, 2012).

WHY NANOPARTICLES ?

Nanoparticles have one dimension that measures 100 nanometres or less. The properties of many conventional materials change when formed from nanoparticles. This is typically because nanoparticles have a greater surface area per weight than larger particles which causes them to be more reactive to some other molecules.

Due to their small dimensions, nanomaterials have extremely large surface area to volume ratio, which makes a large fraction of atoms of the materials to be the surface or interfacial atoms, resulting in more “surface” dependent material properties. When the materials is reduced to nanoscale, materials tend to be single crystals. It has been shown in case of metallic nanocrystalline materials that elastic modulii reduce dramatically. Even though some nanomaterials with slightly large number of atoms (>50-60 atoms) may acquire bulk crystalline materials, it is found that the lattice parameters may not be the same as in the bulk materials. The other physical properties of nanoparticles are:

Color – Nanopartcles of yellow gold and gray silicon are red in color

Silver nanoparticles melt at much lower temperatures (-115^{o}C for 2.5 nm size) than the silver slabs (1064^{o}C)

Absorption of solar radiation in photovoltaic cells is much higher in nanoparticles than it is in thin films of continuous sheets of bulk material – since the particles are smaller, they absorb greater amount of solar radiation.

NANOPARTICLES AND THEIR PROPERTIES

The process of removing toxic and waste metals in the environment includes microorganisms, plants and otherbiological structures; achieved by means of oxidation, reduction orcatalysis of metals with metallic nanoparticles.

Metallic nanoparticles produced by biological methods; are used in the biomedical field for purposes such as protection from harmful microorganisms, bio-imaging, drug transport, cancer treatment, medical diagnosis and sensor construction because of their unique properties such as being insulator, optics, antimicrobial, antioxidant, anti-metastasis, biocompatibility, stability and manipulability. Metallicnanoparticles, which can be used in the industrial field due to their catalytic activity, are of great importance nowadays.

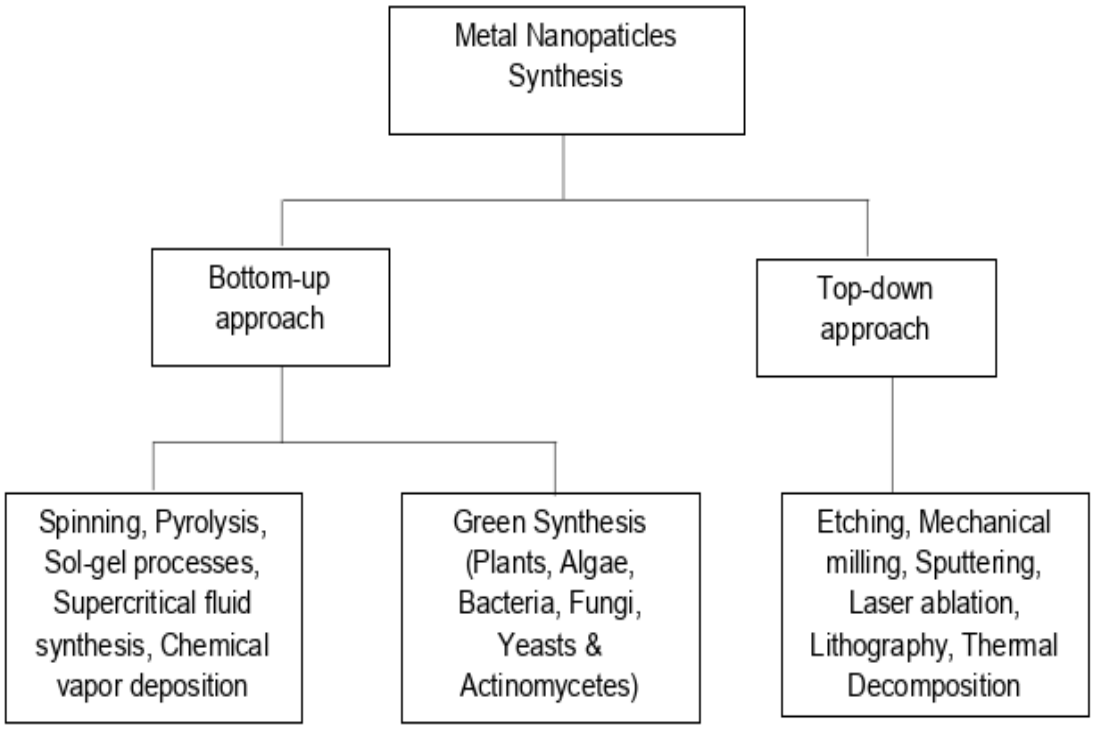
SILVER NANOPARTICLES

Silver nanoparticles have unique optical, electrical and thermal properties and are being incorporated into products that range from photovoltaic to biological and chemical sensors. Examples include conductive inks, pastes and filters which utilize silver nanoparticles for their high electrical conductivity, stability, and low sintering temperatures. The advancement of green synthesis of AgNPs is progressing as a key branch of nanotechnology; where the use of biological entities like micro-organisms, plant extract or plant biomass are being used for the production of AgNPs could be an alternative to chemical and physical methods in an eco friendly manner (Pathak and Hendre, 2015). The synthesis of AgNPs is carried out by the use of various chemical and biological techniques, this will lead to obtain the size and shape of the silver nanoparticles.

Silver nanoparticles have attracted intensive research interest because of their important application in antimicrobial, catalysis and surface-enhanced Raman scaterring (Gokulkrishnan *et al*., 2012). Nanoparticles are important scientific tools have been and are being explored in various biotechnology, pharmacological and pure technological uses. They are a link between bulk materials and atomic or molecular structures. Nanoparticles are unique because of their large surface area and this dominates the contributions made by the small bulk of the material.

The synthesis of silver nanoparticles are being synthesized by two different approaches: (1)Top down procedure (2)Bottom's up procedure. In the top down procedure there will be reduction in the size of Ag metals to form the nanomaterials by various methods including lithography and laser ablation meanwhile the bottoms up procedure includes the dissolvation of silver metals in a solvent and ultimately forming the AgNPs by the reduction of silver nanoparticles by adding reducing agent in it to reduce the chances of accumulation of nanoparticles. Various methods like use of reducing agents, electrochemical techniques, physio-chemical reduction and radiolysis are extensively used for the synthesis of AgNPs which is included in the chemical approaches.

The rapid breakdown of silver nanoparticles releases ionic silver that inactivates vital bacterial enzymes by interacting with essential thiol groups. Silver ions can inhibit bacterial DNA replication, damage bacterial cytoplasm membranes, depleting levels of intracellular adenosine triphosphate (ATP) and finally cause cell death (Parveen *et al*., 2012).



