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# *Croton sparsiflorus* whole plant extract mediated biosynthesis of metallic silver nanoparticles and their antibacterial activity

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## ABSTRACT

In the present study, the potential of aqueous whole plant extract of *Croton sparsiflorus* for biosynthesis of silver nanoparticles (AgNPs) was evaluated. The formation of AgNPs was confirmed by color changes and UV- Visible spectroscopy, which showed absorbance maxima peak at 417nm. The scanning electron microscope (SEM) analysis confirmed the particle size and x-ray diffraction (XRD) confirmed the crystalline character of AgNPs. AFM analysis also confirmed the surface morphology of synthesized AgNPs. Fourier transform infrared (FTIR) authorized the presence of alcoholic and phenolic groups played an important reduction role in the synthesis process. It shows the antibacterial efficiency against gram negative bacteria, *Klebsiella pneumoniae* and *Salmonella paratyphi*. From the results, it is suggested that green synthesized AgNPs could be used effective in future biomedical engineering.

**Keywords:** *Croton sparsiflorus*, Green synthesis, Silver nanoparticles, X-ray diffraction, Antibacterial activity.

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## INTRODUCTION

The synthesis of nanomaterials and the study of their properties and applications is one of the motivating parts in the recent scientific research. Researchers, scientist and engineers have shown excessive curiosity to explore various amazing features of metallic nanoparticles. Such nanomaterials have emerging application in several practical fields including biomedical, catalysis, biosensors and energy storage devices. The major reason behind the intense importance of nano materials is the exposure of many storage and concealed features at the nanoscale[1, 2, 3].

Nanoparticles synthesis is currently a significant area of research, searching for an eco-friendly approach and green materials for current scenario. Number of AgNPs has been developed by a physical, chemical approach and recently developed biological method[4]. Latter it has emerged as a green alternative, because of its cost effective, eco-friendly and easily scaled up nature. It has great potential with natural reductants[5] such as bacteria, fungus and plant extracts [6,7].

*Croton sparsiflorus* Span belonging to the family Euphorbiaceae is a common wild weed of South India. Plant mainly contains steroid, alkaloids and flavonoids. Due to the presence of above active constituent, plant has been reported to possess various pharmacological activities such as antibacterial, antifungal, antitumor, hypotension effect etc, the whole plant of *C.sparsiflorus*is traditionally used as a anti-inflammatory and antipyretic. The active compounds present in the plant material could be used as reductants to react with silver ions and as Seaford's to direct the formation of AgNPs in solution[8]. The aim of the work presented here was to synthesize AgNPs using an eco-friendly method and evaluate the antibacterial effect.

## MATERIALS AND METHODS

### Collection of Plant Material:

*Croton sparsiflorus* Span. was collected from Esanvankulam, Srivaikundam. The collected samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried material was granulated or powdered by using a blender, and sieved to get uniform particles by using sieve No. 60. The final uniform powder was made use for the extraction of active constituents of the plant material.

**Preparation of Extracts for Phytochemical screening:**

**Hot Maceration Method Using Soxhlet Apparatus:**

Required quantity of powder was weighed and transferred to Stoppard flask and treated with aqueous separately until the powder is fully immersed. The flask was shaken every hour for the first six hours and then the extract was filtered through Whatman No.41 filter paper. The extract was subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedures [9,10,11].

**Green Synthesis of Nanoparticles:**

**Preparation of Whole Plant Extract (Reducing Agent):**

Freshly collected was whole plants was washed thoroughly with double distilled water and cut into fine pieces. Twenty gram of fine pieces of whole plant was boiled in 100 ml double distilled water for 20 minutes in a glass beaker. After boiling the extract was filtered using Whatman No. 1.

**Preparation of Precursor:**

Precursors for silver nanoparticles ($AgNO_3$) was purchased from Hi-media chemicals, India and prepared freshly. Precursor for preparing silver nanoparticles was 1 mM of silver nitrate using double distilled water.

**Synthesis of Silver Nanoparticles:**

Ten ml aqueous solution of whole plant extract was slowly added into 20 ml of 1 mM solution of silver nitrate under continuous stirring for 20 mins. The solution was kept warm for 24 hrs at room temperature. Colourless solution changed into pale yellow colour initially and after 24 hrs colour changed from pale yellow to reddish brown which indicates formation of silver nanoparticles. It showed that aqueous silver ions could be reduced by aqueous extract of whole plant to generate extremely stable silver nanoparticles in water. The colloidal solution is then centrifuged at 9000 rpm, supernatant was gathered and protected for further analysis.

