# Green Synthesis of Silver Nano Particle and Comparative Study on Antibacterial Activity of Herbal Extracts & Synthesized Silver Nano Particle

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**Abstract-**Biologically synthesized silver nanoparticles (AgNPs) are being widely used in the field of medicine. Extracellular biosynthesis of silver nanoparticles was carried out by using medicinal plant extracts for the reduction of aqueous silver ions in short period. The silver nanoparticles formation was confirmed by the colour change of plant extracts (AgNPs) and further confirmed with the help of UV-Vis spectroscopy. These Phytosynthesized silver nanoparticles were tested for antibacterial l activity using agar well diffusion method. The test cultures are *Bacillus subtillis, staphylococcus aureus* and *E.coli* species of bacteria. The microbial property of silver nanoparticles was analysed by measuring the inhibition zone. The silver nanoparticles synthesized from fresh and dry herbal extracts. The SNPs synthesized from fresh herbal extracts showed toxic towards pathogens. Whereas the growth of pathogens was inhibited maximum by the AgNPs synthesized from leaf extract of herbals, the results indicate that the silver nanoparticles may have an important advantage over conventional antibiotics.

**Key words-**synthesis of silvernano particle, characterization of silver nanoparticles, herbal extracts,antimicrobialactivity,phytochemicsl analysis

## 1 INTRODUCTION

The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents. Gram positive bacteria such as *Staphylococcus aureus* are mainly responsible for post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning (Mishra p *et al*., 2011[19]). Gram negative bacterium such as *Escherichia coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicaemia (Hussain m et al.,2004[17]; Abou Elkair et al.,2010[22]). Different antibiotics exercise their inhibitory activity on different pathogenic organisms. Multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors. This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents, but the cost production of synthetic drugs is high and they produce adverse effects compared to plant derived drugs (kim *et al*., 2011[10]).

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently,new infections can occur in hospitals resulting in high mortality.

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient.

Nanotechnology is an important field of modern research dealing with synthesis, strategy and manipulation of particle's structure ranging from approximately 1 to 100 nm in size. Within this size range all the properties (chemical, physical and biological) changes in fundamental ways of both individual

atoms/molecules and their corresponding bulk. Nanoparticleshave unique properties as a consequence of their size, distribution and morphology and, therefore, are a very important component in the rapidly developing field of nanotechnology. Silver has been known, for more than 2000 years, as a metal that exhibits good medical properties, silver based compounds being used in numerous antimicrobial applications. Silver ions are highly toxic for microorganisms and, therefore, have multiple roles in the medical field(B.Sadhegi *et al*.,2010[11]).

AgNPs are a very important part of nanotechnology mainly because they do not induce modification on living cells and are unable to cause microbial resistance.AgNPs can be obtained by using conventional or unconventional methods, using two different approaches: “top down” and “bottom-up”. Although there are numerous conventional methods used to obtain AgNPs (e.g.: solution, chemical / photochemical reactions in reverse micelles, thermal decomposition of different silver compounds, electrochemical, sonochemical, radiation and microwave-assisted routes) they usually involve hazardous chemicals, low compound conversions, high energy requirements and wasteful purifications(C Krishnaraj *et al*,2010[16]) .

In recent years green chemistry and biosynthetic methods have become more attractive ways to obtain AgNPs. These unconventional methods use either biological microorganisms (e.g.: bacteria, fungi, marine algae, yeasts) or different alcoholic or aqueous plant extracts. Green synthesis has multiple advantages over classical routes: it is cost effective, eco-friendly and does not require high pressure, energy, temperature or the use of toxic chemical reagents Plant-mediated synthesis of AgNPs is more advantageous compared to the methods that use microorganisms especially because they can be easily improved, are less bio hazardous and do not involve the elaborate stage of growing cell cultures (W.Rout Rajesh*et al*,2009[15]).

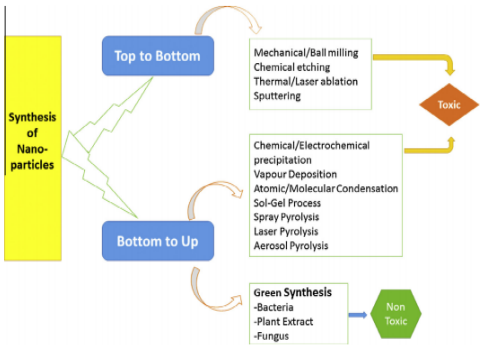



**Fig. 1** Different approaches of synthesis of silver nanoparticles.

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils, as well as in tannin.

The antimicrobial properties of plants have been investigated by a number of researchers worldwide, especially in Latin America. Four different plant species used here are Tulsi, Neem, Elengi, Christmas bush for therapeutic treatments. It was documented that among the compounds extracted from these plants, inhibited the growth of Staphylococus aureus, Escherichia coli, Bacillus (Mishra P *et al*,2011[19]).

