



IJRASET

International Journal For Research in
Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 2 Issue: VIII Month of publication: August 2014

DOI:

www.ijraset.com

Call:  08813907089

E-mail ID: ijraset@gmail.com

Biogenic Synthesis, Characterization of Silver Nanoparticles using *Tabernaemontana Divaricata* and *Polianthes Tuberosa* Flower Extract and Evaluation of Their Antibacterial Activities

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Abstract-The importance of *Tabernaemontana divaricata*, *Polianthes tuberosa* flower and Silver nanoparticles as revealed by various literature resources, we planned to carry out biogenic synthesis of Silver nanoparticles using the above two extracts. Silver nanoparticles were prepared by adopting standard procedure. The formations of Silver nanoparticles from the extracts were identified first by observing the colour changes. The extract colour changes during the formation of Silver nanoparticles from light yellow to yellowish-brown for *Tabernaemontana divaricata* and pale yellow to dark red for *Polianthes tuberosa*. Silver nanoparticle formations were characterized by UV, FT-IR, XRD and SEM. UV absorbance at 460nm and at 430nm for Silver nanoparticles derived from the above two flower extracts. FT-IR stretching frequencies at 509cm^{-1} for both the flower extracts proved the formation of silver nanoparticles. XRD & SEM analysis of silver nanoparticles indicated that they exist in spherical, face centered cubic (fcc) crystalline structure with size range 32nm and 17nm respectively. On comparing invitro bioactivities of *Tabernaemontana divaricata* flower extract, *Polianthes tuberosa* flower extract and their Silver nanoparticles by zone of inhibition studies showed that Silver nanoparticles are highly active against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with the zone of inhibition in 16&11mm and 14&10mm respectively.

Keywords: *Tabernaemontana divaricata*, *Polianthes tuberosa*, Silver nanoparticles, FT-IR, XRD & SEM.

I. INTRODUCTION:

Nanotechnology is one of the most active areas of research in modern materials science [1] and now creating a growing sense of excitement in the life sciences especially biomedical devices and Biotechnology [2] and making an impact in all spheres of human life [3]. Nanomaterials have extensive applications for improving human health and the environment [4&5]. Silver nanoparticles are well known as one of the most universal antimicrobial substances in the field of biology and medicine due to their strong biocidal effect against

microbial species, which has been used for centuries to prevent and treat various diseases, most notably infections [6-8].

Tabernaemontana divaricata is a glabrous, evergreen, dichotomously branched shrub belonging to the family Apocynaceae [9]. *Tabernaemontana divaricata* Flower juice mixed with oil alleviates burning sensation, cures eye sore and skin diseases, leaves juice applied to wounds to prevent inflammation and used in ophthalmia [10]. The most common medicinal use of crude *T. divaricata* extract is its antimicrobial action against infectious diseases such as syphilis, leprosy, and gonorrhoea, as well as its parasitic action against worms,

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dysentery, diarrhea, and malaria [11]. *Polianthes tuberosa* belongs to Agavaceae family, is a perennial plant [12]. *Polianthes Tuberosa* is better known as ornamental flowers, smelled the fragrance of jasmine have some health benefits, ranging from the treatment of insomnia complaints, influenza, and rheumatism. Used in perfume industry as a source of essential oils of aroma compounds [13] and have diuretic and emetic activity. Bulbs are used for anticonorrhoea, diuretic, emetic and for curing rashes in infant [14]. Furthermore, fragrant flowers are added along with stimulants or sedatives to the favourite beverage prepared from chocolate and served either cold or hot as desired. In our present study, a suitable green method for the synthesis of silver nanoparticle were carried out using *Tabernaemontana divaricata* and *Polianthes tuberosa* flower extract as reducing agent. The antibacterial activity of silver nanoparticles were carried out by zone of inhibition studies.

II. EXPERIMENTAL

a) Materials :

Fresh flowers of *Tabernaemontana divaricata* were collected from Sivakasi, and fresh flowers of *Polianthes tuberosa* were collected from local flower market. Silver nitrate of Merck grade was used.

b) Methods:

i) Preparation of the Flower Extracts:

Tabernaemontana divaricata and *Polianthes tuberosa* flowers were washed several times with water to remove the dust particles and then aerial dried to remove the residual moisture. The *T. divaricata* and *P. tuberosa* flower extract used for the reduction of silver ions (Ag^+) to silver nanoparticles (Ag^0) was prepared by placing 50g of washed dried fine cut flowers in 250ml round bottom flask along with 200ml of distilled water. The mixture was then boiled for 2 hours until the color of the aqueous solution changes to light yellow and pale yellow. Then the extract was cooled to room temperature and filtered with Whatman No.1 filter paper. The aqueous flower extract (Fig. 1A & Fig. 2A) were used as a reducing agent for further nanoparticle synthesis. These extracts can be stored at 4°C for one week.