The rapid breakdown of silver nanoparticles releases ionic silver that inactivates vital bacterial enzymes by interacting with essential thiol groups. Silver ions can inhibit bacterial DNA replication, damage bacterial cytoplasm membranes, depleting levels of intracellular adenosine triphosphate (ATP) and finally cause cell death (Parveen *et al*., 2012). Silver nanoparticles resistance of bacteria to bacteriocides and antibiotics has increased due to the development of resistant strains. Some antimicrobial activity of agent are extremely toxic and irritant and much interest in finding ways to formulate new types of safe and cost-effective biocidal materials (Dhrutika *et al*., 2013). Since reducing agents for silver nanoparticles synthesis are often considered toxic or hazardous, the use of green synthesis methods is becoming a priority (Panacek *et al*., 2006).

Silver nanoparticles have more important applications like it is utilized at the same time as discerning covering for lunar energy assimilation and the same as optical receptors intended for biolabeling. Bacterial cell membrane has abundance of sulfur containing proteins with which silver nanoparticles react outside and inside the cell membrane and which affects the viability of bacterial cell leads to increased permeability of bacterial cell membrane (Sharma *et al*., 2015).

NEED OF GREEN SYNTHESIS

Green synthesis is an environmental friendly approach where no toxic chemicals are involved (Logeswari *et al*., 2013). It is a revolutionary technique which leads to new era that unfolds potential of plants in synthesizing stable NPs, increase the life span of NPs synthesized and also overcome the limitations of chemical and physical methods (Kavitha *et al*., 2013), (Malik *et al*., 2014). It is faster and reliable technique comparative to conventional techniques which scale up the process of production of commercially applicable NPs with less or no toxicity. Plants therefore, used for NPs synthesis because

they actively uptake and reduce metal ions in bioremediation and thereby can form complex metal NPs (Singh *et al*., 2014), (Gardea-Torresdey *et al.*, 2002). Green synthesis provides advancement over physical and chemical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, temperature, energy and toxic chemicals (Ravindra *et al*., 2012).

Green synthesis of nanoparticles makes use of environmental friendly non-toxic and safe reagent. **Phytomining** is the uses of hyper accumulating plants to extract a metal from the biomass to return an economic profit (Lamb *et al*., 2001). Green synthesis approaches include mixed-valence polyoxometalates, polysaccharides, Tollens, biological and irradiation method which have advantages over conventional methods involving chemical agents associated with environmental toxicity.

ANTI-MICROBIAL PROPERTY OF SILVER NANOPARTICLES

Silver metal has been utilized generally over the human advancements for various purpose: Numerous social orders utilize silver as gems, ornamentation and fine cutlery. Silver is a well-known antimicrobial agent against a wide range of over 650 microorganisms from different classes such as gram-negative and gram-positive bacteria, fungi or viruses. More recently the metal is finding use in the form of silver nanoparticles (Ahmed *et al*., 2016). In ancient Indian medical system (called Ayurveda), silver has been described as therapeutic agent for many diseases.

The reciprocal action of nanoparticles subsequently breaks the cell membrane and disturbs the protein synthesis mechanism in the bacterial system (Sondi and Sondi, 2004). The increasing concentrations of silver nanoparticles have faster membrane permeability than the lower concentrations and consequently rupture the cell wall of bacteria (Kasthuri *et al*., 2009).

The interactions of bacteria and the metallic silver and gold nanoparticles have been binding with active site of cell membrane to inhibit the cell cycle functions (Kim*et al*., 2007). Silver is generally used in the nitrate form to induce antimicrobial effect but when silver nanoparticles are used, there is a huge increase in the surface area available for the microbes to be exposed to.

Silver nanoparticles have the ability to anchor to the bacterial cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell. There is formation of "pits" on the cell surface, and there is accumulation of the nanoparticles on the cell surface. There have been electron spin resonance spectroscopy studies that suggested that there is formation of free radicals by the silver nanoparticles when in contact with the bacteria, and these free radicals have the ability to damage the cell membrane and make it porous which can ultimately lead to cell death (Danilcauk*et al*., 2006). It has also been proposed that there can be release of silver ions by the nanoparticles (Feng*et al*., 2008). The action of these nanoparticles on the cell can cause the reaction to take place and subsequently lead to cell death. Another fact is that the DNA has sulfur and phosphorus as its major components, thenanoparticles can act on these soft bases and destroy the DNA which would definitely lead to cell death (Moroneset al., 2005). It was found that the nanoparticles dephosphorylate the peptide substrates on tyrosine residues, which leads to signal transduction inhibition and thus the stoppage of growth. It is however necessary to understand that further research is required on the topic to thoroughly establish the claims (Hatchell and Henry, 1996).

The multifunctional AgNPs have a promising activity against spore producing fungus and effectively destroy the fungal growth. The fungal cell membrane structure significant changes were observed by treating it with metallic nanoparticles (Gardea-Terresdey*et al*., 2002).

The antimicrobial properties of silver nanoparticles depend on:

1. Size and environmental conditions (size, pH, ionic strength)
2. Capping agent

2. REVIEW OF LITERATURE

Ocimum sanctum (Tulsi) is a medicinal herb abundantly found and cultured in India, Malaysia, Australia, West Africa, and some of the Arab countries. Tulsi leaves have been traditionally used for treatment of many infections. The antibacterial activity has been reported to be the upshot of essential oil components, mostly eugenols found in it. The present study aims at the synthesis of silver nanoparticles from the aqueous extract of Tulsi leaves. We also attempt to combine the inherent antimicrobial activities of silver metal and Tulsi extract for enhanced antimicrobial activity.

Synthesis can be done in one step using bio logical organism such as bacteria, actinobacteria, yeasts, molds algae and plants, or their products. Molecules in plants microorganisms, such as proteins, enzymes, phenolic compounds, amines, alkaloids and pigments perform nanoparticles ynthesis by reduction.

In traditional chemical and physical methods; reducing agents involved in the reduction of metal ions, and stabilizing agents used to prevent undesired agglomeration of the produced nanoparticles carry a risk of toxicity to the environment and to the cell. Besides, the contents of the produced nanoparticles are thought to be toxic in terms of shape, size and surface chemistry. In the green synthesis method inwhich nanoparticles with biocompatibility are produced, these agents are naturally present in the employed biological organisms.

Actinobacteria, which performs the production of second arymetabolites such as antibiotics, are aerobic, immobile, and mostly filamentous gram-positive bacteria. They are resistant to the most toxic heavy metals owing to their detoxification property. Soluble toxic metal ions are detoxified by either being degraded by intracellular or extra cellular reduction or precipitation. Thus, nanoparticles being antibacterial, antifungal , anticancer, antioxidant, antibio-contamination and having catalytic activity can be produced.