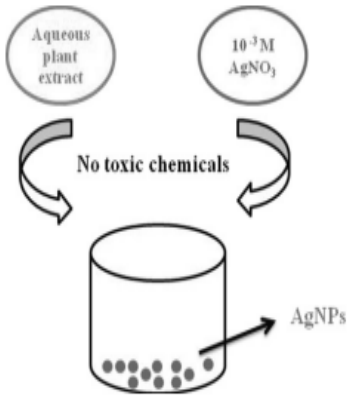



Figure 2. Synthesis of silver nanoparticles (AgNPs) using plant extracts

Ocimum tenuiflorum, also known as Ocimum sanctum, holy basil,or tulsi (also sometimes spelled thulasi), is an

aromatic plant in the family Lamiaceae which is native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics.It is an erect, many-branched subshrub, 30–60 cm (12–24 in) tall with hairy stems and simple phyllotaxic green or purple leaves that are strongly scented.Tulasi (Sanskrit:-Surasa) has been used for thousands of years in Ayurveda for its diverse healing properties(Mishra P *et al*,2011[5]).

It is mentioned in the CharakaSamhita, an ancient Ayurvedic text. Tulsi is considered to be an adaptogen, balancing different processes in the body, and helpful for adapting to stress.Marked by its strong aroma and astringent taste, it is regarded in Ayurveda as a kind of "elixir of life" and believed to promote longevity.Tulasi extracts are used in Ayurvedic remedies for a variety of ailments(R M U S K, 2013[4]. Traditionally, tulasi is taken in many forms: as herbal tea, dried powder, fresh leaf or mixed with ghee. Essential oil extracted from Karpoora tulasi is mostly used for medicinal purposes and in herbal cosmetics.

Fig . 3 Ocimumsanctum

Mimusopselengi is a medium-sized evergreen tree found in tropical forests in South Asia, Southeast Asia and northern Australia. English common names include Spanish cherry, medlar,and bullet wood. Its timber is valuable, the fruit is edible, and it is used in traditional medicine. As the trees give thick shade and flowers emit fragrance, it is a prized collection of gardens. The bark, flowers, fruits, and seeds of Bakulaare used in Ayurvedic medicine in which it is purported to be astringent, cooling, anthelmintic, tonic, and febrifuge(Mishra G *et al*,1968[12]. It is mainly used for dental ailments such as bleeding gums, pyorrhea, dental caries, and loose teeth.

Fig .4Mimusopselengi

Azadirachtaindica, also known as Neem, and Indian Lilac is a tree in the mahogany family Meliaceae. It is one of two species in the genus Azadirachta, and is native to India and the Indian subcontinent including Nepal, Pakistan, Bangladesh, and Sri Lanka. It typically is grown in tropical and semi-tropical regions. Neem trees now also grow in islands located in the southern part of Iran. Its fruits and seeds are the source of neem oil. Products made from neem trees have been used in India for over two millennia for their medicinal properties. Neem products are believed by Siddha and Ayurvedic practitioners to be Anthelmintic, antifungal, antidiabetic, antibacterial, antiviral, c ontraceptive,and sedative. It is considered a major component in siddha medicine and Ayurvedic and Unani medicine and is particularly prescribed for skin diseases(Brinda *et al*,2012[13]).

Fig. 5 Azadirachtaindica

Chromolaenaodorata, It is sometimes grown as a medicinal and ornamental plant. It is used as a traditional medicine in Indonesia, Thailand, Malaysia and parts of Africa including Nigeria. The young leaves are crushed, and the resulting liquid can be used to treat skin wounds in traditional medicine of Thailand the plant is used for the treatment of wounds, rashes, diabetes, and as insect repellent. It has

antifungal and antibacterial properties( Rastogi, R.P*et al*,1998[20]).

The phytoprostane compound chromomoric acid C-I has been identified from Chromolaenaodorata as a strong inducer of the activity of the transcription factor NFE2L2 (Nrf2), a master regulator of a range of genes with defensive, anti-inflammatory, and detoxifying functions.A recent review indicates that the ethno-pharmacological, funcigicidal, nematicidal importance of the plant and its use as a fallow species and as a soil fertility improvement plant in the slash and burn rotation system of agriculture has contributed to its continued use and spread in Nigeria.

Fig. 6 Chromolaenaodorata

## 2 MATERIAL AND METHODS

### 2.1 MATERIALS

#### 2.1 .1 Herbal sample collection

The herbal materials used in this study consisted of Ocimum sanctum (Tulsi), Chromolaenaodorata(Christmas bush), Azadirachtaindica (Neem), Mimusopselengi (Elengi),these were collected from different area in Thrissur.

Table 1 plant materials used in this study

| Herbal/part used | place | Time of collection |
|---|---|---|
| Azadirachtaindica/leaves | garden-Mundur | Morning /January |
| Ocimum sanctum/leaves | Temple garden - Alathur | Morning /January |
| Chromolaenaodorata/leaves | Agricultural land-Alathur | Afternoon/Janaury |
| Mimusopselengi/leaves | Temple garden - Alathur | Morning /January |

#### 2.1.2 Bacteria

Pathogenic strains of Staphylococcus aureus, Bacillus subtilis, and Escherichia coli were obtained from microbiology department at Sahrdaya College, and were maintained on agar medium at 4 ºC for further experiments.

#### 2.1.3 Culture media and chemicals

Types of media were required for carrying out this study, Nutrient agar and Nutrient broth. Also silver nitrate and distilled water was used for AgNP synthesis and extraction process respectively. These media, chemical & solvents were purchased from some company in kerala.