**Characterization of the Synthesized Silver Nanoparticles:**

**UV – Vis Spectroscopy:**

Ultraviolet-visible spectroscopy (UV-Vis) means absorption of spectroscopy in the UV-visible spectral region. The silver and iron nanoparticles were characterized in a Shimadzu V 650 UV- Vis spectrophotometer. The scanning series for the samples was 300-700 nm. The double distilled water was used as a blank reference.

**Fourier Transform Infra-red Spectroscopy (FTIR):**

The nanoparticles were distinguished using a Fourier Transform Infrared Spectrophotometer (FTIR Thermo-scientific iS5). Two milligrams of the sample was mixed with 100 mg Potassium bromide (KBr). Then, condensed to prepare a salt disc approximately 3 mm in diameter and the disc were directly kept in the sample holder. FTIR spectra were verified in the absorption range between 400 and 4000 $cm^{-1}$.

**Scanning Electron Microscope (SEM) Analysis:**

SEM is a kind of electron microscope that projects a sample by scanning it with a tall energy beam of electrons in a faster scan patterns. This film of the sample was arranged on a carbon coated copper grid by immediately dropping a very small amount of the sample on the grid. Extra solution was removed by means of a blotting paper and then the films on the SEM grid were permitted to dry by putting it under a mercury lamp for 5 min.

**X-Ray Diffraction (XRD) Analysis:**

The particle size and nature of the silver nanoparticles were found out using XRD. This was carried out using Shimadzu XRD – 6000/6100 model with 30 kv, 30 mA with Cukα radians at 2θ angle. X-ray powder diffraction is a rapid analytical technique mainly used for phase classification of a crystalline material and can supply information on unit cell dimensions. The analyzed material is finely ground, and the mean bulk composition is found out. The particle or grain size of the particles on the silver and iron nanoparticles was determined using Debye Sherrer's equation.

$$D = 0.94 \lambda / B \cos \theta$$

**AFM Analysis:**

Surface topology of the synthesized silver and iron nanoparticles were studied by 1µm x 1µm Atomic Force Microscopy (AFM Nanosurf 2) analysis, 0.01 g synthesized nanoparticles were mixed with 20 ml of acetone and sonicated for 5-10 minutes using ultrasonicator. The solution was poured on a clean glass slide and was allowed to dry until all the acetone gets evaporated. Now this glass slide is studied using the Atomic Force Microscopy in a non contact mode and the captured image was processed using XEI software.

## RESULTS AND DISCUSSION

Nanoparticles characterization is significant to understand and manage the nanoparticles synthesis and applications. Characterization is achieved by using different variety of techniques such as Scanning and Transmission Electron Microscopy (SEM, TEM, UU-vis spectroscopy, dynamic light scattering (DLS), atomic force microscopy (AFM), powder X-ray diffractometry (XRD), X-ray photoelectron spectroscopy (XPS) and Fourier Transform Infrared spectroscopy (FTIR)[12,13,14,15,16,17]. These techniques are used for determination of different parameters such as particle range, character crystallinity, fractal dimensions, pore size and surface area. In the recent year, researchers have paid more attention on the green synthesis and applications of AgNPs using various plant extracts. The present study was carried out to the biosynthesis AgNPs using whole plant aqueous extract of *C. sparsiflorus*.

**Phytochemical analysis:**

The distribution of different phytochemical constituents in aqueous extract of whole plant of *C. sparsiflorus* was evaluated quantitatively and the results are presented in Table 1. The presence of flavonoid, phenol, saponin, steroid, tannin, glycoside and fixed oil has been confirmed in the aqueous extract of whole plant of *C. sparsiflorus*.

