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PREPARATION OF SILVER NANOPARTICLES FROM HERBAL PLANT

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Abstract:

Recent advances in the field of nanotechnology have found interesting and important applications in clinical medicines and surgery. One promising application is the use of nano sized particles for the delivery of drugs, heat, radiation and other substance to specific types of cells (such as cancer cells). In view of steep rise in the infection caused by multi drugs resistant bacteria in recent years and the reduced efficacy of employed antibiotics-owing to their overuse or misuse, bacteria resistance has dramatically increased. In this respect, silver, which has long been recognized as having inhibitor effects on microbes, has found a major use in the form of nanoparticles. These biologically synthesized nanoparticles have proved to be highly toxic against different multi-drug resistant human pathogens. When synthesized from medicinal plant extract, this device can effectively prevent and combat strains of microbes that have developed resistance to common antibiotic drugs. Further, nanoparticles can also be coated with suitable substrates and used in chemotherapy. In certain cases, formal chemotherapeutic methods are proving to be ineffective, as a result of the development of multiple drug resistance (MDR) in cancer cells by unknown mechanisms. A prospective solution herein, involves the design of nanoparticles with proteins that specifically detect the drug resistant cells and ensure high drug concentration at the designated site. While limiting side effects to neighbouring, healthy tissues. However, conventional procedures of synthesis of silver nanoparticles such as ultraviolet irradiation, aerosol technologies were expensive and sometimes release environmentally hazardous chemicals. To circumvent these problems, biosynthesis of nanoparticles – a greener alternative – is been employed wherever suitable. This project deals with the green synthesis of silver nanoparticles from the leaf extract of common Indian plant, Azadirachta indica (Neem), Ocimum tenuiflorum (Tulsi).

Key words: *Silver nanoparticles, anti microbial activity, Ocimum tenuiflorum, Green synthesis, Azadirachta indica.*

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INTRODUCTION:

Man has been fascinated by nature since he evolved from his primitive ancestors, the apes. To start with, he hunted for food mainly by killing the wild animals, but if there was anything on which he could depend with any confidence towards its availability, it was the plant which provided him with food and they provided him with curative medicine and shelter. Because of this the primitive man was in love with the nature especially with plants because plants were the only source to fight with various diseases. From the plants they found various medicines and treatment practices to treat many diseases which put way for the modern treatment systems to save the human race. Today in this world traditional medicine plays a vital role in providing health care to large section of population, especially in developing countries [1]. Medicinal plants are the important source for the production of synthetic and herbal drugs. Herbs are being used by about 80% of the world population especially in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and minimal side effects. Medicinal plants exhibit phytotherapeutic effects caused by biologically active compounds specific secondary metabolites. The plants have been utilized for basic and curative health care since time immemorial. The use of plants as food and medicines started ever since man started life on the planet. The plant kingdom is a virtual goldmine of potential drug targets and other active drug molecules waiting to be discovered [2].

Nanotechnology deals with the synthesis of nanoparticles with controlled size, shape, and dispersity of materials at the nanometer scale length. Nano-technology is a rapidly growing field in which research deals with the synthesis, design, and particle structures manipulation which are ranging from 1-100 nm. Nanoparticles possess high surface area to volume ratio. Nanoparticles such as silver, gold, cadmium sulfide, zinc sulfide, and zinc oxide play important role in various fields. Silver nanoparticles are having good history in the field of antimicrobial properties. The silver nanoparticles are vigorously involved in the antimicrobial activity against a lot of disease causing food borne and water borne pathogenic bacteria and fungus. Various nanoparticles have been synthesized by using plant extracts which includes silver, gold, and copper oxide. Use of plant extracts for nanoparticles synthesis is favorable over the other biological material as it removes the long process of maintenance of cell culture. Among various metal nanoparticles, silver nanoparticles obtain more attention due to its good conductivity, stability and antimicrobial activity. The biological activity of silver nanoparticles depends on various factors such as size, shape, size, surface chemistry, distribution, particle composition, particle morphology, capping, agglomeration, etc. Nanoparticles physicochemical properties increase the bioavailability of therapeutic agents. Therefore, development of silver nanoparticles with controlled structures that are uniform in morphology, size, and functionality is important for its various applications [3].