### 2.2 METHODS

#### 2.2.1 Preparation of herbal extracts

##### 2.2.1.1 Aqueous extraction of dried herbal leaves

For aqueous extraction, the leaves of ofOcimum sanctum (Tulsi), Chromolaenaodorata(Christmas bush), Azadirachtaindica (Neem), Mimusopselengi (Elengi) were collected from the sources and leaves were washed for 2-3 times with tap water and final with distilled water. Fresh leaves were dried under sunlight. Then 5 g of leaves were mixed with 50 mL of water and boiled on slow heat for 20 min. After cooling, it was filtered through Whatman Filter paper no. 1. And the supernatant was collected.

##### 2.2.1.2 Aqueous Extraction of fresh herbal leaves

5 gm of fresh leaves of Ocimum sanctum, Chromolaenaodorata, Azadirachtaindica, Mimusopselengi are collected from the sources and leaves were washed for 2-3 times with tap water and final with distilled water and grinded by adding 50 ml water and boiled on slow heat for 20 min. After cooling, it was filtered through Whatman Filter paper no. 1. and the supernatant was collected.

### 2.3 Synthesis of silver nanoparticles from herbal extracts

#### 2.3.1 Preparation of .1M AgNO3 solution

The aqueous solution of .1M silver nitrate (AgNO3) was prepared by adding 1.69gm of silver nitrate to 100ml of distilled water, stored in a dark place and used for the synthesis of silver nanoparticles.

### 2.3.2 Synthesis of silver nanoparticles from dried herbal extracts

1 ml of herbal extract was added into 2 ml of aqueous solution of .1M silver nitrate for reduction into Ag+ ions and kept for 15-20 minutes at 70-75°C. This aqueous extract acts as reducing and stabilizing agent for .1M of AgNO3. The prepared AgNPs were further used for antibacterial activity assay.

### 2.4 Media preparation, sterilization and inoculation

### 2.4.1Nutrient broth

Table 2 Nutrient broth

| | |
|---|---|
| Nutrient medium | 13 g |
| Distilled water | 1000ml |

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium in 1000ml distilled water and boiled to dissolve the medium completely.The medium wasdispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

### 2.4.2 Nutrient agar

Table 3 Nutrient agar

| | |
|---|---|
| Nutrient medium | 13 g |
| Agar | 25g |
| Distilled water | 1000ml |

One litre of nutrient agar was prepared by dissolving 13 g ofcommercially available nutrient medium (HiMedia) and 25g agar in 1000ml distilled water and boiled to dissolve the medium completely. The medium wasdispensed as desired and sterilized by autoclaving at 15 lbspressure(121ºC) for 15 minutes

### 2.4.3 Culturing of microorganisms

To the sterile nutrient broth microorganisms were inoculated and flasks were kept on orbital shaker for 24hrs at room temperature.

### 2.5 Antibacterial assay of herbal extracts and AgNP

### 2.5.1 Agar- well diffusion method

Agar well-diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA)plates were swabbed with 12hours old - broth culture of respective bacteria. (Escherichia coli, Staphylococcus aureus, Bacillus subtilis). Here 3 Wells were boredand 20 µl of the herbal extracts (namely aqueous) in 1st well , 20µl of silver nano particle produced from herbal extracts in the 2nd well and 20 µl of aqueous solution of AgNO3 in 3rd well were added using sterile syringe into the wells and allowed to diffuse at room temperature for 2hrs. The plates were then incubated at 37°C for 24 hours. The antibacterialactivity was assayed by measuring the diameter of the inhibition zoneformed around the well.

### 2.5.2 Determination of minimum inhibitory concentration

The minimum inhibitory concentration is defined as the lowest concentration able to inhibit any visible bacterial growth on the culture plates. This was determined from readings on the culture plates after incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism.

The Minimum Inhibitory Concentration (MIC) was determined for the 4 plants whichshowed antibacterial activity against E. coli, Bacillus substilis, Staphylococcus aureus.

### 2.6 Phytochemial analysis

To analyse qualitative and quantitative phytochemicals and evaluate in vitro antioxidant properties of various alcoholic and aqueous extracts of leaf and root parts of Hypochaerisradicata. Methods: Preliminary phytochemicals analysis for alkaloids, cardiac glycosides, flavonoids, glycosides, phenols, resins, saponins, steroids, tannins, terpenoids and triterpenoids and quantitative phytochemicals analysis for alkaloids, total phenolics, total flavonoids, tannins, saponins and ascorbic acid were made by following standard procedures. In vitro antioxidant properties were evaluated by assessing DPPH• , NO• and ABTS•+ , radical scavenging abilities and assaying the reducing power, β-carotene and antihemolytic activities by adapting standard methods.

The analysis was carried out in a lab care keralam ltd (confederation for Ayurvedic renaissance-keralam limited) is a public limited company established as a cluster of Ayurveda industries with the support of ayush department of government of india and equity partnership from government of kerala through the kerala state industrial infrastructure development corporation (kinfra).

2.6.1 Test for phytochemical constituents

Test for Alkaloides

0.5 ml of each extract was stirred with 5cm3 of 1% aqueous hydrochloric acid on a steam bath. 1cm3 each of the filtrate was treated, and then it was treated separately with a few drops of Drangedrorfts reagents, Meyer's reagent and Wagner's reagents. A deep brown creamy precipitate indicates a positive test (Manga and Oyeleke, 2008).

