Available Online at

http://www.ijcpa.in

January-March 2017

DOI : http://dx.doi.org/10.21276/ijcpa

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

eISSN: 2348-0726 ; pISSN : 2395-2466

Research Article | Volume-4 | Issue-2 | Article ID: 1263

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *TRAGIA INVOLUCRATA* STEM EXTRACT AND ANALYSIS OF THEIR ANTIMICROBIAL PROPERTY

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Received: 24 February 2017 / Revised: 18 March 2017 / Accepted: 22 March 2017 / Available online: 31 March 2017

ABSTRACT

Plant extract from Tragia involucrata is used for the synthesis of silver nanoparticles from silver nitrate solution. The profile of synthesized silver nanoparticles was evaluated by using UV-Visible spectrophotometer, X-ray diffractometer, energy dispersive X-ray spectroscope, scanning electron microscope and Fourier transform infrared spectroscopy. Green synthesized silver nanoparticles and aqueous extract of plant showed zone of inhibition against Escherichia coli, Staphylococcus aureus species of bacteria and Candida albicans fungi species.

***Keyword**: Biosynthesis, Silver nanoparticles, Tragia involucrata stem extract, Characterization, Antimicrobial activity.*

1. INTRODUCTION

Nanotechnology is emerging as a rapidly growing field with its uses in science and technology for the purpose of manufacturing new materials at the nanoscale level [1]. Nanoparticles exhibit distinctive visible properties relative to bulk material and produce quantum effects [2-4]. The application of nanosilver usually ranging from 1 to 100nm, is uprising area of nanoscience. A quest for an environmentally sustainable synthesis process has led to a few biomimetic approaches. Biomimetic refers to applying biological principles in materials formation. One of the fundamental processes in biomimetic synthesis involves bio reduction. Biological methods of nanoparticle synthesis using microorganisms, enzymes, fungus and plant extracts have been suggested as possible ecofriendly alternatives to chemical and physical methods. Sometimes the synthesis of nanoparticles using plants or parts of plants can prove advantageous over other biological processes by eliminating the elaborate processes of maintaining microbial cultures[5-10].

The present study is conducted to synthesize and characterize silver nanoparticles and their antimicrobial activity from widely available plant Tragia *involucrata* belonging to the family Euphorbiaceae. The efficacy of this plant is well known in Indian traditional medicine and it is used for treatment of eczema, wound and headache [11-12]. This is a simple green and cost-effective method for the rapid and facile synthesis of silver nanoparticles.

2. MATERIALS AND METHODS

2.1 Collection of Plant material

Tragia involucrata (Family: Euphorbiaceae) was collected freshly on the road sides of Papanasam, Tamilnadu, India. The plant was identified and authenticated by personnel of Rabinet Herbarium, St. Joseph's college, Thiruchirappalli, Tamilnadu. The stem of the plants was collected and shade dried.

Figure 1: Voucher specimen photocopy of *Tragia involucrata*

2.2 Preparation of stem extract

The plant stem was rinsed with water thrice to remove the fine dust materials. The plant stem was air dried for 20 days and then they were dried in air hot oven at $60^0$for 36 hours. The plant stem was ground to a fine powder. 25g of stem powder was added in 250ml deionized water in 500ml Erlenmeyer flask and it was boiled for approximately 15 minutes and were filtered. The filtrate was used as plant extract.

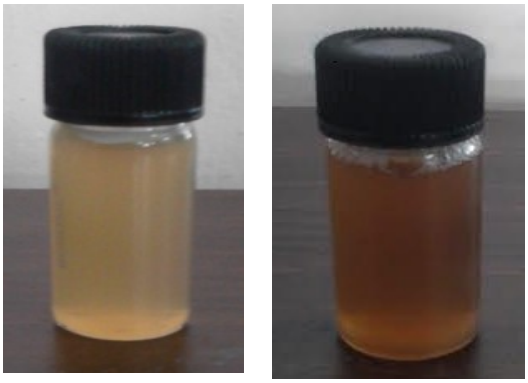
2.3 Synthesis of silver nanoparticles

10ml of plant extract was added to the aqueous solution of 1mM silver nitrate. The appearance of reddish brown color after 3 hours indicates the formation of silver nanoparticles. The completion of the reaction was monitored by UV-Visible spectroscopy. The synthesized silver nanoparticles were separated by centrifuging at 13,000 rpm for 15 mins and filtered. The filtrate was stored at 4^0C for further use.