MICROORGANISMS

Escherichiacoli or E. coli is a bacterium that can be found in human intestines Scientists have studied E. coli very extensively, and know more about how E. coli cells work than any other organism. E. coli is not always harmful. In fact, the only known harmful strand is O157.E.coli is a prokaryotic organism. E. coli normally grow in soil and in the large intestines of many mammals, including humans. Most strains of E. coli do not cause disease, but instead help animals get vitamins and digest food. Some strains of E coli cause sickness in people. E. coli are not usually in food or water. When food has not been prepared with clean equipment, E. coli can grow in the food. When E. coli are found in water, this may mean that the water has touched sewage. It is named after The odor Escherich, who discovered it in 1885. It was officially named after him in 1919.

Staphylococcus is a genus of Gram-positive bacteria in the family Staphylococcaceae in the order Bacillales. Under the microscope, they appear spherical (cocci), and form in grape-like clusters. Staphylococcus species are facultative anaerobic organisms (capable of growth both aerobically and anaerobically). The name was coined in 1882 by Scottish surgeon and bacteriologist Alexander Ogston (1844-1929), following the pattern established five years earlier with the naming of Streptococcus. Staphylococcus includes at least 40 species. Of these, nine have two subspecies, one has three subspecies, and one has four subspecies. Most are harmless and reside normally on the skin and mucous membranes of humans and other organisms. Staphylococcus has been found to be a nectar-inhabiting microbe. Found worldwide, they are a small component of soil microbial flora.

The antimicrobial activity was screened against both gram-negative and gram-positive microorganisms. It was observed that O. sanctum leaf extract can reduce silver ions into silver nanoparticles within 8 min of reaction time. Thus, this method can be used for rapid and ecofriendly biosynthesis of stable silver nanoparticles of size range 4–30 nm possessing antimicrobial activity suggesting their possible application in medical industry.

The mean diameter of the inhibition zone and the Minimum Inhibitory Concentration (MIC) were 27.6 mm and 3.12 µg/mL for S. aureus and 19.3 mm and 12.5 µg/mL for E. coli, respectively.

3. MATERIALS AND METHODS

3.1 CHEMICALS, MATERIALS AND EQUIPMENTS:

Silver nitrate was purchased from Kemint chemicals, near Tharekaad, Palakkad. The bacteriological media were available in the Department of Biotechnology, Mercy College, Palakkad. All media and solutions were prepared in double-distilled Milli Q water. The experiments were performed in triplicates, and mean values are presented in results.

Other materials used:

- Deionized water
- Potassium ferricyanide
- Sodium Chloride
- Potassium Chloride
- Disodium hydrogen phosphate
- Potassium dihydrogen phosphate
- Hydrochloric acid
- Trichloroacetic acid
- Ferric chloride
- Centrifuge tubes
- Pipette

- Water bath
- Vortex shaker

3.2 PREPARATION OF PLANT EXTRACT:

One gram of fresh leaves from various species of Tulsi (Ocimum Sanctum(Krishna Tulsi), Ocimum tenuiflourum(Rama Tulsi) and Ocimumgratissimum(Kattu Tulsi)) were washed thoroughly with double-distilled water and dried in shade. The dried leaves were thencrushed to fine powder and mixed with 50 mL doubled-distilled water.To 20 mL of Tulsi extracts of various species kept separately, 20 mL of Silver Nitrate, 2 drops of 1% Ammonium hydroxide solution and Sodium borohydrate is added and kept in an ultrasonicator (magnetic stirrer) at 70^{o}C for 90 minutes. Finally, these mixtures were poured onto different petriplates and kept overnight for evaporation. The resultant were collected in separate vials.

3.3 MICROORGANISMS AND MEDIA:

Common human pathogenic bacterial strains of Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 were used for assessment of antibacterial activity of lab-synthesized AgNPs. The strains were obtained from National Collection of Industrial Microorganisms (NCIM), Pune (India). Nutrient agar was used for growth and maintenance of bacterial strains. Nutrient broth was used for preparation of suspension cultures.

Preparation of nutrient agar

Suspend 28 g of nutrient agar powder in 1 litre of distilled water and heat this mixture while stirring to fully dissolve all components. When all the components are fully dissolved, autoclave the dissolved mixture at 121 degrees Celsius for 15 minutes.Once the nutrient agar has been autoclaved, allow it to cool but not solidify.Then pour nutrient agar into each plate and leave plates on the sterile surface until the agar has solidified. Replace the lid of each Petridish and store the plates in a refrigerator.

Preparation of nutrient broth

Add 13g to 15g of nutritious broth powder in 1L of distilled water , Mix and completely dissolve the components and then sterilise by autoclaving at 121 ° C for 15 minutes.

3.4 SYNTHESIS OF SILVER NANOPARTICLES AND THE EVALUATION OF REDUCING POTENTIAL OF EXTRACT BY FRAP ASSAY:

The quantitative evaluation of the reducing potential of ethanoic extract of various species of Tulsi leaves were carried out as per the method reported by (Chung et al; 2002).Antioxidant compounds cause the reduction of ferric form to the ferrous form because of their reductive capabilities. Prussian blue coloured complex is formed byadding $FeCl_3$.Therefore the reduction can be determined by measuring the formation of Pearl's precession blue at 700nm absorbance [Chung et al;2002].Inthis assay, the colour of the test solution changes to green or blue depending on the reducing power of the antioxidants in the extracts. A higher absorbance indicates high ferric ion reducing antioxidant power.

3.5 ANALYSIS OF BIOREDUCED SILVER NANOPARTICLES

3.5.1 UV-Vis SPECTROSCOPY:

UV-Vis spectroscopic analysis was carried out on Shimadzu UV 1800. Cuvette of path length 10mm was used. The measurements were carried out as a function of reaction time at room temperature.

3.5.2 FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY:

The binding properties of AgNPs synthesized by Tulsi leaf extract were investigated by FTIR analysis. FTIR measurements were taken on Bruker vertex 70. Dried and powdered AgNPs were palleted with potassium bromide (KBr) (1:10 proportion). The spectra were recorded in the wave number range of 450-2500 cm and analyzed by subtracting the spectrum of pure KBr.

3.6 ASSESSMENTOF ANTIBACTERIAL ACTIVITY:

In order to examine the anti bacterial activity of the AgNPs on selected bacteria, the Agar well diffusion method was used. Nutrient agar (NA) was swabbed (sterile cotton swabs) with 8 hour old – broth culture of respective bacteria. Wells (10mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. Stock solution of each Leaf extract was prepared at a concentration of 1 mg/ml in different leaf extacts viz. Ethanol, Water. About 100 µl of different concentrations of leaf solvent extracts were added sterile syringe into the wells and allowed to diffuse at room temperature for 2hrs. Control experiments comprising inoculums without leaf extract were set up. The plates were incubated at 37°C for 18-24 h for bacterial pathogens. The diameter of the inhibition zone (mm) was measured and the activity index was also calculated. Triplicates were maintained and the experiment was repeated rice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

3.7 ASSESSMENT OF ANTIOXIDANT ACTIVITY:

The total antioxidant activity can be measured by FRAP assay. This method is based on the principle of the absorbance of the reaction mixture of various species of Tulsi plant. As the absorbance increase, the antioxidant activity also increases.