Test for Flavonoids

1ml (1cm3) of each extract was dissolved in 2mls of sodium hydroxide solution. The appearance of a yellow solution which disappeared on addition of hydrochloric acid indicates the presence of flavonoids (Manga and Oyeleke, 2008).

Detection of Phenols

Ferric chloride test: 10mg extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates that the presence of phenol.

Detection of Saponins

About 0.5mg of the extract was shaken with five ml of distilled water. Formation of frothing (appearance of creamy miss of small bubbles) shows that the presence of saponins.

Detection of Steroids

Two ml of acetic anhydride was added to five mg of the extracts, each with two ml of H2SO4. The colour was changed from violet to blue or green in some samples indicate that the presence of steroids.

Detection of Tannins

A small quantity of extract was mixed with water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green colour was formed. It indicates that the presence of tannins.

Test for Triterpenoids:

Liebermann Burchard test - Crude extract was mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers, the formation of deep red color in the lower layer would indicate a positive test for triterpenoids.

Test for Glycosides:

Keller Killiani Test – Test solution was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

Test for Carbohydrate:

Benedict's test – Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

## 3 RESULTS AND DISCUSSION

The antimicrobial activity of herbal extracts with silver nanoparticles is compared with the antimicrobial activity of

herbal extracts and silver nano particles, against the three microbial pathogens by agar well diffusion method successfully. Four different types of herbal plants are used here to test the antimicrobial activity.These different herbal plants are Ocimum sanctum, Chromolaena odorata, Azadirachta indica, Mimusops elengi. And the different microbial pathogens used here are staphylococcus aureus, Bacillus subtilis, E.coli.

Table 4: Antibacterial activity of dried herbal extract at different concentrations

| Conc. (mg/ml) | Azadirachtaindica(Neem) | | | Ocimum sanctum (Tulsi) | | | Chromolaenaodorata (Christmasbush) | | | Mimusopselengi (Elengi) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B | S | E | B | S | E | B | S | E | B | S | E |
| 50 | - | - | - | - | - | - | - | - | - | - | - | - |
| 75 | - | - | - | - | - | - | - | - | - | - | - | - |
| 100 | - | - | - | - | - | - | - | - | - | - | - | - |
| 125 | - | - | - | - | - | - | - | - | - | - | - | - |
| 150 | - | - | - | - | - | - | - | - | - | - | - | - |
| 175 | + | + | + | + | + | + | + | + | + | + | + | + |



Table 5: Antibacterial activity of fresh herbal extract at different concentrations

| Conc. (mg/ml) | Azadirachtaindica(Neem) | | | Ocimum sanctum (Tulsi) | | | Chromolaenaodorata (Christmasbush) | | | Mimusopselengi (Elengi) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B | S | E | B | S | E | B | S | E | B | S | E |
| 50 | - | - | - | - | - | - | - | - | - | - | - | - |
| 75 | - | - | - | - | - | - | - | - | - | - | - | - |
| 100 | + | + | + | + | + | + | + | + | + | + | + | + |

**Synthesis of AgNPs**

The green synthesis of silver nanoparticles through plant extracts were carried out. On mixing the herbal extracts with silver nitrate solution (.1 M), a change in the colour from pale yellow to dark brown was observed. Similar results were also reported by many researchers. The brown color confirms that it was due to the reduction of Ag+ which indicates the formation of Ag nanoparticles. Silver nanoparticles bear a characteristic yellow brown colour due to the excitation of surface plasmon vibrations.

AgNO3 Solution AgNP production from Ocimum sanctum AgNP production from Chromolaenaodorata

AgNP production from Azadirachtaindica AgNP production from Mimusopselengi

Fig. 7synthesisofAgNPs



**Characterization of Ag nanoparticles**

UV–VIS spectral analysis: In our results peak specific for the synthesis of silver nanoparticles was obtained at 450 nm by UV Visible spectroscope in the form of a sharp peak which was specific for the synthesis of AgNPs. It is well known that colloidal silver nanoparticles exhibit absorption at the wavelength from 390 to 420 nm due to Mie scattering . Hence, the band at 430-450 nm can be attributed to the property of Mie scattering. This may not include the protecting agent, because the Mie scattering responds only to the silver metal.

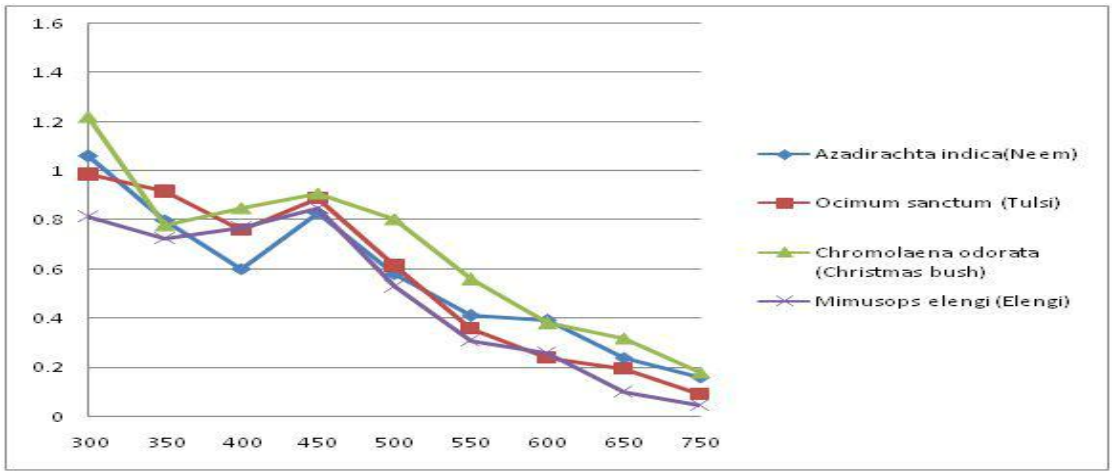



Fig.8 : UV-VIS absorption spectra of AgNPs produced from fresh herbal extracts at a range of 300-750 nm.

