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Green Synthesis of Silver Nanoparticles, Characterization and Antimicrobial Activity Studies by Using Gomphrena Serrata Leaf Extract

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Abstract: **As part of our project study we proposed to synthesize silver nanoparticles from the leaf extract of the medicinal plant Gamphrena Serrata and to study its biological activity.We synthesized silver nanoparticles, and they were characterized using UV-Visible spectroscopy and Scanning Electron Microscope (SEM). Fourier Transform Infrared Spectroscopy (FTIR) and X-ray diffraction studies were done for silver nanoparticles. The biological activity of the silver nanoparticles was determined by using well diffusion method. The UV-Visible spectroscopy showed the formation of nanoparticles in a size range of 400-460 nm. FTIR analysis of silver nanoparticles and leaf extracts showed the formation of aldehydes, alkenes, amines, alcohols, etc., which confirmed the presence of the compounds present in plant extracts. SEM image showed the formation of nanoparticles of size 2 micrometer. Phytochemical analysis of plant extracts showed the presence of carbohydrates, phenols, flavonoids, saponins, tannins and terpenoids. The results of present study showed that the silver nanoparticle synthesized from the plant extract of Gomphrena serrate has many bioactive compounds and it was found to have significant biological activities. The silver nanoparticles showed antibacterial activities against both gram positive (Staphylococcus aureus) and gram negative (Escherichia coli) microorganisms. It concludes that the nanoparticle extract can be used as a potential resource for therapeutic purpose.**

Index Terms: Aantimicrobial Activity, FTIR, Gomphrena Serrata, Silver Nanoparticle Synthesis, SEM, XRD.

I. INTRODUCTION

Nowadays the focus of the researchers is on the use of the traditionally available plants because they are medicinally and pharmacologically important and they possess valuable bioactive molecules. From ancient times these medicinal plants have been used and their effectiveness has been increasing day by day in the current world. The compounds available naturally are considered as environmentally friendly and also more effective than the synthetic drug. These medicinal Plants represent a foundation for many pharmaceutical treatments of many human diseases (Akhila et al., 2012). Different constituents present in plant materials which serve as both reducing and capping agents in the synthesis of silver nanoparticles. The green method of nanoparticle synthesis targets mostly on silver nanoparticles as they are striving towards the edge level utilities in every aspect of science and technology including the medical fields. Colloidal silver is a current area of interest due to its distinctive properties viz, good conductivity, catalytic activity, chemical stability and antimicrobial activity (Ashok Kumar et al., 2015). Silver nanoparticles (AgNPs) have been extensively investigated due to their unique size-dependent properties which make them useful in a variety of applications including optical/chemical sensors, electronic devices, catalysts, therapeutic and contrasting agents (Tishima et al., 2008, Daniel et al., 2008, Gao et al., 2009).

Gomphrena serrate belongs to the Amaranthaceae family and the various species present in this family are used in traditional folk medicine for the treatment of various infections, inflammation and fever as well as in nutrition. *Gomphrena* species found all over the world. The phytoconstituents present in it are flavonoids, phytosterols, phenolics and terpenoids (Muhammad et al., 2013).

There is investigation in to the green synthesis of silver nanoparticles as they possess useful properties, like non-toxic nature, less expensive, due to their size and are useful in the field of catalysis, electronics, and in medicine (Herrera et al., 2005). As the conventional methods require the use of harsh organic solvents/surfactants and strong reducing agents (e.g., borohydride or hydrazine) (Liu et al., 2009) and produce large quantities of hazardous wastes and energy consuming, and hence, we considered this method as more economic, alternative

and safe one for the synthesis of nanoparticles. This method controls the size and shape of the particles and these procedures produce stable nanoparticles by avoiding the use of hazardous chemicals.

One of the important uses of this method is that the plant extracts are readily available, safe and nontoxic with various phytochemical constituents which help in the reduction of silver ions (Huang et al., 2007).