To1ml petroleum ether; chloroform andmethanol (20-100 microlitre /mL),0.9 mL 96% ethanol, 5ml of distilled water, 1.5mlof 1MHCl , 1.5 ml of 1% potassium ferricyanide, 0.5 ml of 1% Sodium dodecyl sulphate and 0.2% ferric chloride were added. The mixture was then kept in water bath at 50°C for 20 minutes. After cooling the O. D (optical density) of the mixture was measured at 700nm. The increase in absorbance is used to measure the reducing power of the plant extracts solution.

Ascorbic acid is served as the positive control.

Percentage of Ferric reducing activity of the test solution was calculated using the following formula;

Inhibition % = (1- A/A)×100

where A is the absorbanceof the control (blank;withoutextract)and A, in the absorbance of the sample or extracts.

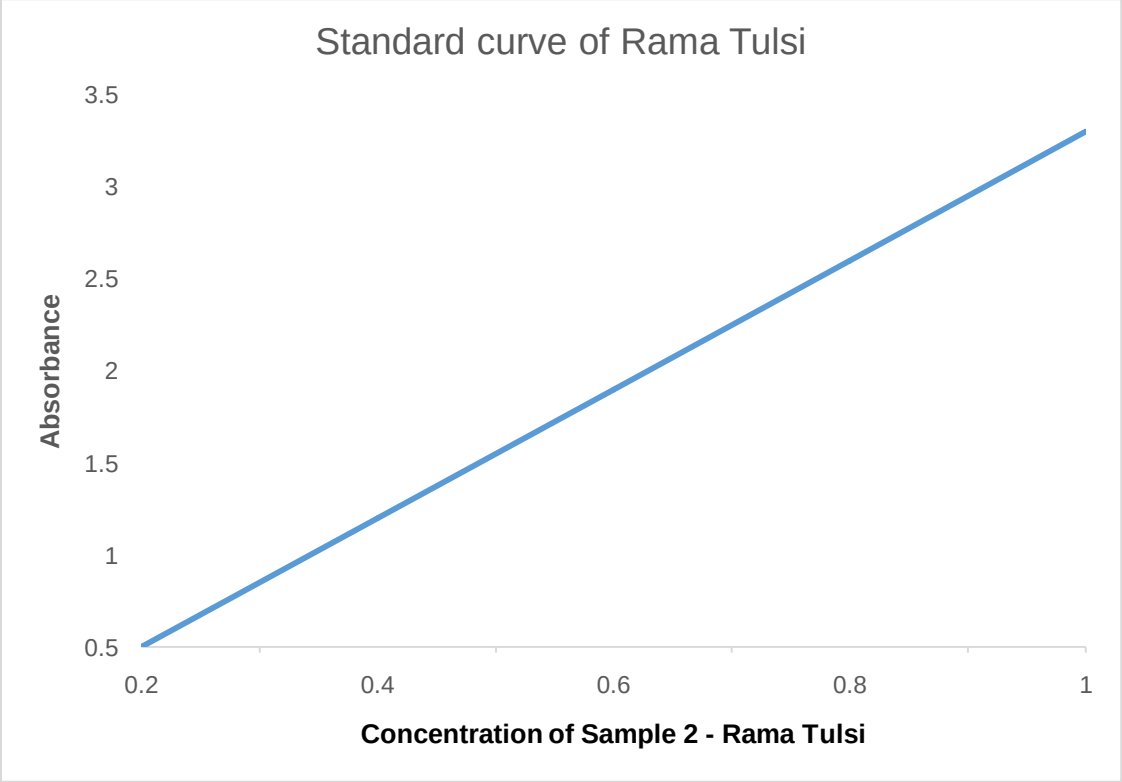
4. RESULT AND DISCUSSION

4.1 FRAP ASSAY BY UV-Vis ABSORPTION SPECTRUM FOR THE ANALYSIS OF OCIMUM SPECIES

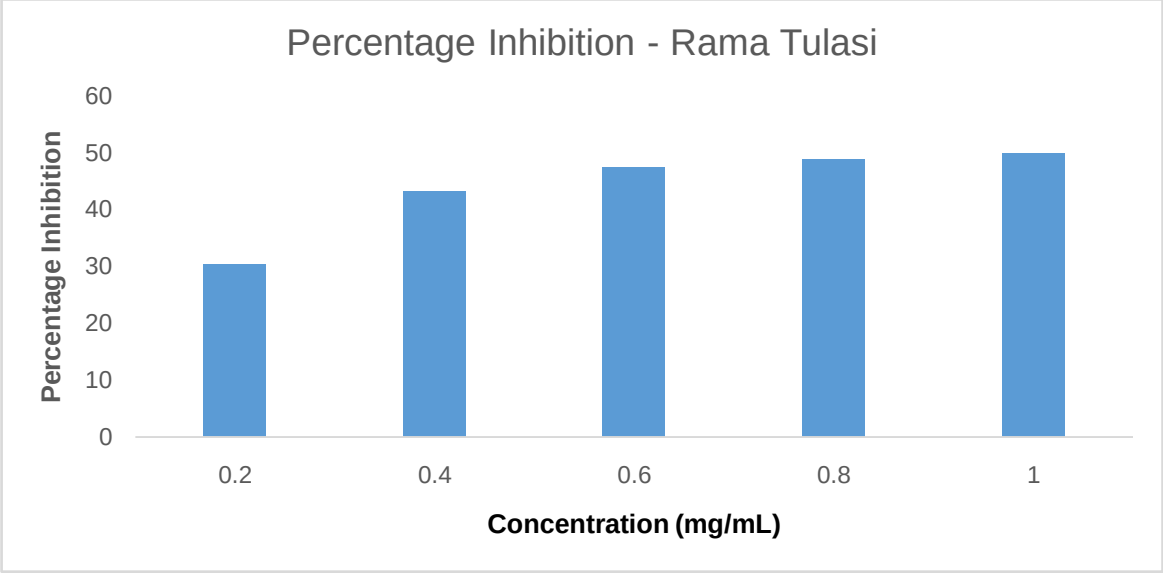
4.1.1 FRAP ASSAY OF OCIMUM TENUIFLORUM (RAMA TULSI)

SAMPLE	CONCENTRATION (mg/mL)	ABSORBANCE	ABSORBANCEOF BLANK	PERCENTAGE INHIBITION
S1	0.2	0.5	0.348	30.4
S2	0.4	1.2	0.682	43.2
S3	0.6	1.9	1	47.4
S4	0.8	2.6	1.328	48.93
S5	1	3.3	1.654	49.88

The result shows that as the sample concentration increases, the percentage of inhibition increases, that is antioxidant activity increases. With an increase in the concentration of silver nanoparticles of Rama tulsi, there will be an increase in the active sites available for inhibiting conversion of ferrous to ferric, so the percentage of inhibition efficiency increases.