Figure 8 shows UV-Vis absorption spectrum of silver nanoparticles. though the plasmon band is broad due to the presence of components in leaf extracts which are also being read in the spectrophotometric range, it is observed that the silver

surface plasmon resonance (SPR) occurs at 450 nm and steadily increases in intensity as a function reaction time. there is no change in peak position, suggesting that nucleation of silver nanoparticles starts with initiation of reaction time only, and the size remains unchanged throughout the course of reaction. According to Mie theory, only a single SPR band is expected in the absorption spectra of spherical nanoparticles whereas, the number of peaks increases as anisotropy increases . In the present study, SPR band reveals spherical shape of silver nanoparticles.

**Antibacterial activity of AgNPs produced from herbal extracts** The inhibitory action of silver compounds and silver ions had been historically recognized and applied as a useful therapeutic agent for preventing wound infections. The inhibitory action of silver on bacterial cells is related to the strong interaction of silver with thiol groups present in key respiratory enzymes in bacteria .Whereas, Nano crystalline silver shows the most effective inhibitory action with a rapid inhibition rate. In the present study, herbal extracts was taken for synthesis of AgNPs because of its medicinal values. Various studies have been done by many researchers which confirm that herbal extract was found to be good antibacterial agent against pathogenic and non-pathogenic organisms.

In this study, we are comparing the antibacterial activity of silver nano particle produced from fresh and dried leaves of herbals.

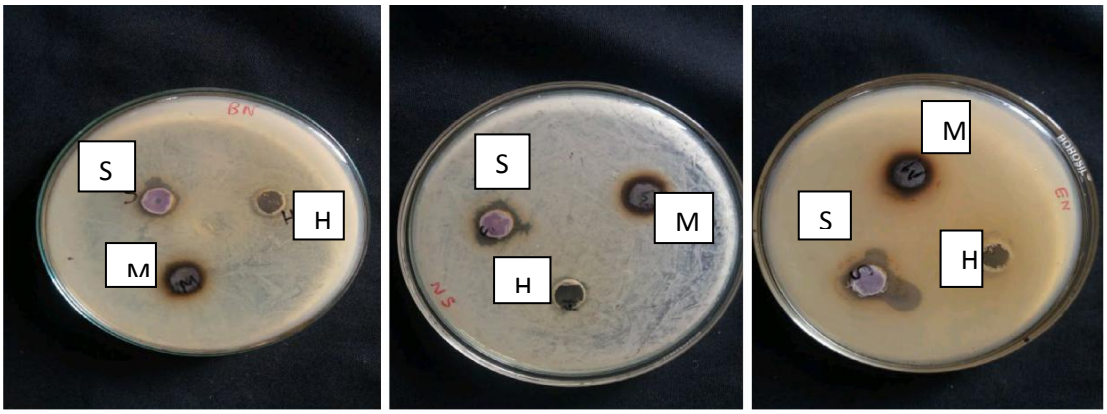
Table 6: Antimicrobial activity of dried herbal extracts &AgNP produced from dried herbal extracts against bacterial pathogens

| | Azadirachtaindica(Neem) | | | Ocimum sanctum (Tulsi) | | | Chromolaenaodorata (Christmasbush) | | | Mimusopselengi (Elengi) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Zone of inhibition(mm) | | | | | | | | | | | |
| | B | S | E | B | S | E | B | S | E | B | S | E |
| AgNO3 solution (0.1M) | 13±1 | 15±2 | 16±2 | 20±2 | 18±3 | 11±2 | 16±1 | 14±3 | 13±3 | 14±1 | 11±4 | 11±1 |
| AgNP | 13±2 | 14±3 | 10±2 | 10±1 | 11±3 | 11±3 | 8±0 | 8±0 | 10±1 | 8±0 | 12±2 | 9±0 |
| Herbal extract (100mg/ml) | 9±1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Values are mean± SD of three replicates

AgNP=silver nano-particle,B= Bacillus subtilis,S=Staphylococcus aureus,E=Escherichia coli