ii) Synthesis of Silver Nanoparticles:

5mM aqueous solution of Silver nitrate (AgNO_3) was prepared and used for the synthesis of silver nanoparticles. 10ml of *Tabernaemontana divaricata* and *Polianthes tuberosa* flower extract was added to 90 ml of aqueous solution of 5mM Silver

nitrate for reduction into Ag^+ ions and kept at room temperature. As a result, a dark red solution was formed indicating the formation of silver nanoparticles (Fig. 1B & Fig. 2B) and it was further confirmed by UV-Vis spectrum analysis.

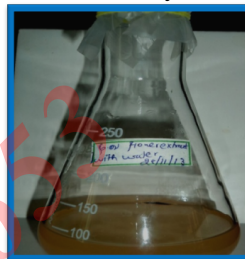


Fig. 1A. Photographs showing Pure *T. divaricata* flower Extract

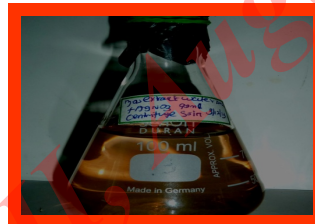


Fig. 1B Colour changes after adding flower Extract to AgNO_3 solution.

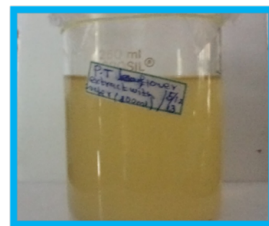


Fig. 2A Photographs showing Pure *P. tuberosa* Flower Extract

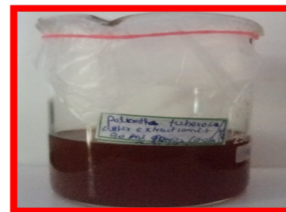


Fig. 2B Silver nanoparticle formation using *P. tuberosa* Flower Extract

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iii) Separation of Silver nanoparticles:

The synthesized AgNP's was separated by means of centrifugation (Spectrofuge 7M) at 10,000 rpm for 30 mins. The pellets was redispersed and again centrifuged for 30 mins. The supernatant solution thus obtained was stored at -4°C .

iv) Characterization of AgNPs:

Characterisation of silver nanoparticles was carried out using UV-visible absorption spectrophotometer 2400PC with a resolution of 1 nm between 300 and 900 nm possessing a scanning speed of 300 nm/min. FT-IR measurements were carried out on a Shimadzu FT-IR 8400S Model and the spectra was scanned in the range of 4000-400 cm^{-1} range at a resolution of 4 cm^{-1} . The sample were prepared by dispersing the AgNPs uniformly in a matrix of dry KBr, compressed to form an almost transparent disc. KBr was used as a standard analyze the samples [49]. The particle size and nature of the AgNPs were determined using XRD PW3050/60 X-pert PRO operating at a voltage of 45 kV, a current of 40 mA with Cu K α radiation at 2θ angle ranging from 10° to 80° [15]. A thin film of the silver nanoparticle was made by dipping a glass plate in a solution and carried out for X-ray diffraction studies. SEM analysis was done by using a JSM 6701F – 6701Model .

v). Antibacterial Assay

Several loopful of microorganisms were inoculated into 5 ml of sterilized peptone water and the turbidity was compared and adjusted with 0.5 McFarland Nephelometric standard (Baron and Finegold, 1990) [16]. Mueller Hinton Agar surface was inoculated and uniformly spread by using a swab impregnated with standardized inoculum. After 15 minutes of inoculation, wells (6 mm diameter) were made by well puncture and the wells were filled with 50 ml of each different extract and AgNP's [17].

III. RESULTS

Biogenic synthesis of silver nanoparticles using *Tabernaemontana divaricata* and *Polianthes tuberosa* flower extracts were confirmed by UV-Visible Spectroscopy, Fourier Transform Infrared Spectroscopy, X-ray Diffraction and Scanning Electron Microscopy studies. The formation of silver nanoparticles can be observed by the changes in the color of the solution from light yellow color to yellowish - brown color for *Tabernaemontana divaricata* flower extract and pale yellow to dark red for *Polianthes tuberosa* flower extract . Color of silver colloid is attributed to surface Plasma resonance (SPR) arising

due to the collective oscillation of free conduction electrons induced by an interacting electromagnetic field. Fig. 3 & 4 showed the UV absorption spectra of the silver nanoparticles . Surface Plasmon Resonance bands of the colloids are centered at 460 nm for *T.divaricata* and *P.tuberosa* for 430nm. The bands are broad for AgNP's and the intensity increases compared to the UV absorption spectra of *T.divaricata* and *P.tuberosa* flower extracts indicated the formation of silver nanoparticles.