UV-Visible absorption spectra were measured using a Lambda 25 spectrophotometer. Scanning electron microscopy (SEM) analysis of synthesized silver nanoparticles was done using a Hitachi S-4500 SEM machine. Crystalline metallic silver nanoparticles were examined using an X-ray diffractometer (Shimadzu, XRD-6000) equipped with Cu k α radiation source using Ni as filter at a setting of 30kv/30mA. FTIR spectra of the sample was analyzed using a Perkin-Elmer spectrum instrument by KBr pellet method.

2.4 Antimicrobial activity

Antimicrobial activity of aqueous extract was determined by disc diffusion method[13] for bacterial species like S*taphyloccus aureus*, *Escherichia coli* and fungal specie *Candida albicans*. The culture was inoculated by spread plate method. Chloramphenicol and Fluconazole was used as standard solutions. The plates were then incubated for 24 hours at 37^0C.



2A **2B**

Figure 2: The conversion of silver nitrate to nano silver by *Tragia involucrata* (2A) At starting stage, (2B) After three hours

Characterization of silver nanoparticles

3. RESULTS AND DISCUSSION

3.1 UV-Visible spectroscopy

The silver nanoparticles were characterized by UV-Visible spectroscopy, one of the most widely used techniques for structural characterization of silver nanoparticles[14]. The absorption spectrum(Figure 3) of the reddish-brown silver nanoparticle solution prepared with the proposed method showed a surface plasma absorption band with a maximum of 455nm, indicating the presence of spherical silver nanoparticles. This peak in the region confirms the formation of nano particles from earlier reported literatures[15].

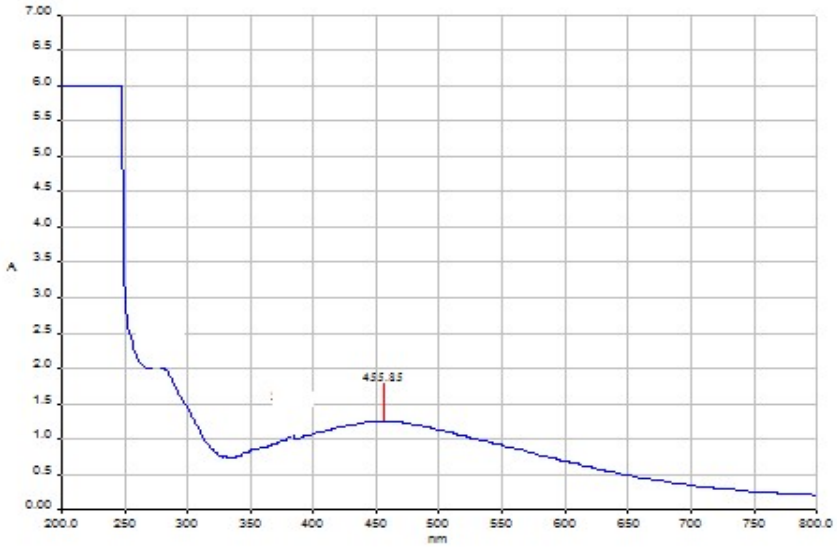


Figure 3: UV-Visible spectra of silver nanoparticles

The SEM measurements were carried out to determine the morphology and shape of silver nanoparticles. The SEM micrograph (Figure-4) revealed that, the silver nanoparticles are more or less spherical shape. The particle sizes of silver nanoparticles synthesized by *Tragia involucrata* stem extract were within 100nm.