EFFECT OF ANTIOXIDATION

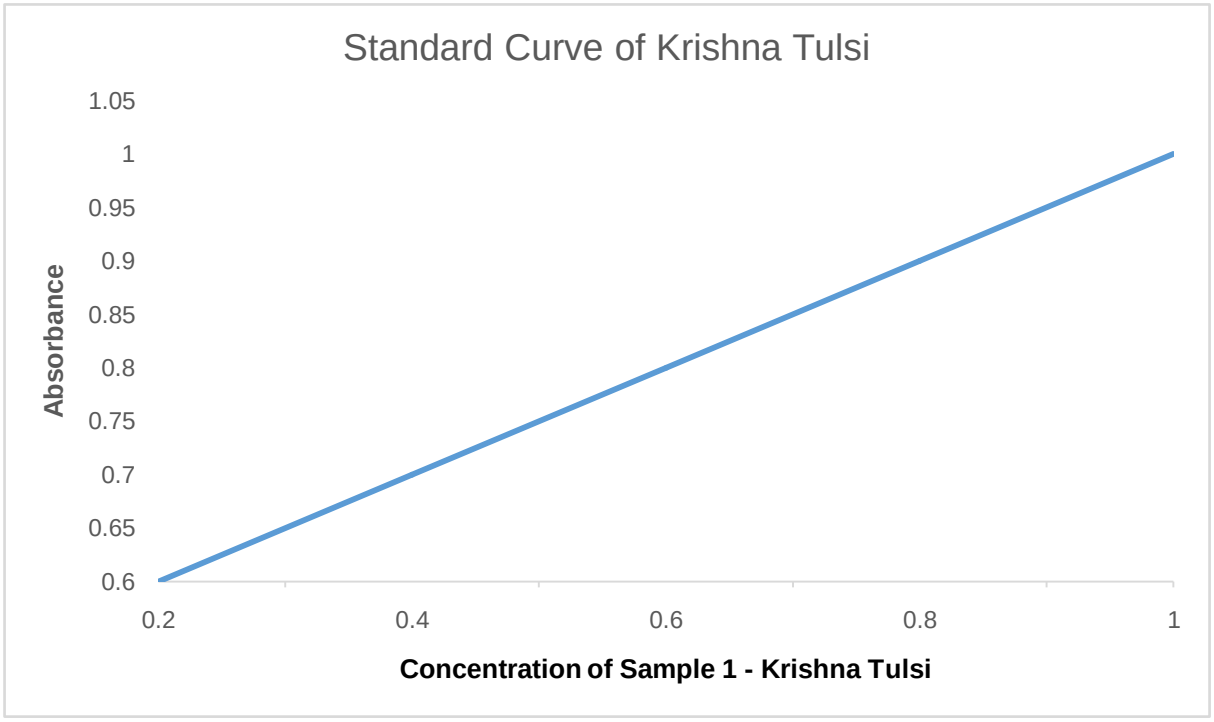


It is clear from the graph that with a concentration of 0.4mg/ml,the percentage of inhibition is 43%. But when the concentration is increased to1mg/ml;the percentage of inhibition is increased by an efficiency of 49.88%.

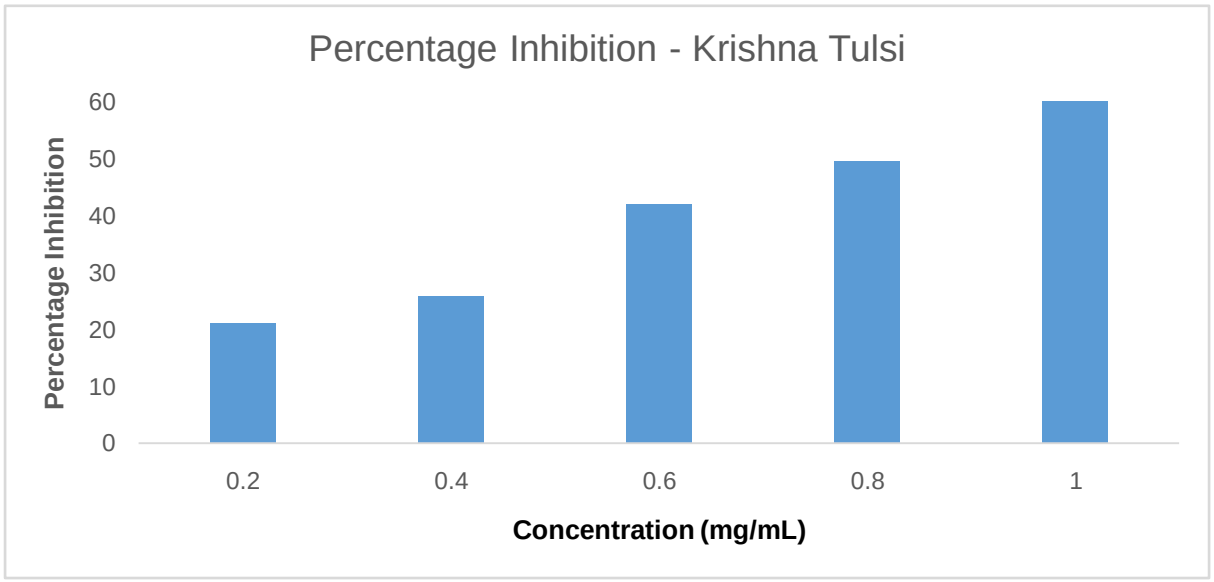
4.1.2 FRAP ASSAY OF OCIMUM SANCTUM (KRISHNA TULSI)

SAMPLE	CONCENTRATION (mg/mL)	ABSORBANCE	ABSORBANCEOF BLANK	PERCENTAGE INHIBITION
S1	0.2	0.6	0.348	21
S2	0.4	0.7	0.682	25.8
S3	0.6	0.8	1	42
S4	0.8	0.9	1.328	47.55
S5	1	1	1.654	65.4

The result shows that as the sample concentration increases, the percentage of inhibition increases, that is antioxidant activity increases. With an increase in the concentration of silver nanoparticles of KrishnaTulsi,there will be an increase in the active sites available for inhibiting conversion of ferrous to ferric,so the percentage of inhibition efficiency increases.