Even though various groups of phytochemical constituents were identified from this plant, work on synthesis of nanoparticles using leaf extract has not been carried out. Therefore, in this chapter, we report synthesis of silver nanoparticles, reducing Ag+ ions present in the aqueous solution of silver nitrate by using Gamphrena serrata leaf extract. Silver nanoparticles (AgNPs) have been widely studied and proved as an effective antimicrobial agent. The mechanism is similar as the metallic silver, but it is more effective as an antimicrobial agent than other silver compounds. Silver nanoparticles and silver ions released by silver nanoparticles interact with the biomolecules (Sondi et al., 2004). The emphasis of the current work based on the green routed silver nanoparticle synthesis using medicinal plant Gomphrena serrata and study its biological activity.

II. EXPERIMENTAL

A. Materials and Methods

1) Chemicals

Silver Nitrate, Hydrochloric acid, Sodium hydroxide, Sulphuric acid, Chloroform, Fehling's A, Fehling's B, Million's reagent, Ninhydrin, 1,1-Diphenyl2-picryl hydroxyl (DPPH), DMSO, purchased from National Scientfic products, Guntur. All the chemicals purchased were of analytical grade.

2) Collection of plant material

The leaves of Gomphrena serrate were collected from the Campus of Bapatla Engineering College, Bapatla, Guntur district of Andhra Pradesh, India.

B. General procedure

1) Preparation of Gomphrena serrata leaf extract:

Freshly obtained leaves of the Gomphrena serrata plant were carefully washed to remove any traces of dirt. They were then thoroughly rinsed with deionized water before chopping them into smaller pieces. 10 gm of such chopped leaves were weighed and put in a 250 ml wide neck Borosil conical flask. 200 ml double distilled water was added to the flask containing freshly cut leaves and boiled for 20 min. Then the raw extract obtained was filtered in hot condition with Wattmann filter paper to remove fibrous impurities. The filtered extract was stored in refrigerator at 4 ◦C for further studies (Bhaskaran et al., 2013). In each and every steps of the experiment, sterility conditions were maintained for the effectiveness and accuracy in results without contamination. The Gpmphrena plant, the leaf extract and silver nanoparticles are shown in Figure 1.

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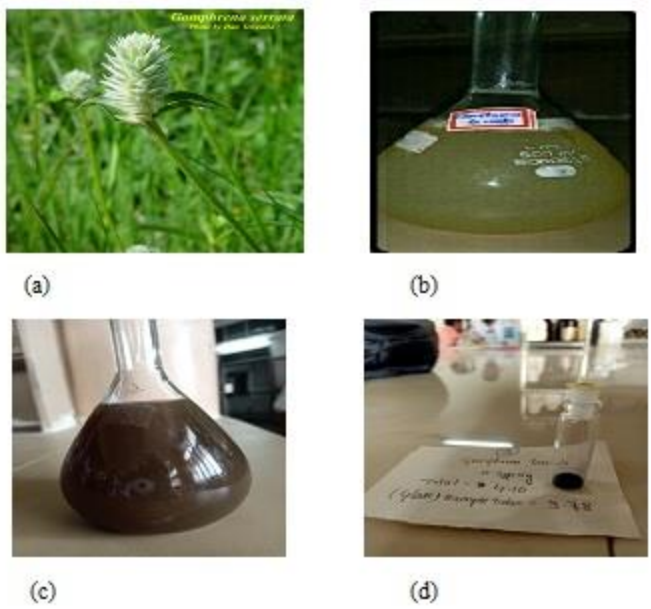

Fig. 1**.** a) Gomphrena Serrata plant b). Gomphrena serrate leaf extract c). Suspended silver nanoparticles in the aqueous medium d). Silver nanoparticles of Gomphrena serrata.

2) Phytochemical Analysis

The plant extract was analyzed with different chemical tests to find the presence of various phytoconstituents by given methods.

a) Test for flavonoid

The crude extract is mixed with 10 ml distilled water; 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate solution then 1 ml concentrated sulphuric acid is added. Indication of yellow color shows the presence of flavonoid.

b) Test for terpenoid

The crude extract is mixed with 2 ml of chloroform and 3 ml of concentrated sulphuric acid was added and mixed thoroughly. A reddish brown color was formed which shows the presence of terpenoids.

c) Test for saponin

The crude extract is mixed with 5 ml distilled water in a test tube then it was shaken thoroughly. The formation of stable foam which indicate the presence of saponins.

d) Test for carbohydrate

The crude extract is mixed with 2 ml of Fehling solution and gently boiled. In the bottom of the test tube a brick red

precipitate is appeared which indicate the presence of reducing compounds.

e) Test for tannin

The crude extract was mixed with water and heated on water bath. The mixture was filtered and added freshly prepared ferric chloride to the filtrate. A dark green solution indicates the presence of tannins.