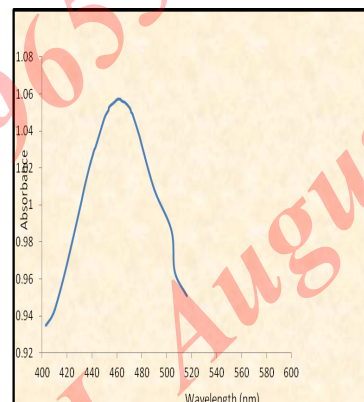


Fig. 3- UV- Visible Spectra of Silver nanoparticles synthesized using *T.divaricata* flower extract

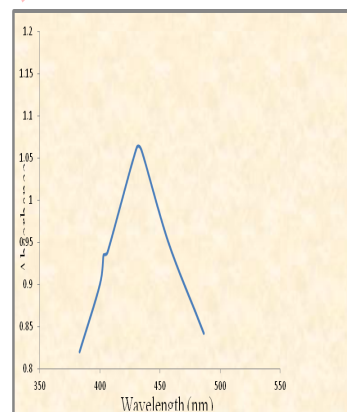


Fig. 4- UV-Visible Spectra of Silver nanoparticles synthesized using *P.tuberosa* flower extract

The FT-IR spectra of *Tabernaemontana divaricata* and *Polianthes tuberosa* flower extract, as given in Fig.5A&6A

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showed a broad band at 3425 cm^{-1} due bonded O-H and at 1635 cm^{-1} weak band indicated bonded carbonyl. FT-IR spectra of the silver nanoparticles obtained using T.divaricata flower extract at 3441 cm^{-1} (weak) and at 3448 cm^{-1} (weak)for the P.tuberosa flower extract indicated that the bonded O-H in both extracts had broken and involved in binding with silver during the formation of AgNP's . The formation of strong peak at 1735 cm^{-1} indicated that during the formation of silver nanoparticles the carbonyl group which was found to be weakly bonded in the extract was found to be freed during the formation of silver nanoparticles. Formation of the silver nanoparticles was confirmed by the appearance of stretching band at 509 cm^{-1} , 424 cm^{-1} & 509 cm^{-1} corresponds to (M-O) stretching as given in Fig.5B&6.

Fig. 5B - FT-IR Spectrum of T.divaricata flower extract mediated silver nanoparticles

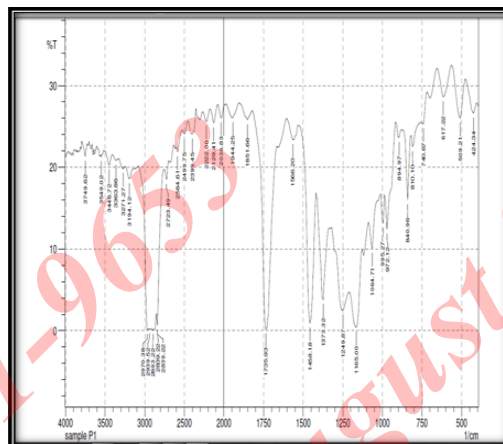


Fig. 6- FT-IR Spectrum of P.tuberosa flower extract mediated silver nanoparticles