EFFECT OF ANTIOXIDATION

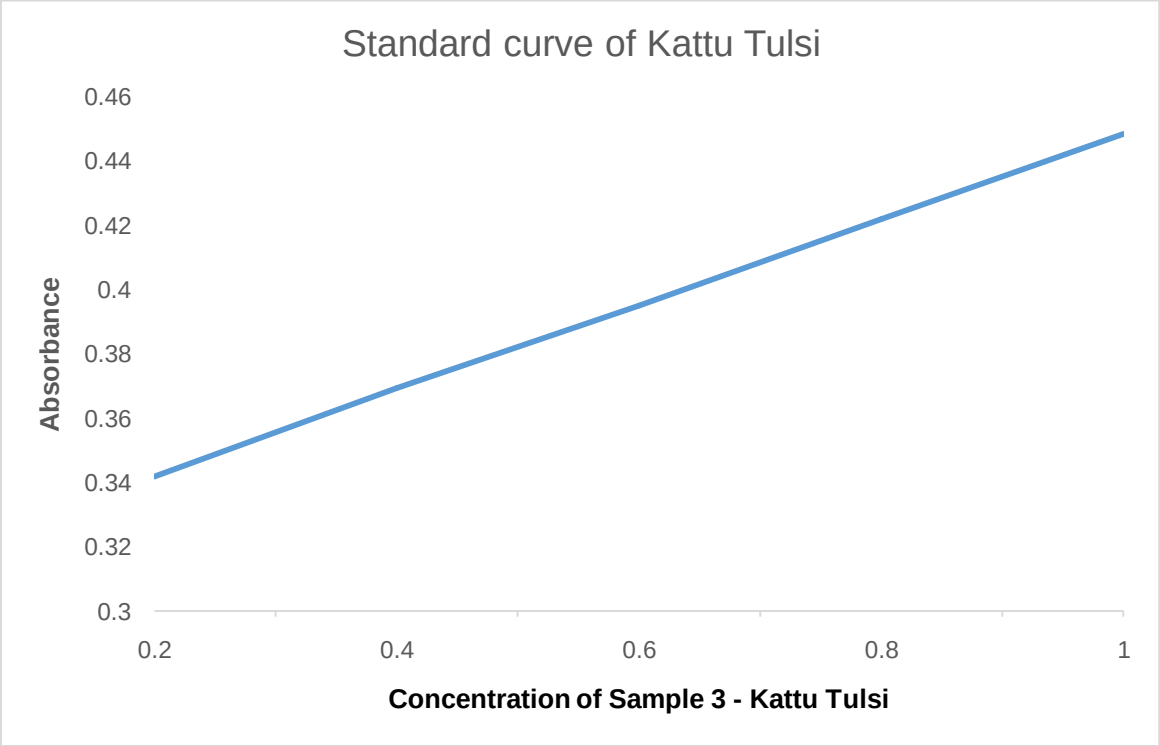


It is clear from the graph that with a concentration of 0.4mg/ml,the percentage of inhibition is 25.8%. But when the concentration is increased to1mg/ml;the percentage of inhibition is increased by an efficiency of 65.4%.

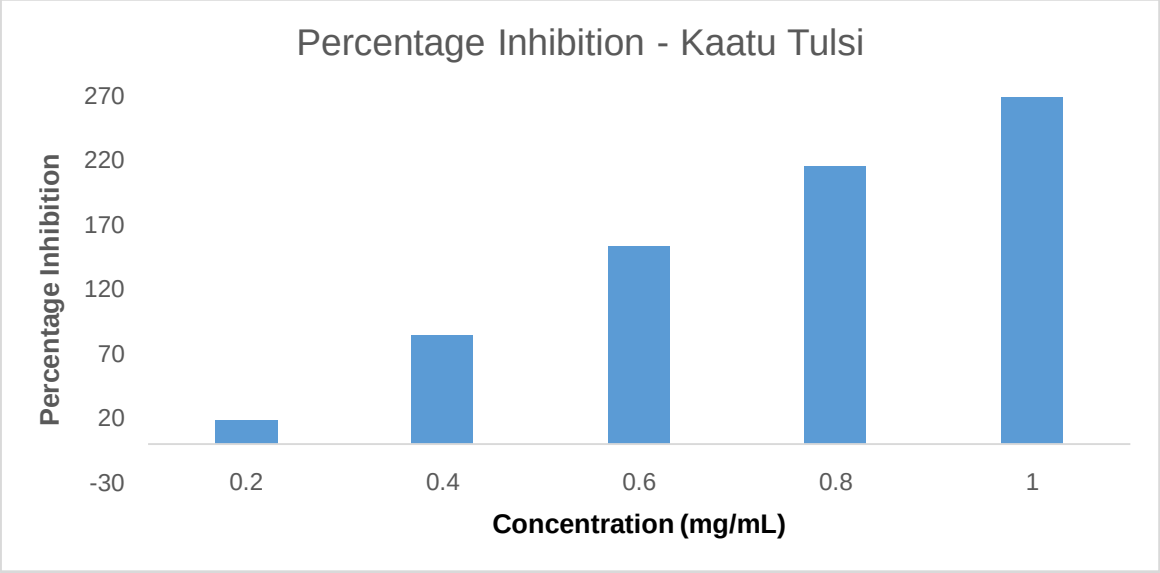
4.1.3 FRAP ASSAY OF OCIMUM GRASSITUMUM (KATTU TULSI)

SAMPLE	CONCENTRATION (mg/mL)	ABSORBANCE	ABSORBANCEOF BLANK	PERCENTAGE INHIBITION
S1	0.2	0.3418	0.348	18.1
S2	0.4	0.3693	0.682	84.67
S3	0.6	0.3949	1	153.22
S4	0.8	0.4217	1.328	214.91
S5	1	0.4483	1.654	268.94

The result shows that as the sample concentration increases, the percentage of inhibition increases, that is antioxidant activity increases. With an increase in the concentration of silver nanoparticles of KattuTulsi,there will be an increase in the active sites available for inhibiting conversion of ferrous to ferric,so the percentage of inhibition efficiency increases.



EFFECT OF ANTIOXIDATION



It is clear from the graph that with a concentration of 0.4mg/ml,the percentage of inhibition is 84.67%. But when the concentration is increased to1mg/ml;the percentage of inhibition is increased by an efficiency of 268.94%.