3) Biosynthesis of silver nanoparticles

90 ml of 0.1 mmol of aqueous Silver nitrate ($AgNO_3$) was prepared in a conical flask, to which 10 ml of plant extracts were added, and the flask was covered with aluminium foil to prevent photoreaction of silver nitrate. The reaction mix showed colour change after 30 min, still for better synthesis the mixture was kept for incubation at room temperature for 72 h.

4) Separation of Silver Nanoparticles

After incubation, the silver nanoparticles were separated by centrifuging the sample at 13,000 rpm for 10 min under refrigeration and washed three times with deionised water. The resultant pellets were collected and suspended thrice in distilled water and centrifuged to remove unbound biomass residues for better Ag nanoparticles for further characterization studies. The silver nanoparticles were dried and stored.

5) Characterization of AgNPs

The optical property of the AgNPs was carried out by UV – visible absorption spectroscopy (Shimadzu – Bio Spec – Nano, Japan). A volume of 100µl of synthesized AgNPs were diluted with 900 µl of distilled water and subjected to spectral analysis in the wavelength range from 200-800 nm. The elemental composition of the synthesized AgNPs was confirmed by scanning electron microscopy. The Scanning electron microscopy spectroscopy (SEM) and Ultraviolet-visible spectroscopy were carrying out to confirmation of Nano scale, uniformity and Nano structure. The X-ray powder diffraction (XRD) was carried out using Rigaku X-ray diffractometer (Rigaku, Japan). The scanning was performed in the region of 2θ=30°–80° at 0.041°/min with a time constant of 2 seconds. The Fourier transform infrared spectrum (FTIR) of the AgNPs was obtained on a Bruker FTIR Spectrometer ALPHA II in the diffuse reflectance mode at a resolution of 4 cm-1 in KBr pellets.The FTIR spectrum was used to identify the functional groups in the plant extract responsible for the reduction of silver ions for the synthesis of silver nanoparticles (Sahayaraj et al., 2011).

III. RESULT AND DISCUSSION

A. Phytochemical screening of G. serrate leaf extract

On the basis of therapeutic potential of secondary metabolites, the phytochemical characters of the Gomphrena serreta leaf investigated and recorded. The qualitative phytochemical analysis of aqueous extract of G. serreta leaf was found to contain triterpenoids, tannin, saponins, glycosides, carbohydrate, terpenoids, anthraquinone (Lavanya et al., 2017).

1) UV- Visible Absorption Spectroscopy

The addition of the tuber extract to 1 mM-solution of $AgNO_3$ changed the color from greenish white to dark brown in about 1 hour. The intensity of the color increased after 72 h of incubation and the reduction of pure Ag^+ ions to Ag was monitored by measuring the UV–Vis spectrum of the reaction media. Figure 2 shows the UV spectra of silver nanoparticles..

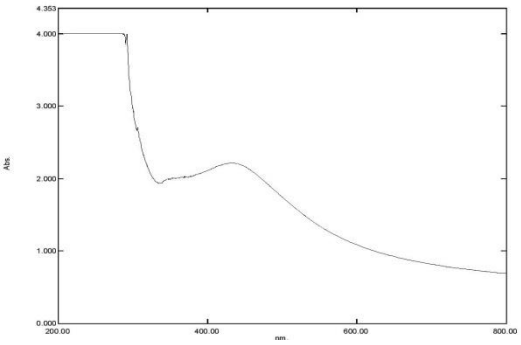

Fig. 2. UV Analysis of Silver nanoparticles of Gomphrena serrate

The optical properties of the synthesized silver nanoparticles were analyzed by this technique. The silver nanoparticles synthesized from Gomphrena serreta leaf extract showed a distinct peak at 430 nm, which are characteristic for AgNPs. Similar characteristic peak at 432 nm has been reported using Gomphrena serreta leaf extract [13]. Silver nanoparticles synthesized from *Andrographispaniculata* showed an absorption band at 410 nm (Sankar et al., 2015).