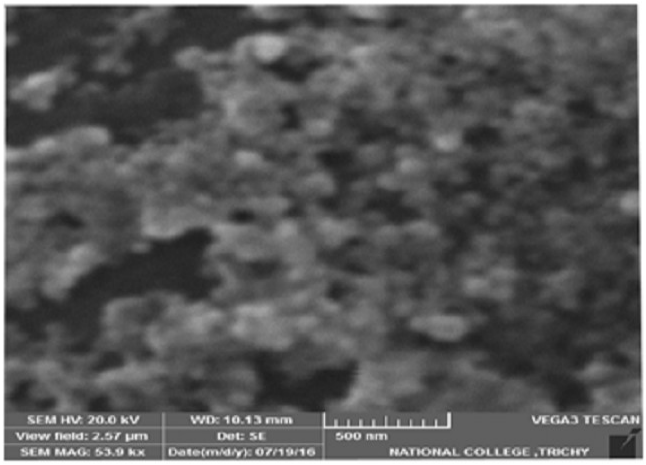


Figure 4: SEM image of silver Nanoparticles

In energy dispersive X-ray spectra, the peak obtained at energy of 3keV confirmed silver (Figure 5). The emission energy at 3keV illustrated the reduction of silver ion.

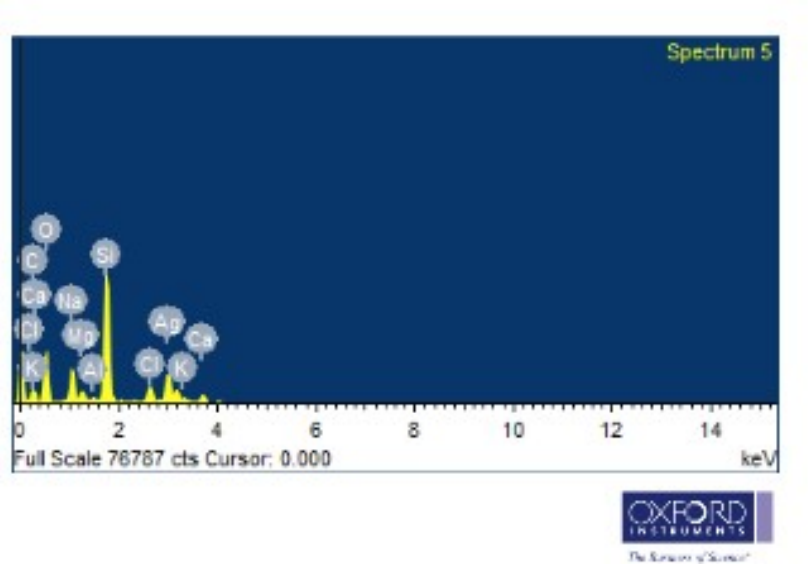


Figure 5: EDX spectra of silver Nanoparticles formed in Tragia involucrate stem extract

3.2 FTIR chemical analysis

Results of the FTIR study of biosynthesized silver nanoparticles showed sharp absorption peaks located 3460,2172,1647,1358,1100,881,576,471 cm^{-1} (Figure 6). The absorption peak at 1647cm^{-1} may be assigned to the amide I bond of proteins arising from carbonyl stretching in proteins, and the peak at 3460 $cm^{-1}$is assigned to OH stretching in alcohols and phenolic compounds. The absorption peak at 1647cm^{-1} is close to that reported for native proteins, which suggest that proteins are interacting with biosynthesized silver nanoparticles and also their secondary structure was not affected during reaction with Ag^{+}ions or after binding with Ag^{0} nanoparticles[16].This FTIR spectroscopic study confirmed that the carbonyl group of amino acid residues has a strong binding ability with silver, suggesting the formation of a layer covering silver nanoparticles and acting as a capping agent to prevent

agglomeration and provide stability to the medium. These results confirm the presence of possible proteins acting as reducing and stabilizing agents.