REFERENCES

1. Ahmed, S., Ahmad, M., Swami, B. L. and Ikram, S. 2016. A review on plant extracts mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. Journal of Advanced Research 7:17-28.
2. Asha, M. K., Prashanth, D., Murali, B., Padmaja, R., & Amit, A. (2001). Anthelmintic activity of essential oil of Ocimum sanctum and eugenol. Fitoterapia, 72(6), 669-670.
3. A. W. Bauer, W. M. Kirby, J. C. Sherris, and M. Turck, "Antibiotic susceptibility testing by a" standardized single disk method," American Journal of Clinical Pathology, vol. 45, no. 4,pp. 493-496, 1966.
4. Byrappa K, Ohara S, Adschiri T. Nanoparticles synthesis using supercritical fluid technology – towards biomedical applications. Advanced Drug Delivery Reviews (2008) 60:299-327. Doi:10.1016/j.addr.2007.09.001.
5. Cerjak H. Book note: introductions to nanoparticles and nanomaterials . Powder Metallurgy (2014) 57:82.
6. Cicek S, Gungor AA, Adiguzel A, Nadaroglu H. Biochemical Evaluation and Green Synthesis of Nano Silver Using Peroxidase from Euphorbia (Euphorbia amygdaloides) and Its Antibacterial Activity. Journal of Chemistry (2015). Doi:10.1155/2015/486948.
7. C. W. Hanson and W. J. Martin, "Modified agar dilution method for rapid antibiotic susceptibility testing of anaerobic bacteria, Antimicrobial Agents and Chemotherapy, vol. 13, no. 3, pp. 383-388, 1978.
8. Dhrutika, P., Miral, P. and Krishnamurthy, R..2013. Silver nanoparticles biosynthesis and its antimicrobial activity. Cibtech Journal of Bio-protocol. 2 (1): 50-57.
9. Danilcauk, M., Lund, A, Saldo, J., Yamada, H. and Michalik, J.2006. "Conduction electron spin resonance of small silver particles". Spectrochimaca. Acta. Part A., 63:189-191.

10. Feng, Q., Wu, J., Chen, G.Q., Cui, F.Z., Kim, T.N. and Kim, J.O. 2008. "A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus". Journal of Biomedical Material Research.52:662-668.
11. Gao Y, Wei 2, Li F, Yang ZM, Chen YM, Zrinyi M, et al. Synthesis of a morphology controllable Fe304 nanoparticle/hydrogel magnetic nanocomposite inspired by magnetotactic bacteria and its application in H2O2 detection. Green Chemistry (2014) 16:1255-61. Doi:10.1039/c3gc41535j.
12. Gardea-Torresdey, J.L., Parson, J.G., Gomez, E., Peralta-Videa, J., Troiani, H.E., Santiago, P. and Yacaman, M.J. 2002. Formation and growth of Au nanoparticles inside live alfalfa plants. Nano Letters. 2:397-401.
13. Gardea-Torresdey, J.L., Parson, J.G., Gomez, E., Peralta-Videa, J., Troiani, H.E., Santiago, P. and Yacaman, M.J. 2002. Formation and growth of Au nanoparticles inside live alfalfa plants. Nano Letters. 2:397-401.
14. Geetha, R. K., & Vasudevan, D. M. (2004). Inhibition of lipid peroxidation by botanical extracts of Ocimum sanctum: in vivo and in vitro studies. Life sciences, 76(1), 21-28.
15. Geonmonond R$, Da Silva, AGM, Camargo PH. Controlled synthesis of noble metal nanomaterials: motivation, principles, and opportunities in nanocatalysis. Anais Da Academia Brasileira De Ciencias (2018) 90:719-44. Doi:10.1590/00013765201820170561.
16. Gokulakrishnan, R., Ravikumar, S. and Raj, J.A. 2012. In vitro antibacterial potential of metaloxide nanoparticles against antibiotic resistant bacteria pathogens. Asian Pacific Journal of Tropical Disease. 2(5):411-413.
17. Hannan, J. M. A., Marenah, L., Ali, L., Rokeya, B., Flatt, P. R., & Abdel-Wahab, Y. H. A. (2006). Ocimum sanctum leaf extracts stimulate insulin secretion from perfused pancreas, isolated islets and clonal pancreatic β-cells. Journal of Endocrinology, 189(1), 127-136.
18. Hatchett, D. and Henry, S. 1996. "Electrochemistry of sulfur adlayers on low-index faces of silver". Journal of Physical Chemistry. 100(23):9854-9859.
19. Hussain I, Singh NB, Singh A, Singh H, Singh SC. Green synthesis of nanoparticles and its potential application. Biotechnology Letters (2016) 38:545-60. Doi:10.1007/s10529-015-2026-7.
20. Ince S. Determination of retention, characterization and antimicrobial properties of Ag, Au and Pt nanoparticles by green synthesis method using yolks of quail eggs. Master Thesis. Erzurum (2017).
21. Iravani S. Green synthesis of metal nanoparticles using plants. Green Chemistry (2011) 13:2638-50. Doi:10.1039/c1gc15386b.
22. Jain S. Osimum sanctum as a herbal medicine. A review. Int J. Maxi Res. 2015; 1(1):3-12.
23. Karaduman I, Gungor AA, Nadaroglu H, Altundas A, Acar S. Green synthesis of gamma-Fe203 nanoparticles for methane gas sensing.Journal of Materials Science-Materials in Electronics (2017)28:16094-105. Doi:10.1007/s10854-017-7510-5.
24. Karthikeyan, K., Ravichandran, P., & Govindasamy, S. (1999). Chemopreventive effect of Ocimum sanctum on DMBA-induced hamster buccal pouch carcinogenesis. Oral oncology, 35(1), 112-119.
25. Kasthuri, J., Kathiravan, K. and Rajendiran, N., 2009. Phyllanthin assisted biosynthesis of silver and gold nanoparticles: a novel biological approach. Journal of Nanoparticles. Research.11:1075-1085.
26. Kavitha, K.S., Syed, B., Rakshith, D., Kavitha, H U., Yashwanta, R H C., Harini, B. P. and Satish, S. 2013. Plants as green source towards synthesis of nanoparticles. International Research ajournal Biological Science. 2(6):66-76.