2) Scanning Electron Microscope Analysis (SEM)

The scanning electron microscopy (SEM) analysis was done to investigate the morphology of the sample at different magnifications which indicate that the AgNPs to be in nanostructure (Lin et al., 2014). The nanoparticles obtained from this leaf extract are generally were shown to be random and non-uniform with agglomeration. It is reported in the literature that these particles to be spherical in morphology as shown in Figure 3. In this SEM image, some of the nanoparticles show large size due to the aggregation of small size of nanoparticles. Polydispersed nanoparticles were observed in SEM image which is shown in Figure 3. The surfaces of aggregated nanoparticles were shown to be rough. This aggregation of nanoparticles may be due to the insufficiency of capping agent in the leaf extract to synthesis of nanoparticles.

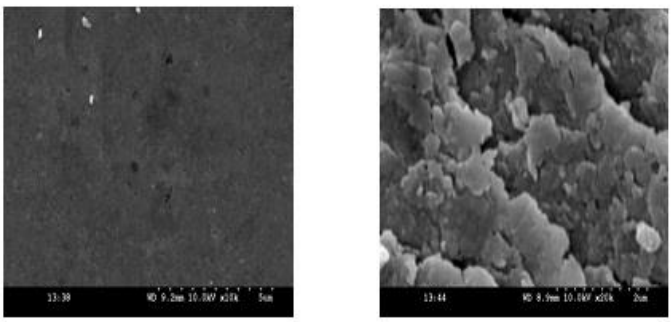

Fig. 3. SEM Analysis Silver nanoparticles of Gomphrena serrate

3) FTIR analysis

The FTIR spectrum of biosynthesized silver nanoparticles (AgNPs) of Gomphrena serreta was recorded . The FTIR spectra, fig. 4 showed the characteristic peaks of OH, alkene,

aldehyde groups which may be involved in the reduction and stabilization of silver nanoparticles. The presence of OH, aldehyde and alkene in the extract acted as the capping and stabilizing agent for the synthesis of silver nanoparticles (Netai et al., 2017). Our results are in accord with the above studies showing the presence of several functional groups in Gomphrena serreta leaf extract which were expressed in the synthesized silver nanoparticles possibly rendering capping and stabilization to the particles. Some pronounced peaks were observed at 3,337, 3,009, 1,743, 1,091, and 733 cm^{-1} in the 4,000–400 cm^{-1} region. The corresponding peaks were associated with the stretching vibrations of -C–O, C–H, C=C, CH_2, and O–H, respectively. The peaks could be attributed to the phytochemicals present in the tuber extract, such as reducing sugars, flavonoids, saccharides, and proteins. The FTIR spectrum of the AgNPs is shown in Figure 5.

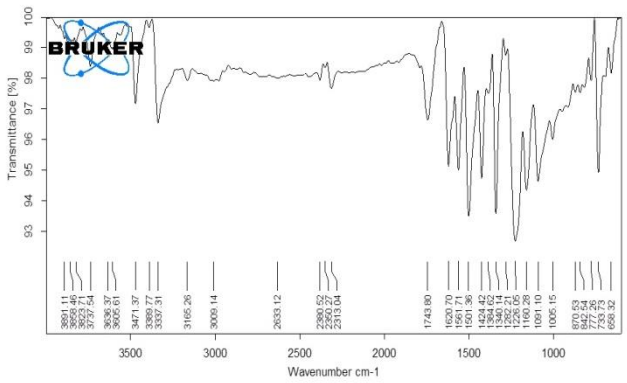

Fig. 4**.** FTIR Analysis Silver nanoparticles of Gomphrena serrate

4) X-ray powder diffraction studies

The crystal nature of the biosynthesized AgNPs from the Gomphrena serreta leaf extract was examined by XRD technique. The XRD profiles of the synthesized AgNPs are depicted in figure 5. The diffraction peaks at 2θ values of 38.13°, 46.72°, 64.44° and 76.93° can be index to (111), (002), (022) and (113) planes of face centered cubic structure of AgNPs (Ponarulselvam et al., 2012). This confirmed the presence of silver and the highly crystalline nature of the particles (Wani et al., 2011). The results are in agreement with several studies that reported the cubic nature of biologically synthesized AgNPs.The pattern of AgNPs synthesized from Gomphrena serreta extract showed some additional peaks, which might be due to the presence of organic molecules in the extract and these peaks are shown in Figure 5. Thus, the results of X-ray diffraction pattern validated the presence of organic molecules that facilitate the synthesis of nanoparticles.