**Characterization of silver nanoparticles UV-vis spectroscopic analysis:**

The changing colour of the reaction mixture during the reaction time is the primary indication of nanoparticles synthesis[18]. The formation of brown colour in the reaction mixture was observed after 1 hr. (Fig 1) The brown colour formation is the characteristic of AgNPs formation and changes in colour due to size of nanoparticles also depends on their surface plasmon resonance (SPR)[19,20]. It is an efficient and rapid method, which was very well explained by other researches who worked with different plant systems [21, 22]. In the present results are in conformity with

Bonde *et al.*[23] who reported the formation of AgNPs within 1hr of incubation. However, Chinnappan *et al.*[24] reported colour change after 24 hr indicating the slow reduction of the $AgNO_3$by the aqueous flower extract of *Bauhinia purpurea*. An extract of *Ocimum sanctum* leaves was found to reduce silver ions to nanoparticles within 8min[25]. The dissimilarity in the rates of bio-reduction observed may be due to the differences in the activities of the enzyme present in the plant materials. The UV-Vis spectroscopic peak of 417 nm was observed and confirmed AgNPs synthesis. (Fig 2).The reduction was ascribed to the phenolics, terpenoids and flavonoids present in the whole plant extract. The characteristic peak around 400-450 nm is specific for AgNPs and shifting of peak from shorter wavelength to higher wavelength indicates the formation of smaller to bigger nanoparticles respectively[26]. Pal *et al.*[27] reported that the absorption spectrum of spherical silver nanoparticles present a maximum between 400 and 450 nm when the particle size diminishes or increases respectively.

**Table 1: Preliminary phytochemical screening of aqueous extract *C. sparsiflorus***

| Phytochemicals | Aqueous |
|---|---|
| Alkaloid | - |
| Anthraquinone | - |
| Catechin | - |
| Coumarin | - |
| Flavonoid | + |
| Phenol | + |
| Quinone | - |
| Saponin | + |
| Steroids | + |
| Tannin | + |
| Terpenoids | + |
| Sugar | + |
| Glycoside | + |
| Xanthoprotein | + |
| Fixed oil | + |

**+ Present - Absent**

**Figure 1: Synthesis of silver nanaoparticles**

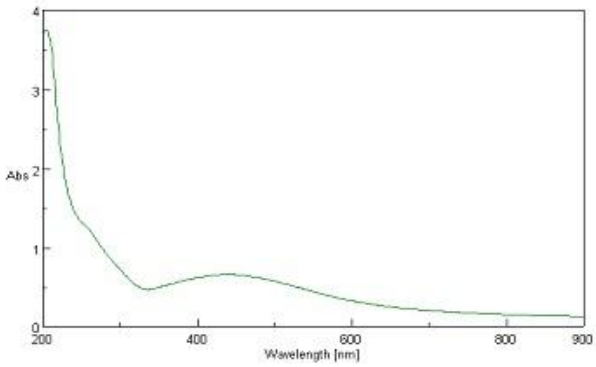


**Figure 2: UV-Vis analysis of synthesized silver nanoparticles of *C. sparsiflorus***

**Fourier Transform Infra-Red Spectroscopy (FTIR):**

The FTIR Spectrum was used to identify the functional groups of the active components based on the peak value in the region of infra -red radiation (Frequency $cm^{-1}$) 3500 – 3200 ( O–H stretch , H – bonded alcohols , phenols ) , 3300 – 2500 ( O–H stretch carboxylic acids) , 3000 – 2850 (C–H stretch alkanes), 1650 – 1550 ( > N –H bend secondary amine), 1600 –1585 (C–C stretch (in ring aromatics), 1500 – 1400 (C – C stretch in ring aromatics) 1390 – 1350 ( C – H rock alkanes ), 1320 –1000 ( C – O stretch esters , ethers ) , 1360 -1290 ( N – O symmetric stretch nitro compounds) , 1250 -1020 ( C – N stretch aliphatic amines ), 910 -665 ( N– H "opp" amines ) , 690 – 400 ( C– Br stretch alkyl halides ) etc , were observed. Peak values of different compounds present in their frequencies and functional groups are showed in table 2. Absorption spectra of whole plant powder and silver and iron nanoparticles from FTIR analysis is depicted in figure 3a and 3b. Figure 3a shows the FTIR spectrum of the whole plant powder , which clearly shows peaks at 3842 and 3736 $cm^{-1}$ corresponds to the O-H stretching of hydroxyl groups, peak at 3414 $cm^{-1}$ assigned as O-H stretching in alcoholic and phenolic compounds , peak at 2915 and 2346 $cm^{-1}$ represent O – H of carboxylic acid, peak at 2915 $cm^{-1}$ and 2346$cm^{-1}$ assigned as C – H stretching of alkanes , peaks at 1629 $cm^{-1}$ represents > N – H bend of secondary amine , peaks at 1451 $cm^{-1}$ represent C –C stretching of aromatics, peak at 1316 $cm^{-1}$ assigned as N-O asymmetric stretching of nitro compound, peaks at 1033$cm^{-1}$ corresponds to C–O stretching of esters and ethers , peaks at 779$cm^{-1}$ represent as N – H wag 1*2* amine, peak at 661cm $^{-1}$ represent as C–H "opp" of aromatics, peaks at 519 and 468 $cm^{-1}$ assigned as C-Br stretching of alkyl halides. Figure 3b in biosynthesized silver nano-particle peak at 3788 and 340 $cm^{-1}$ was assigned as O – H stretching in alcoholic and phenolic compounds , peak at 2925, 2361 and 2340 $cm^{-1}$ represent O-H stretching of carboxylic acid , peak at 1628 cm-1 represent C-> N-H bond of secondary amine, peak at 1384$cm^{-1}$ represent C – H rock of alkanes, peak at 1047 $cm^{-1}$ correspond to C-O stretching of esters and ethers, peak at 602 $cm^{-1}$, represent C-Br stretching of alkyl halides.