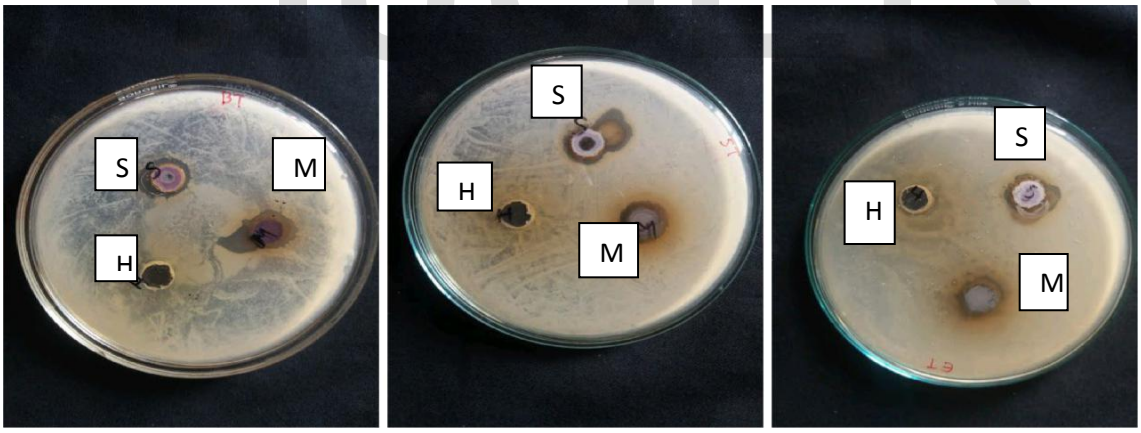
Azadirachtaindica (NEEM)




(a) (b) (c)

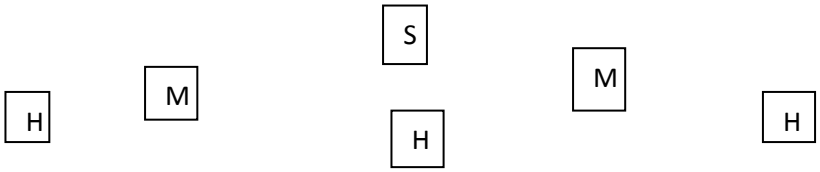
Ocimum sanctum (TULSI)





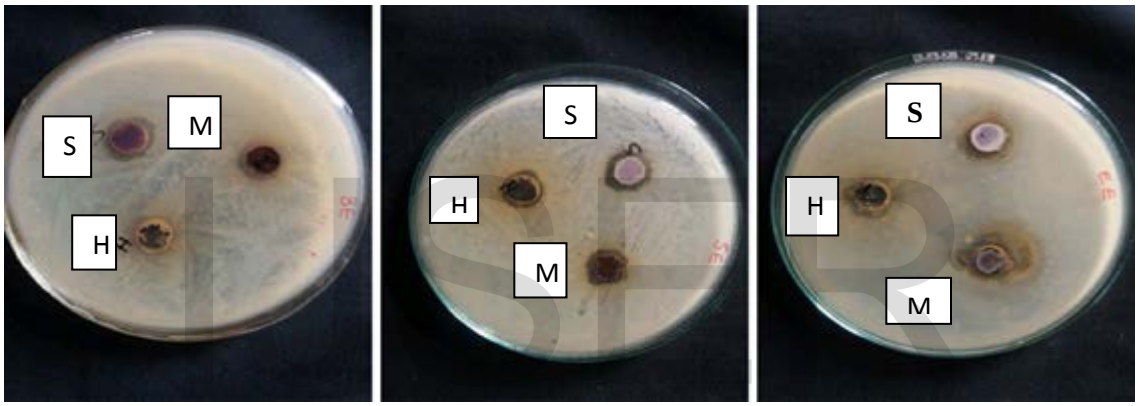

(a) (b) (c)

Chromolaenaodorata (CHRISTMAS BUSH)




(a) (b) (c)

Mimusopselengi (ELENGI)




(a) (b) (c)

Fig.9: Antibacterial activity of fresh herbals against (a)= Bacillus subtilis, (b) =Staphylococcus aureus,

(c) = Escherichia coli, S=0.1M AgNO3 solution, H=herbal extract, M=AgNP

Table 7: Antimicrobial activity of fresh herbal extracts &AgNP produced from fresh herbalextracts against bacterial pathogens

| | Azadirachtaindica(Neem) | | | Ocimum sanctum (Tulsi) | | | Chromolaenaodorata (Christmasbush) | | | Mimusopselengi (Elengi) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Zone of inhibition(mm) | | | | | | | | | | | |
| | B | S | E | B | S | E | B | S | E | B | S | E |
| AgNO3 | 16±4 | 15±1 | 22±2 | 21±5 | 20±5 | 16±2 | 13±2 | 16±3 | 15±0 | 14±0 | 13±1 | 19±6 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| solution (0.1M) | | | | | | | | | | | | |
| AgNP | 12±2 | 14±3 | 13±0 | 23±2 | 14±4 | 15±0 | 17±0 | 17±5 | 17±5 | 13±4 | 12±1 | 24±6 |
| Herbal extract (100mg/ml) | 8±0 | 0 | 0 | 12±2 | 12±1 | 15±0 | 0 | 8±0 | 0 | 8±0 | 12±2 | 11±4 |

Values are mean± SD of three replicates

AgNP=silver nano-particle, B= Bacillus subtilis, S=Staphylococcus aureus,

E=Escherichia coli

Based on the information got from this study the antibacterial effect of four herbal extracts can change depending on the state of the material used whether it is dry or fresh. Fromthis study, extracts of fresh herbals are more effective in the antibacterial activity than the extracts of dried herbals.