Azadirachta indica, commonly known as neem, nimtree or Indian lilac, is a tree in the mahogany family Meliaceae. It is one of two species in the genus *Azadirachta*, and is native to the Indian subcontinent, i.e. India, Nepal, Pakistan, Bangladesh, Sri Lanka, and Maldives. It is typically grown in tropical and semi-tropical regions. The opposite, pinnate leaves are 20–40 centimetres (7.9–15.7 in) long, with 20 to 31 medium to dark green leaflets about 3–8 centimetres (1.2–3.1 in) long. The terminal leaflet often is missing. The petioles are short. Neem leaves are dried in India and placed in cupboards to prevent insects eating the clothes, and also in tins where rice is stored. Neem leaves are dried and burnt in the tropical regions to keep away mosquitoes. These flowers are also used in many Indian festivals like Ugadi. See below: Association with Hindu festivals in India. As an ayurvedic herb, neem is also used in baths. 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, anti fungal, anti diabetic, anti viral, anti bacterial, contraceptive and sedative. It is considered a major component in Siddha medicine and Ayurvedic and Unani medicine and is particularly prescribed for skin diseases. Neem oil is also used for healthy hair, to improve liver function, detoxify the blood, and balance blood sugar levels. Neem leaves have also been used to treat skin diseases like eczema, psoriasis, etc. [4,5].

Ocimum tenuiflorum (synonym *Ocimum sanctum*), commonly known as holy basil, tulasi (sometimes spelled thulasi) or tulsi, is an aromatic perennial plant in the family Lamiaceae. It is native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics. Tulasi is cultivated for religious and supposed traditional medicine purposes, and for its essential oil. It is widely used as a herbal tea, commonly used in Ayurveda, and has a place within the Vaishnava tradition of Hinduism, in which devotees perform worship involving holy basil plants or leaves. Tulsi leaves are an essential part in the worship of Vishnu and his avatars, including Krishna and Rama and other male Vaishnava deities such as Hanuman, Balarama, Garuda and many others. Tulsi is a sacred plant for Hindus and is worshipped as the avatar of Lakshmi. It is believed that water mixed with the petals given to the dying raises their departing souls to heaven. Holy basil is an erect, many-branched subshrub, 30–60 cm (12–24 in) tall with hairy stems. Leaves are green or purple; they are simple, petioled, with an ovate, up to 5 cm (2.0 in) long blade which usually has a slightly toothed margin; they are strongly scented and have a decussate phyllotaxy [6,7].

Conventional procedures for nanoparticle synthesis involve toxic chemicals that are hazardous to the environment. It is hence necessary to pursue safer alternatives such as biologically inspired synthesis of nanoparticles. The biosynthesis of silver nanoparticles (AgNP's) using microorganisms like bacteria, fungi. Enzymes are also ecofriendly methods of synthesis. But this requires highly aseptic conditions that are industrially not viable. Further the rate of synthesis is also slow when compared to the plant mediated synthetic procedures. The plant mediated synthesis is more advanced than the other methods as it has achieved a better yield, controlled size, shape and greater stability of nanoparticles. With the help of spectral data few plant metabolites such as ascorbic acid, citric acid, phyllanthin, to name a few, have been identified as the capping agents responsible for the biogenic synthesis of AgNP's. Silver nanoparticles are of great interest today due to its wide range of biomedical applications and biocidal effect on microorganisms. It is also been found effective in the control and treatment of dreadful diseases like cancer. The biosynthesis of AgNP's is environmentally benign as the plant extracts containing complex bimolecular such as proteins, alkaloids, terpenoids, facilitate the reduction of Ag^{+} to $Ag^{(0)}$. This review focuses on the green synthesis of AgNP's using plant extract as a simple, cost effective and ecofriendly alternative [8,9,10].

Fig. 1: *Azadirachta indica* plant

Fig. 2: *Azadirachta indica* leaves

Fig. 3: *Ocimum tenuiflorum* plant

Fig. 4: *Ocimum tenuiflorum* leaves

Fig. 5: *Azadirachta indica* dried leaves

Fig. 6: *Ocimum tenuiflorum* dried leaves

The main objective is to prepare silver nanoparticles from herbal plants; in the present research, we have prepared silver nanoparticles from dried leaves of Tulsi, Neem and their mixture.