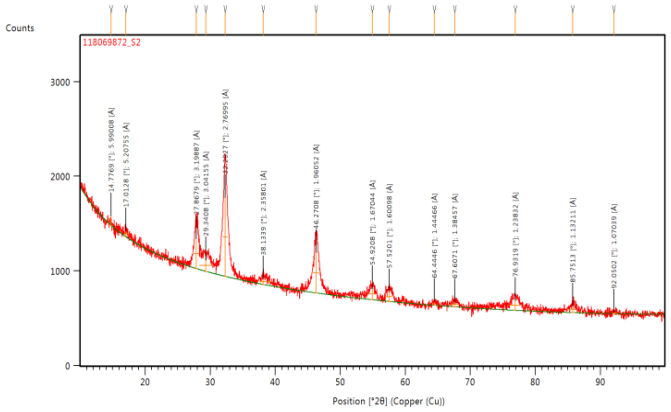

Fig. 5. XRD Analysis of AgNPs of Gomphrena serrate

5) Antimicrobial activity of silver nanoparticles

The antimicrobial activity of the given compound was determined using well diffusion method against sixi.e.*Candidaalbicans* (CA), Staphylococcus aureus (SA), *Escherichia coli* (E.coli), Klebsiellaplanticola (K.P), Bacillus subtilis (BS), Micrococcus luteus(MLS-16) different pathogenic bacterial strains obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India. The pathogenic reference strains were seeded on the surface of the Petri plates containing Muller-Hinton agar with 0.1 ml of previously prepared microbial suspensions individually containing 1.5×10^8 cfu ml^{-1} (equal to 0.5 McFarland standards). Wells of 6.0 mm diameter were prepared in the medium plates using a cork borer and the test compounds dissolved in 10 % DMSO at a dose range of 250 µg/mL were added in each well under sterile conditions in a laminar air flow chamber. A standard antibiotic solution of Ciprofloxacin is taken as positive control, while the well containing DMSO served as negative control. The plates were incubated at 37 °C for 24 h for the different bacterial strains. After that the results have noticed that compound named S2 in figure (Gomphrena serrate) has showed activity. The results of the analysis are given in Table 1 and Figure 6.

Table 1: Activity study of AgNPs of Gomphrena serrate

S.NO	Name of the Test Organism	Zone of inhibition (mm)
		S2
1	Bacillus subtilis	≥ 20
2	Micrococcus luteus	≥ 10
3	Staphylococcus aureus	≥ 15
4	Klebsiellaplanticola	≤ 10
5	Escherichia coli	≤ 5
6	Candida albicans	≥ 10

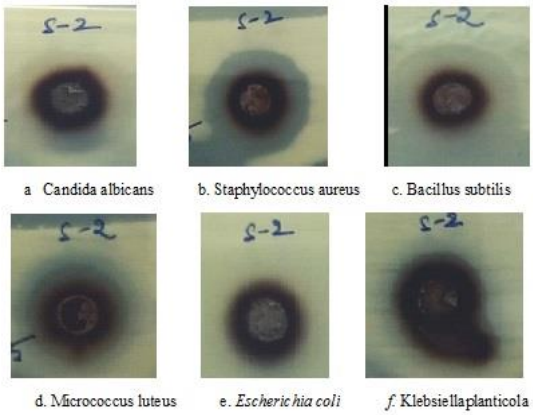

Figure 6: Antimicrobial activities of silver nanoparticle of Gomphrena serrate

CONCLUSION

When It was concluded on the basis of the above studies that various techniques applied for characterizing the silver nanoparticles exhibited the fruitful capping of the phyto organic components on silver and formed dispersed and spherical nanoparticles. The biological potential of these particles is due to the presence of these functional groups on their surface. The silver nanoparticles exhibited a stability profile of good quality. The suitable structures for the particles were characterized successfully. The Gomphrena serreta leaf extract has antifungal activity. Further, the method used for the synthesis of silver nanoparticles is of low cost, eco-friendly, and for designing new drugs this method could be applied in the medical health care.

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DECLARATION OF INTEREST:

The authors report no conflicts of interest.

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