FT-IR analysis confirmed that the bio reduction of silver nitrate in the silver oxide nanoparticles is due to the reduction by capping material of *C. sparsiflorus* whole plant extract. FT-IR analysis in present study showed presence of various medicinally important phytoconstituents on the basis of different functional groups.

**Table 2: FT-IR analysis of powder and synthesized silver nanoparticles of *C. sparsiflorus***

| S.No. | Frequency ($cm^{-1}$) | Chemical Bond | Phytoconstituents Present | Peak Observed (Plant Powder) | Peak Observed (Silver NPS) |
|---|---|---|---|---|---|
| 1. | 3850-3500 | O-H Stretch | Hydroxyl group | 3842, 3736 | 3788 |
| 2. | 3500-3200 | O-H Stretch | Alcohols or Phenols | 3414 | 3409 |
| 3. | 3300 – 2500 | O-H Stretch | Carboxylic acid | 2915, 2346 | 2925,2361 |
| 4. | 3000-2850 | C-H Stretch | Alkanes | 2846 | 1628 |
| 5. | 1650-1550 | >N-H bond | Secondary amine | 1629 | _ |
| 6. | 1600-1585 | C-C Stretch (in ring) | Aromatics | ___ | _ |
| 7. | 1500-1400 | C-C Stretch | Aromatics | 1451 | ____ |
| 8. | 1390-1350 | C-H rock | Alkanes | __ | 1384 |
| 9. | 1360-1290 | N-O Symmetric Stretch | Nitro Compound | 1316 | __ |
| 10. | 1320-1000 | C-O stretch | Esters, Ethers | 1033 | 1047 |
| 11. | 1250-1020 | C-N Stretch | Aliphatic amines | ___ | __ |
| 12. | 910-665 | N-H wag | 1*, 2* amines | 779 | _ |
| 13. | 900-675 | C-H “oop” | Aromatics | 661 | _ |
|  | 690-400 | C-Br Stretch | Alkyl halides | 519,468 | 602 |