MATERIALS AND METHODS:

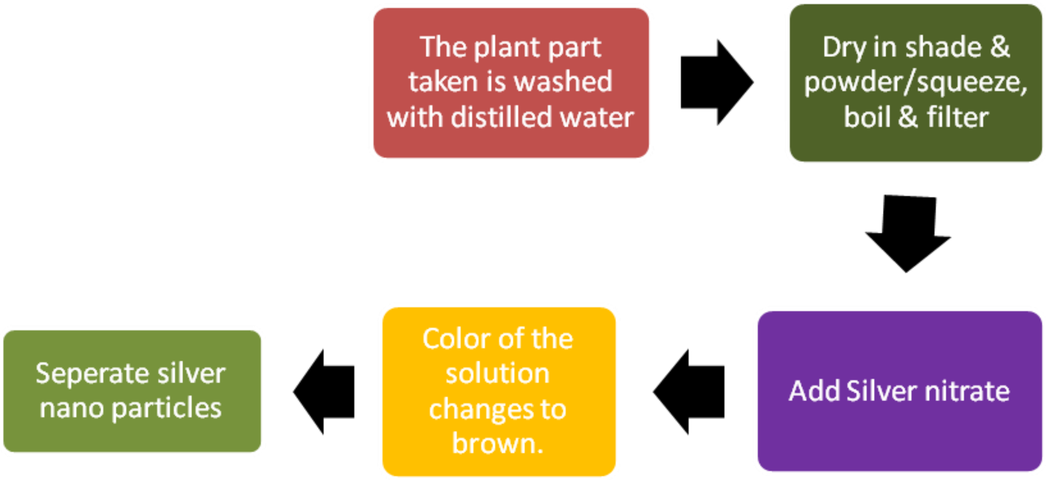

Fig. 7: Graphical representation of synthesis

Collection of plant leaves:

The leaves of Neem and Tulsi were collected from surrounding places of Nagole, Hyderabad Dist. The leaves collected were thoroughly washed (twice or thrice) under running tap water and then with double distilled water to remove adhering substances if any. The clean plant sources are dried in shade for 3-5 days. The dried leaves were powdered and stored in air tight container and protected from light.

Preparation of leaf extract:

Approximately 10-12g of dried powder/ finely cut plant part has then been boiled with 100ml of double sterilized, distilled water for 2-3 minutes. The boiled extract was then filtered with whatmann no.1 filter paper. The extract was then stored at low temperature in refrigerator for further use.

Incubation in $AgNO_3$:

To the 10ml of 0.001M $AgNO_3$ varying amounts of plant extract from 1-5ml can be added separately and vice versa. The set up should be incubated in dark at room temperature for at least 2-3 days. The change in the colour of the solution to brown indicates the synthesis of AgNP's11 which can be monitored by measuring UV-visible spectra of the solution. The size and shape of the AgNP's formed is affected by the change in the pH of the reaction mixture and also by the change in the concentration of the plant extract. The particle size is expected to be lesser in basic medium and larger in the acidic medium. If the pH is changed, the electrical charges on the biomolecules may change which might affect their capping and stabilizing ability and subsequently the growth. Increase in the concentration of plant extract decreases the particle size. In higher extract concentration the biomolecules cap the nanoparticles surfaces protecting them from coagulation, so comparatively a higher extract concentration is essential for the synthesis of symmetrical nanoparticles. Temperature and contact time of the reaction mixture also affect the synthesis of AgNP's.

Fig. 8: $AgNO_3$, Neem extract, Tulsi extract

Fig. 9: Before incubation, in 10ml of $AgNO_3$

RESULTS AND DISCUSSION:

The light coloured solution containing neem, tulsi and their mixture respectively was turned dark. Dark coloured solution indicates the presence of silver nanoparticles.