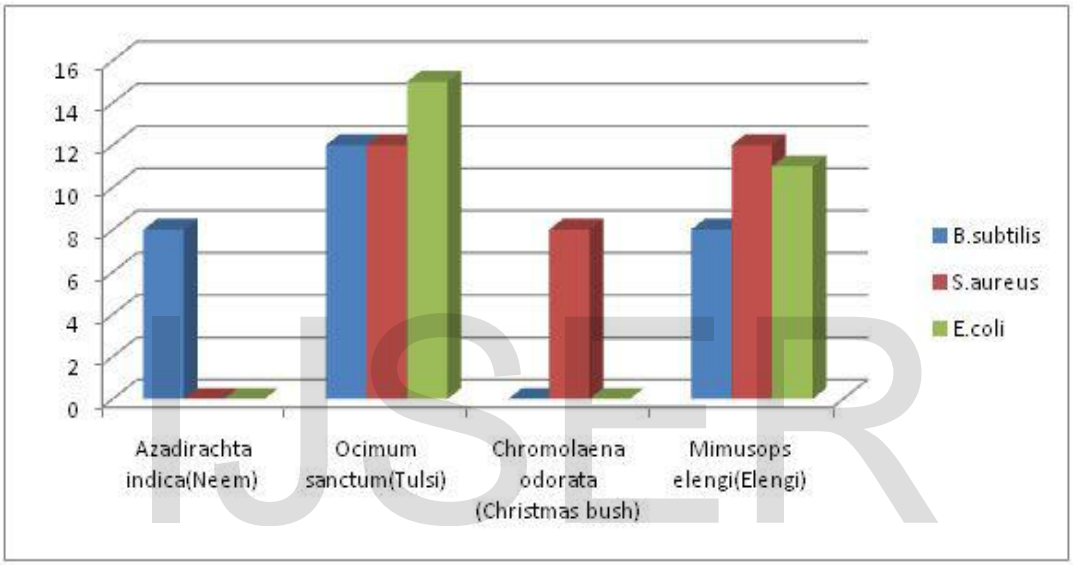





Fig .10: Antibacterial activity of fresh herbal extracts

From the fig.10,the graph shows the antibacterial activity of aqueous extracts of fresh herbals against bacterial pathogens, in which the extract of Azadirachtaindica(Neem) is only active against B.subtilis. The graph shows the fresh herbal extract of Ocimum sanctum is more effective against E.coli, also it has activity against S.aureus& B.subtilis.Aqueous extract of fresh Ocimum sanctum is found to be more effective in controlling gram negative bacteria than gram positive bacteria. The extract was more effective against the test organisms, may be due to the presence of phenolic compounds, terpenoids, alkaloids, flavonoids and steroids etc.Chromolaenaodorata fresh leaf extract showed antimicrobial activity against S.aureus and not shown activity against B.subtilis&E.coli . The results obtained from this study showed that the aqueous extract of fresh Mimusopselengi was found to be effective against both gram positive and gram negative bacterial strains, may be due to the presence of phenolic compounds, terpenoids, alkaloids, flavonoids and steroids etc.The extract shown more activity against S.aureuscompared to E.coli& B.subtilis.

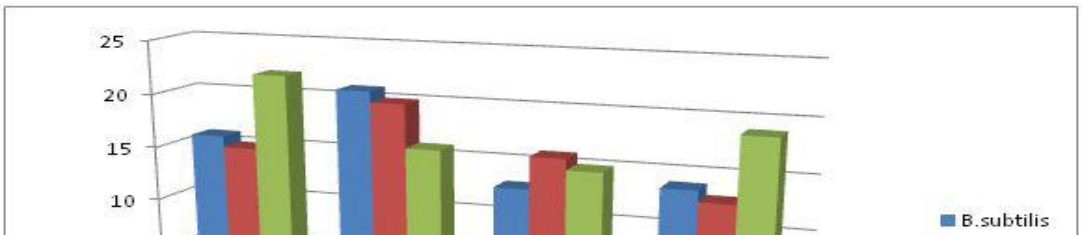


Fig.11: antibacterial activity of AgNP produced from fresh herbal extracts

From the fig , the graph shows that the AgNP produced from Azadirachtaindica shows activity against B.subtilis, S.aureus&E.coli ,then also it is more effective against E.coli rather than other two bacteria. Investigation on the antibacterial activity of AgNPsagainst Bacillussubtilis, Staphylococcus aureus and E. coli reveals the potential of Ocimum sanctum extract stabilized AgNPs to be used as antimicrobial agent in medical field as well as food and cosmetic industries. AgNP produced from the fresh Chromolaenaodorata leaf extract showed activity against pathogens. but it shows more effectiveness against S.aureus. AgNP produced from fresh Mimusopselengi leaf extract also showed activity against ,it is more effective against S.aureus.