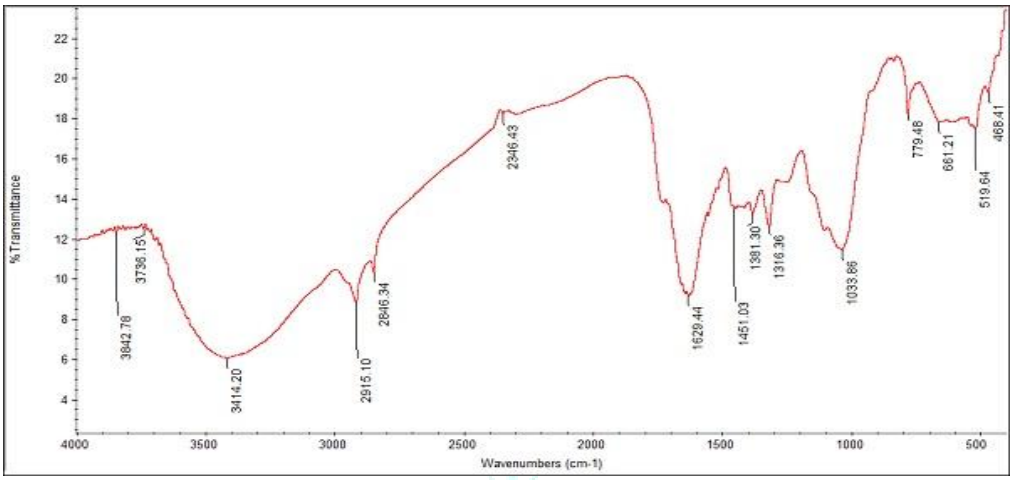


**Figure 3a: FT-IR spectra of whole plant powder of *C. sparsiflorus***

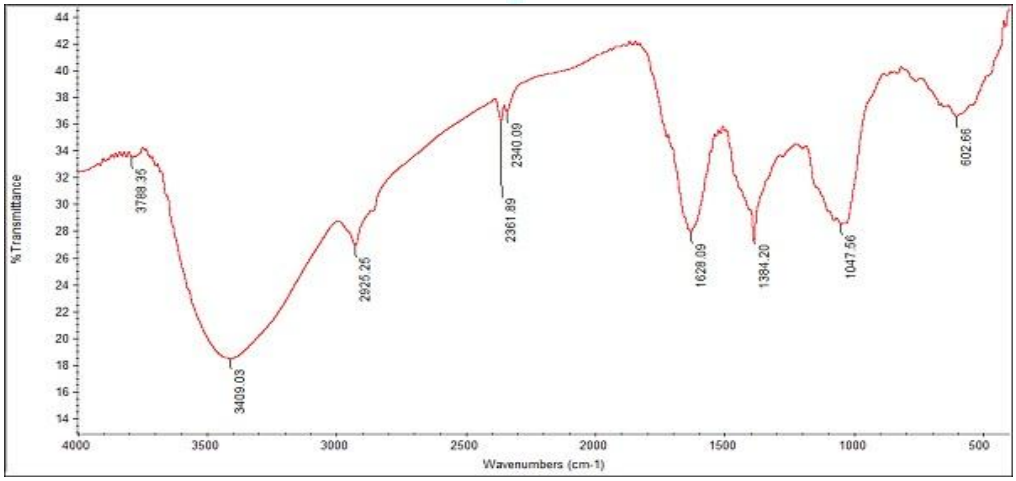


**Figure 3b: FT-IR spectra of synthesized silver nanoparticles of *C. sparsiflorus***

**SEM and AFM analysis:**

SEM image is employed to predict the morphology of the AgNPs. The SEM was shown uniform distribution of AgNPs. The SEM images (Fig 4) has shown separate AgNPs as well as particle agglomeration. This indicates that the particle shape is spherical. The particle size of metallic silver nanoparticles were found about 10-40nm.

Surface topology of the synthesized AgNPs was studied by 1µm x 1µm atomic force microscopy (AFM) analysis. (Fig 5). AFM was used as the primary method to monitor AgNPS dissolution and agglomeration pattern. The topograpy matrix data should be treated in each profile line (2D) or overall profiles extending the analysis of surface (3D). The topograpy of Micrographs clearly indicate that the formulated AgNPs possess mixed type of sponge and granular like shape.