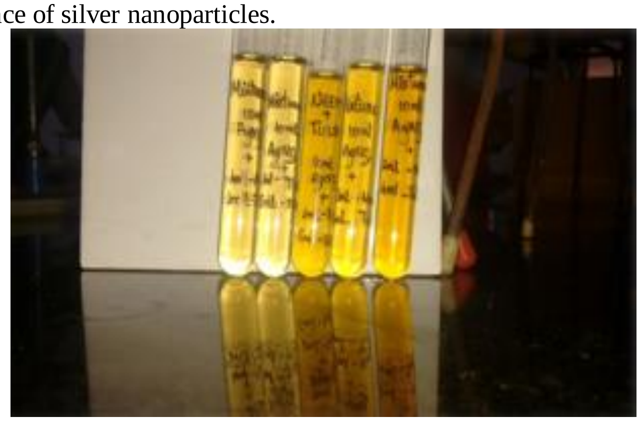

Fig. 10: After incubation-Mixture of Neem and Tulsi extract

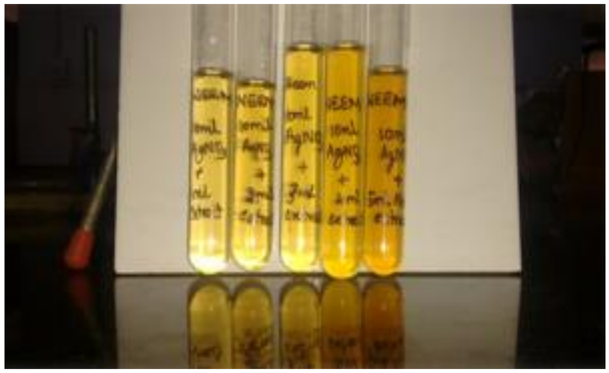

Fig. 11: After incubation-Neem extract

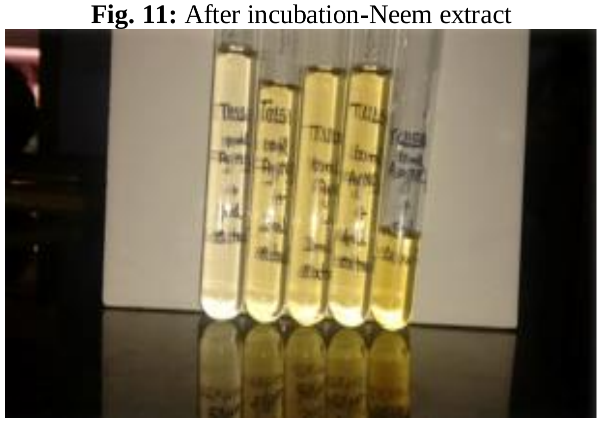

Fig. 12: After incubation-Tulsi extract

Recently, there has been increasing study of AgNPs synthesis to develop several applications such as catalysis, biosensing, imaging, and antibacterial activity. Green synthesis is an alternative method developed to produce metal nanoparticles by using natural compounds or plant components. These are environmentally friendly processes that avoid the toxicity of chemicals. Algae, bacteria, fungi and plants have been used to synthesize NPs without the need for additional reducing and stabilizing agents. The synthesized AgNPs with therapeutic agents can be further capped with more potent drug for greater activity. The AgNPs thus prepared when injected into an infected biological system enters the cells of the microbe, when the positively charged AgNPs accumulate on the negatively charged cell membrane. It brings about a substantial conformational change in the membrane ultimately loses permeability control leading to inflow of the AgNPs. The nanoparticles interact with sulphur containing proteins present in the bacterial membrane as well as with the phosphorous containing DNA. Ag^{+} from the AgNPs then binds to the functional group of proteins, resulting in protein denaturing which causes considerable change to the DNA resulting in cell death. The AgNPs show efficient antimicrobial property due to their extremely large surface area, which provides better contact with microorganisms. Smaller particles having a large surface area available for interaction will have a stronger bactericidal effect than with larger particles.

Toxicity of AgNPs:

Doing a storage period of several days at room temperature coated silver nanoparticles can undergo dissolution releasing toxicity silver ions. The toxicity of silver ions rises from unfavorable binding interaction with the DNA/nucleic acid and cell wall components of the host cells. Small size nanoparticles have been reported to have not only logged but also to have passed through protein channels and nuclear membrane pores in the size range of 9-10nm. Such infiltration of AgNPs presents problems due to their interaction with structures internal to these members. Hence, AgNPs of stipulated size are being used for clinical procedure presently.

CONCLUSION:

The rapid synthesis of nanoparticles through leaf mediated synthetic procedure has been demonstrated. As discussed above, these obtained nano particles have powerful application in the biomedical field and this simple procedure has several advantages such as cost-effective, compatibility for medical and pharmaceutical application as well as large scale commercial production. However, plant uptake and utilization of Silver nanoparticles (AgNPs) require more detailed research on many issues like uptake potential of various species, process of uptake and translocation and the activities of the AgNPs at the cellular and molecular levels.

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Conflict of interest
There is no conflict of interest.

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