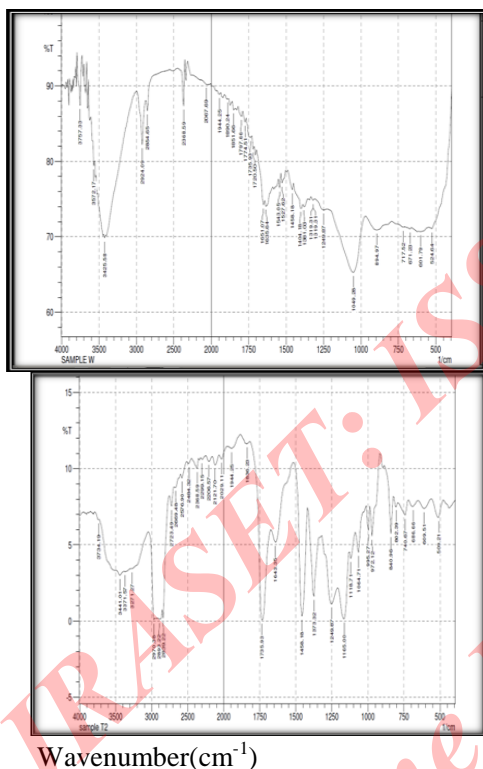


Fig. 5A - FT-IR Spectrum of T.divaricata flower extract

The biosynthesized silver nanostructure was further confirmed by the characteristic peaks observed in the XRD pattern. The particle size and nature of the AgNPs were determined using XRD PW3050/60 X-pert PRO operating at a voltage of 45 kV, a current of 40 mA with Cu K α radiation at 2θ angle ranging from 10° to 80° . A thin film of the silver nanoparticle was made by dipping a glass plate in a solution and to carried out for X-ray diffraction studies. The XRD spectrums of silver nanoparticles were given in Fig.7& 8. All diffraction peaks correspond to the characteristic face centered cubic (FCC) silver lines. These diffraction lines observed at 2θ angle 32.78, 38.03, 58.51 and 75.12 respectively, have been indexed as (111), (200), (221) and (321) planes respectively(JCPDS 03-065-3289)for T.divaricata flower and P.tuberosa flower containing four main characteristic diffraction peaks for Ag were observed at $2\theta = 38.12, 44.30, 64.45$ and 77.41 which correspond to the (111), (200), (220) and (311) crystallographic planes of face-centered cubic (fcc) Ag crystals, respectively (JCPDS 01-089-3722). The typical XRD pattern revealed that the sample contains a cubic structure of silver nanoparticles . The average particle size of silver nanoparticles formed in the process was estimated from Debye-Scherrer's equation ($d = (k\lambda \times 180) / \beta \cos \theta \Pi$) by determining the width of the (111) Bragg's reflection .The average size of silver nanoparticles were

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found to be 36nm and 18nm corresponding to T.divaricata and P.tuberosa flower extract mediated AgNPs respectively.

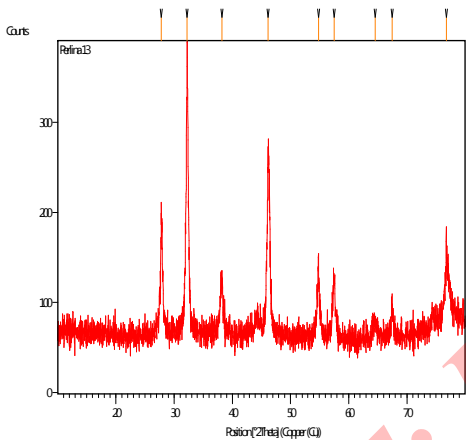
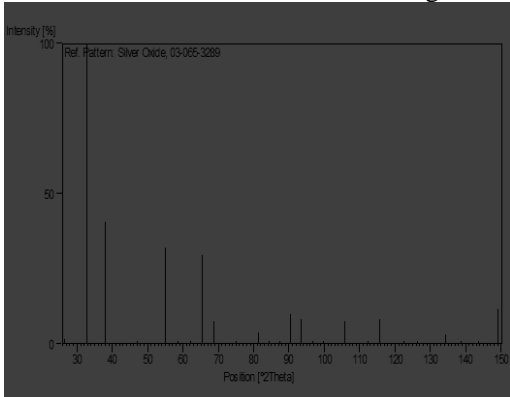


Fig. 7- XRD Spectrum of Silver nanoparticles using T.divaricata flower extract.

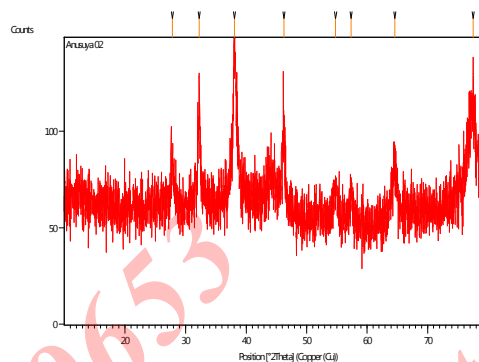
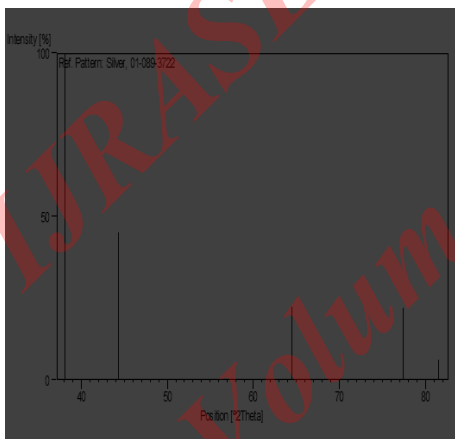


Fig. 8: XRD Spectrum of Silver nanoparticles using P.tuberosa flower extract.