Phytochemical composition:

The results of phytochemical screening, presented in table showed the presence or absence of alkaloids, flavanoids, triterpenoids, saponins, glycosides, phenols, sterols, tannins and carbohydrate in the different leaf extracts.



| Sl.No. | Parameters | Result | Specification | Detection Limit | Test Method |
|---|---|---|---|---|---|
| 1 | Alkaloids | Present | - | - | Experimental Phytopharmacognosy |
| 2 | Flavanoids | Absent | - | - | Experimental Phytopharmacognosy |
| 3 | Phenol | Present | - | - | Experimental Phytopharmacognosy |
| 4 | Saponins | Present | - | - | Experimental Phytopharmacognosy |
| 5 | Sterols | Absent | - | - | Experimental Phytopharmacognosy |
| 6 | Tannins | Present | - | - | Experimental Phytopharmacognosy |
| 7 | Triterpenoids | Absent | - | - | Experimental Phytopharmacognosy |
| 8 | Glycosides | Present | - | - | Experimental Phytopharmacognosy |
| 9 | Carbohydrate | Present | - | - | Experimental Phytopharmacognosy |

Table 8: phytochemical analysis of Azadirachtaindica(Neem)

| Sl.No. | Parameters | Result | Specification | Detection Limit | Test Method |
|---|---|---|---|---|---|
| 1 | Alkaloids | Present | - | - | Experimental Phytopharmacognosy |
| 2 | Flavanoids | Present | - | - | Experimental Phytopharmacognosy |
| 3 | Phenol | Present | - | - | Experimental Phytopharmacognosy |
| 4 | Saponins | Absent | - | - | Experimental Phytopharmacognosy |
| 5 | Sterols | Present | - | - | Experimental Phytopharmacognosy |
| 6 | Tannins | Present | - | - | Experimental Phytopharmacognosy |
| 7 | Triterpenoids | Absent | - | - | Experimental Phytopharmacognosy |
| 8 | Glycosides | Absent | - | - | Experimental Phytopharmacognosy |
| 9 | Carbohydrate | Present | - | - | Experimental Phytopharmacognosy |

Table9:Phytochemical analysis ofOcimum sanctum (tulsi)

| Sl.No. | Parameters | Result | Specification | Detection Limit | Test Method |
|---|---|---|---|---|---|
| 1 | Alkaloids | Present | - | - | Experimental Phytopharmacognosy |
| 2 | Flavanoids | Present | - | - | Experimental Phytopharmacognosy |
| 3 | Phenol | Present | - | - | Experimental Phytopharmacognosy |
| 4 | Saponins | Present | - | - | Experimental Phytopharmacognosy |
| 5 | Sterols | Absent | - | - | Experimental Phytopharmacognosy |
| 6 | Tannins | Present | - | - | Experimental Phytopharmacognosy |
| 7 | Triterpenoids | Absent | - | - | Experimental Phytopharmacognosy |
| 8 | Glycosides | Present | - | - | Experimental Phytopharmacognosy |
| 9 | Carbohydrate | Present | - | - | Experimental Phytopharmacognosy |

Table 10: phytochemical analysis of Chromolaenaodorata (Christmas bush)

| Sl.No. | Parameters | Result | Specification | Detection Limit | Test Method |
|---|---|---|---|---|---|
| 1 | Alkaloids | Present | - | - | Experimental Phytopharmacognosy |
| 2 | Flavanoids | Absent | - | - | Experimental Phytopharmacognosy |
| 3 | Phenol | Present | - | - | Experimental Phytopharmacognosy |
| 4 | Saponins | Absent | - | - | Experimental Phytopharmacognosy |
| 5 | Sterols | Absent | - | - | Experimental Phytopharmacognosy |
| 6 | Tannins | Present | - | - | Experimental Phytopharmacognosy |
| 7 | Triterpenoids | Absent | - | - | Experimental Phytopharmacognosy |
| 8 | Glycosides | Present | - | - | Experimental Phytopharmacognosy |
| 9 | Carbohydrate | Present | - | - | Experimental Phytopharmacognosy |

Table 11: Phytochemical analysis of Mimusopselengi(Elengi)

## 4 CONCLUSIONS

This study showed that leaf extracts and AgNP produced from the herbal extracts of A. indica (Neem), Ocimumsanctum (tulsi), Chromolaenaodorata (christmasbush), Mimusopselengi (elengi) has a potent antibacterial activity against various strains of bacterial pathogens. Fresh leaves extract is greater in their

effectiveness compared to dry leaves.It is recommended to isolate and separate the bioactive compounds responsible for this antibacterial activity using advanced scientific techniques.

Based on the information got from this study the antibacterial effect of extracts can change depending on the state of the material used whether it is dry or fresh. Investigation on the antibacterial activity of AgNPs against Bacillus subtilis, Staphylococcus aureus and E. coli reveals the potential of different herbal extract stabilized AgNPs to be used as antimicrobial agent in medical field as well as food and cosmetic industries. The extract was more effective against the test organisms, may be due to the presence of phenolic compounds, terpenoids, alkaloids, flavonoids and steroids etc.

Some herbs tested in this work could be used as natural preservatives for prolonging shelf-life of food. Some of them showed moderate antimicrobial effect, thus they rather could be used in mixture with other herbs. Susceptibility of individual bacteria to extracts was very dissimilar. It is important to research antimicrobial effects of herbs and other plants in many various forms, like different extracts or essential oils, as a natural source of antimicrobial compounds.

## 5 FUTURE PROSPECTS

The formulations of plant extract with silver nanoparticle worked out in this project to find the antimicrobial activity can be useful in various fields of biotechnology. So we are aimed to use these formulations for the food preservation technologies and in the development of drugs against the food borne microbial pathogens. And can be used in the development of drugs against the microorganism's present in the wound sites in body .And also these formulations can be applied to produce different healthcare products like hand washes, soaps.

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