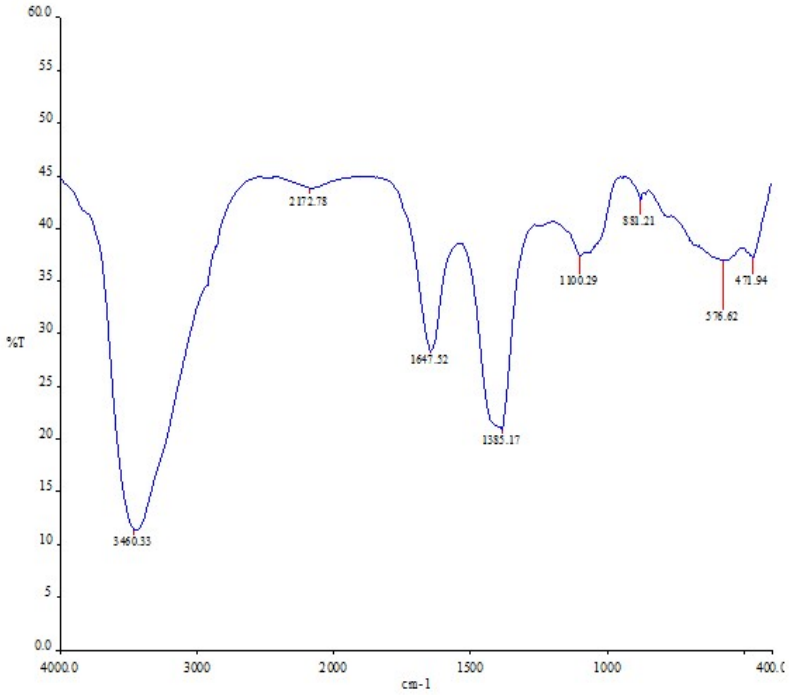


Figure 6: FTIR spectrum of silver nanoparticles formed by *Tragia involucrata* stem extract

3.3 XRD Studies

The XRD patterns confirm that the synthesized silver nanoparticles from *Trajia involucrata* stem extract have cubic structure according to JCPDS data. The peaks are observed at 2θ values 27.21, 32.40, 38.33, 46.39 corresponding to the plane of 110,111,200,211 show the presence of cubic phases in the final product. Apart from these peaks responsible for silver nano particles the recorded XRD pattern shows additional unassigned peaks also noted. This may be due to the formation of the crystalline bio organic compounds/metaloproteins that are present in the *Trajia involucrata* stem extract. Similar observations are reported [17,18].

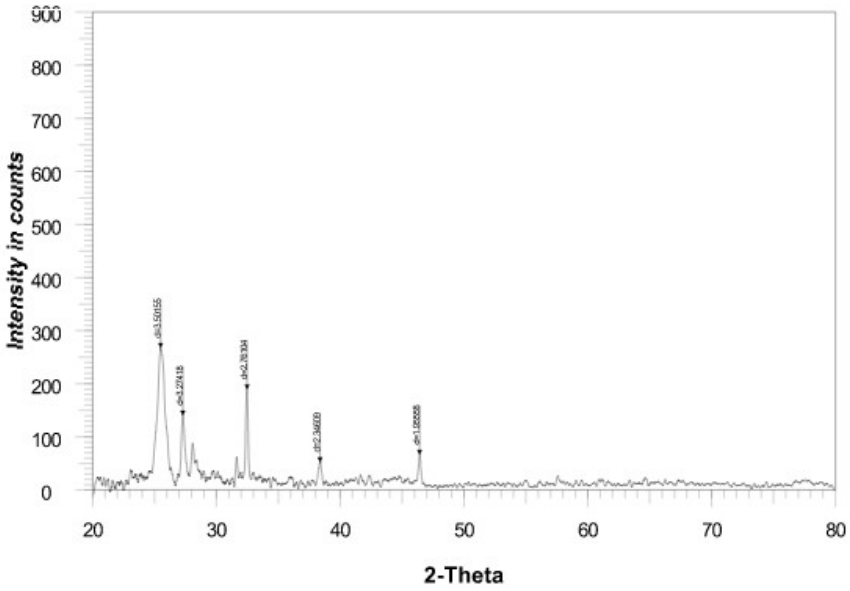


Figure 7: XRD spectrum of silver nanoparticles formed by *Tragia involucrata* stem extract

3.4 Antimicrobial analysis

`The antimicrobial activity of silver nanoparticles was tested against *Escherichia coli*, *Staphylococcus aurous* species of bacteria and *Candida albicans* fungi specie. The silver nanoparticles showed significant antimicrobial activity was comparable to that of standard drug (Figure-8). Antimicrobial effect of silver nanoparticles was found to be dose dependent. The clear inhibitory zone was appeared against *Escherichia coli, Staphylococcus aurous* species of bacteria and *Candida albicans* at 30µl concentration of sample. The synthesized nano particles possess antibacterial and antifungal effect more than aqueous extract of the plant and silver nitrate. The values are more or less near the standard value. This suggests that the synthesized nanoparticle showed good antibacterial activity against human pathogens (Table- 1).