27. Kelm, M. A., Nair, M. G., Strasburg, G. M., & DeWitt, D. L. (2000). Antioxidant and cyclooxygenase inhibitory phenolic compounds from Ocimum sanctum Linn. Phytomedicine, 7(1), 7-13.
28. Kim, J.S., Kuk, E., Yu, K.N., Jong-Ho, K., Park, S.J., Lee, H.J. and Kim, S.H. 2007. Antimicrobial effects of silver nanoparticles. Nano medicine 3:95-101.
29. Korbekandi H, Iravani S, Abbasi S. Production of nanoparticles using organisms. Critical Reviews in Biotechnology (2009) 29:279-306. Doi:10.3109/07388550903062462.
30. Lamb, A. E., Anderson, C. W. N. and Haverkamp, R. G. 2001. The extraction of gold from plants and its application to phytomining. Chemistry of New Zealand.65: 31-33.
31. Li XQ, Xu HZ, Chen ZS, Chen GF. Biosynthesis of Nanoparticles by Microorganisms and Their Applications. Journal of Nanomaterials (2011). Doi:10.1155/2011/270974.
32. Logeswari, P., Silambarasan, S. and Abraham, J. 2013. Ecofriendly synthesis of silver nanoparticles from commercially available plant powders and their antibacterial properties. Scientia Iranica, 20(3): 1049-1054.
33. Makarov VV, Love AJ, Sinitsyna OV, Makarova SS, Yaminsky IV Taliansky ME, et al. "Green" Nanotechnologies: Synthesis of Metal Nanoparticles Using Plants. Acta Nature (2014) 6:35-44.
34. Malik, P., Shankar, R., Malik, V., Sharma, N. and Mukherjee, T. K. 2014. Green chemistry based benign routes for nanoparticle synthesis. Journal of Nanoparticles. 1-14.
35. Manivasagan P, Venkatesan J, Sivakumar K, Kim SK. Actinobacteria mediated synthesis of nanoparticles and their biological properties: A review. Critical Reviews in Microbiology (2016) 42:209-21. Doi:10.3109/1040841x.2014.917069.
36. [36] Moghaddam AB, Namvar F, Moniri M, Tahir PM, Azizi S, Mohamad R. Nanoparticles Biosynthesized by Fungi and Yeast: A Review of Their Preparation, Properties, and Medical Applications. Molecules (2015) 20:16540-65. Doi:10.3390/molecules200916540.
37. [37] Morones, J., Elechiguerra, J.L., Camacho, A., Holt, K., Kouri, J.B., Ramirez, J.T. and Yacaman, M.J.2005. "The bactericidal effect of silver nanoparticles". Nanotechnology. 16:2346-2353.
38. [38] Mukhopadhyay NK, Yadav TP. Some Aspects of Stability and Nanophase Formation in Quasicrystals during Mechanical Milling. Israel Journal of Chemistry (2011) 51:1185-96. Doi:10.1002/ijch.201100145.
39. Nadaroglu H, Onem H, Gungor AA. Green synthesis of Ce203 NPS and determination of its antioxidant activity. Let Nanobiotechnology (2017) 11:411-9. Doi:10.1049/iet-nbt.2016.0138.
40. Nadaroglu H, Gungor AA, Ince S, Babagil A. Green synthesis and characterisation of platinum nanoparticles using quail egg yolk. Spectrochimica Acta Part -Molecular and Biomolecular Spectroscopy (2017) 172:43-7. Doi:10.1016/j.saa.2016.05.023.
41. N. Ahmad, S. Sharma, Md. Alam, V. N. Singh, S. F. Shamsi, B. R. Mehta, A. Fatma Colloid Surf. B 81, 81 (2010).
42. Narayanan KB, Sakthivel N. Biological synthesis of metal nanoparticles by microbes. Advances in Colloid and Interface Science (2010) 156:1-13. Doi:10.1016/j.cis.2010.02.001.
43. N. Saifuddin, C. W. Wong, and A. A. N. Yasumira, "Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation," E-Journal of Chemistry, vol. 6, no. 1, pp. 61-70, 2009.

44. Panacek, A., Kvitek, L., Prucek, R., Kolar, M., Vecerova, R., Pizurova, N., Sharma, V., Nevecna. T. and Zboril, R. 2006. Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. Journal of Physical Chemistry B 110:16248-16253.
45. Park S, Sung HK, Kim Y. Green Synthesis of Metal Nanoparticles. Using Sprout Plants: Pros and Cons. Journal of Nanoscience and Nanotechnology (2016) 16:4444-9. Doi:10.1166/jnn.2016.10970.
46. Parveen, A., Ashish, S. R. and Srinath< R. 2012. Biosynthesis and characterization of silver nanoparticles from Cassia auriculata leaf extract and in-vitro evaluation of anti-microbial activity. International Journal of Applied Biology and Pharmaceutical Technology. 3 (2): 222-228.
47. Pathak, S. R. and Hendre, S. A. 2015. Sunlight induced green synthesis of silver nanoparticles using sundried leaves extract of Kalanchoepinnata and evaluation of its photocatalytic potential. Der Pharmacia Lettre, 7 (5): 313-324.
48. Pearce JM. Make nanotechnology research open-source. Nature (2012) 491:519-21. Doi:10.1038/491519a.
49. Ravindra, B. M., Seema, L. N., Neelambika, T. M., Gangadhar, S. M., Nataraja, K. and Vijaya, K. S. 2012. Silver nanoparticles synthesized by in-vitro derived plants and Callus culture of Clitoriaternatea; evaluation of antimicrobial activity. Research in Biotechnology. 3 (5): 26-38.
50. Schrofel A, Kratosova G, Safarik I, Safarikova M, Raska I, ShorLM. Applications of biosynthesized metallic nanoparticles – A review. Acta Biomaterialia (2014) 10:4023 42. Doi:10.1016/j.actbio.2014.05.022.
51. Shah M, Fawcett D, Sharma S, Tripathy SK, Poinern GE. Green Synthesis of Metallic Nanoparticles via Biological Entities. Material (2015) 8:7278-308. Doi:10.3390/ma8115377.
52. Sharma, C. K., Sharma, M., Verma., O. and Sharma, V. 2015. Green synthesis of different nanoparticles and their potential applications in different fields. A critical review. International Journal of Pharma and Bio Sciences 6(3): 555-567.
53. Siddiqi KS, Husen A. Fabrication of Metal and Metal Oxide Nanoparticles by Algae and their Toxic Effects. Nanoscale Research Letters (2016) 11. Doi:10.1186/s11671-016-1580-9.
54. Singh, A., Singh, S. and Singh, N. 2014. Green chemistry; Sustainability an Innovative Approach. Journal of Applied Chemistry. 2(2): 77-82.
55. Singh P, Kim YJ, Zhang DB, Yang DC. Biological Synthesis of Nanoparticles from Plants and Microorganisms. Trends in Biotechnology (2016) 34:588-99. Doi:10.1016/j.tibtech.2016.02.006.
56. Sondi, I. and Salopek-Sondi, B. 2004. Silver nanoparticles as antimicrobial agent: a case study on E.coli as a model for Gram-negative bacteria. Journal of Colloid and Interface Science. 275:177-182.
57. S. Mondal, B. R. Mirdha, and S. C. Mahapatra, "The science behind sacredness of Tulsi (Ocimum sanctum linn.)," Indian Journal of Physiology and Pharmacology, vol. 53, no. 4, pp. 291-306, 2009.
58. Y.Cui, C. M. Lieber Science 291, 851 (2001).