Scanning electron microscopy analysis of the silver nanoparticles showed that the synthesised silver nanoparticles existed in polydisperse spherical shape and in the range of 10 to 80 nm with average size of 32 nm and 17nm corresponding to T.divaricata and P.tuberosa respectively. SEM image as given in Fig.9A&B using T.divaricata &P.tuberosa flower extracts confirm the formation of silver nanoparticles.

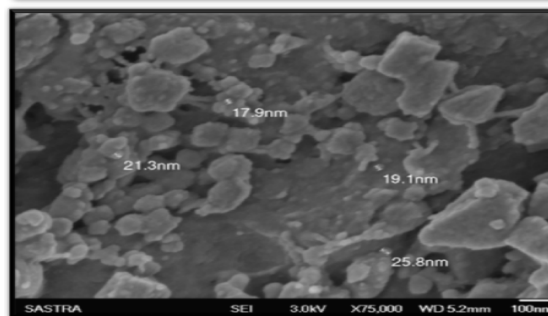
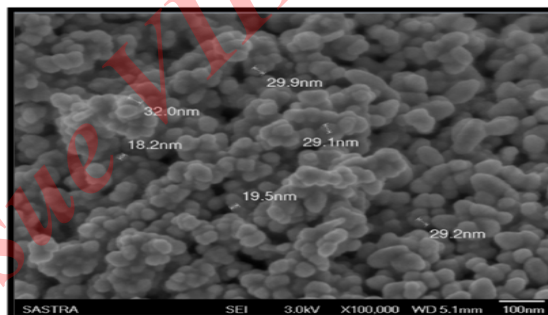


Fig. 9A&B- SEM Spectrum of Silver nanoparticles using T.divaricata & P.tuberosa flower extracts

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In vitro antimicrobial activity of silver nanoparticle synthesized using *T.divaricata* and *P.tuberosa* flower extract were tested against the microorganisms like *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* by well puncture method were given in Table 1. On comparing the *T.divaricata* flower extract and *P.tuberosa* flower extract and their Silver nanoparticles by measuring the zone of inhibition proved that Silver nanoparticles are highly active against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with the zone of inhibition in 16&11mm and 14&10mm than the

flower
extracts.

Microorganism	Tabernaemontana divaricata	Polianthes tuberosa		
	Zone of inhibition			
	Flower Extract	Ag nanoparticle	Flower Extract	Ag nanoparticle
<i>Escherichia coli</i>	-	8	12	9
<i>Staphylococcus aureus</i>	-	16	13	14
<i>Pseudomonas aeruginosa</i>	-	13	-	10
<i>Bacillus subtilis</i>	-	9	11	8

Table 1- Antibacterial activities of silver nanoparticles mediated using *t.divaricata* & *p.tuberosa* flower extracts.

IV. CONCLUSIONS

Biogenic synthesis of Silver nanoparticles using *Tabernaemontana divaricata* and *Polianthes tuberosa* flower extracts were performed by adopting standard procedure were characterized by UV-vis, FT-IR, XRD and SEM studies. Due to surface plasmon resonance during the reaction with the ingredients present in the flower extracts Color changes which result in the formation of silver nanoparticles. The typical XRD

pattern revealed that the average size of silver nanoparticles was found to be 36nm and 18nm corresponding to *T.divaricata* and *P.tuberosa* flower extract mediated AgNPs respectively and exist in face-centered cubic (fcc) Ag crystals. SEM analysis showed that the synthesised silver nanoparticles existed in polydisperse spherical shape and in the range of 10 to 80 nm with average size of 32 nm and 17nm corresponding to *T.divaricata* and *P.tuberosa* respectively. In vitro antimicrobial activity of silver nanoparticle showed that Silver nanoparticles are highly active against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with the zone of inhibition in 16&11mm and 14&10mm than their mediated flower extracts.

Acknowledgement:

The authors are thankful to Manonmaniam Sundaranar University, Department of Physics for XRD and SAstra University Thanjaur for SEM analysis.

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