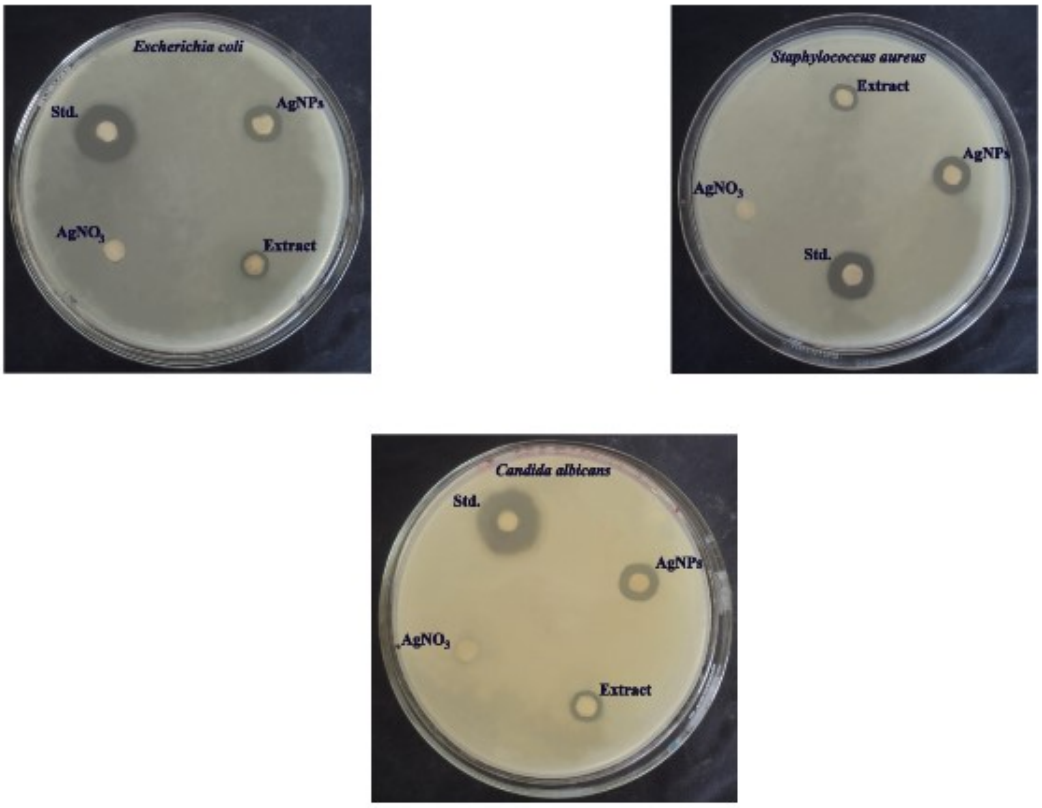


Figure 8: Antibacterial activity of silver nano particles, $AgNO_3$ and plant extract

Table 1: Antimicrobial activity of AgNPs, $AgNO_3$ and Plant extract

Sample	Concentrations	*Escherchia Coli* (mm)	*Staphylococcus aureus* (mm)	*Candida albicans* (mm)
$AgNO_3$	30µl	0.80±0.05	0.60±0.04	0.70±0.04
Plant extract	30µl	1.50±0.10	1.10±0.07	1.30±0.09
AgNPs	30µl	3.80±0.26	3.30±0.23	3.50±0.24
Standard (Chloramphenicol)	30µl	4.20±0.29	3.90±0.27	-----
Standard (Fluconazole)	30µl	---	---	4.00±0.28

Values are expressed as Mean ± SD for triplicates

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 accumulation of the nanoparticles on the cell surface[19].

4. CONCLUSION

The silver nanoparticles have been produced by *Tragia involucrata* stem extract, which is an economical, efficient and ecofriendly process. UV-visible spectrophotometer, XRD, FTIR, SEM and EDAX techniques have confirmed the reduction of silver nitrate to silver nanoparticles. The zones of inhibition were formed in the antimicrobial screening test indicated, that the silver nanoparticles synthesized in this process has the efficient antimicrobial activity against pathogenic bacteria and fungi. The biologically synthesized silver nanoparticles could be of immense use in medical field for their efficient antimicrobial function.

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