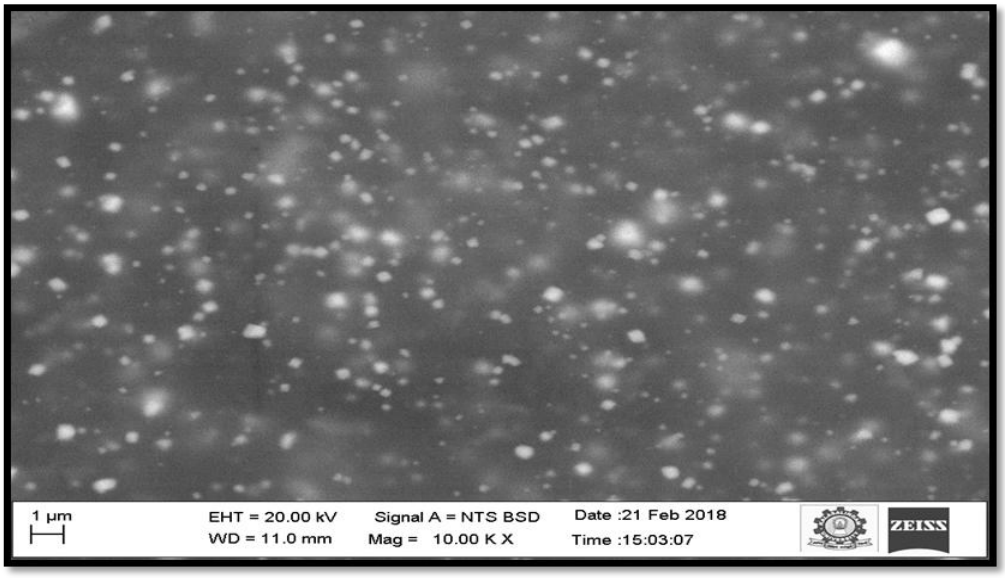


**Figure 4: SEM image of silver nanoparticles of *C. sparsiflorus***

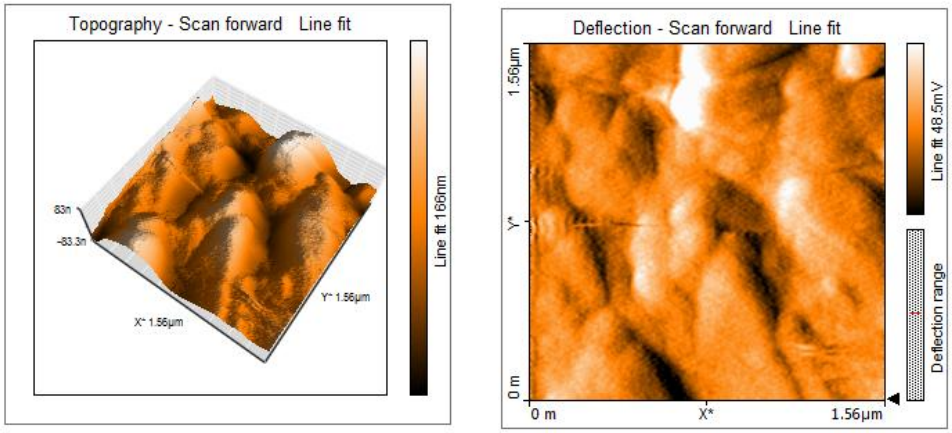


**Figure 5: AFM structure of silver nanoparticles of *C. sparsiflorus***

**XRD Analysis:**

The XRD analysis of phytogenic AgNPs for their crystal structure is shown in Fig 6. The diffraction intensities of phytogenic AgNPs were recorded in the range of 20-80°. The peak arise in XRD at 27.41°, 28.02°, 29.62°, 32.42°, 44.51°, 46.39°, 54.96°, 57.62°, 67.74° and 76.83° that can be imposed to (111), (200), (200), (210), (300), (221), (222), (320), (321) and (322) Bregg's reflection which facts of formation of pure silver materials with face centered cubic crystalline structure. The average particles size measured according to Debye-Scherer's equation calculated is found to be 36.93 nm.

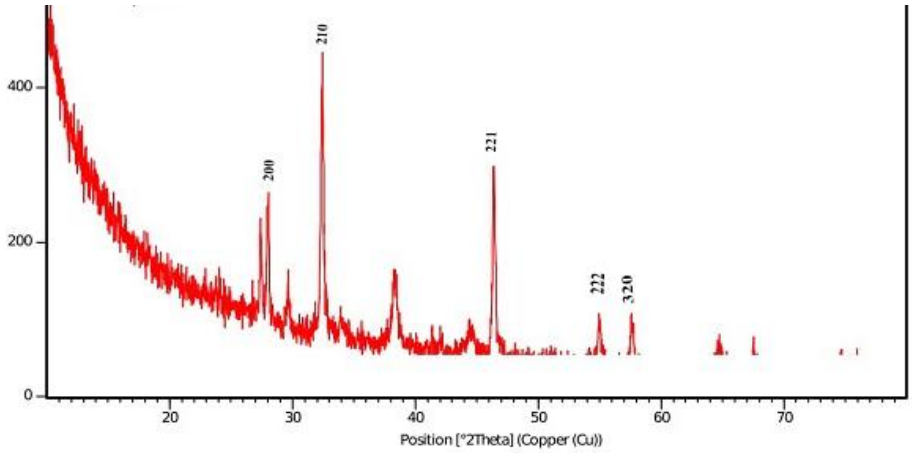


**Figure 6: XRD analysis of synthesized silver nanoparticles of *C. sparsiflorus***

**Antibacterial Array:**

Dose-dependent growth inhibition of bacterial strains was used to assess the antibacterial activity of AgNPs. Table 3 shows the growth inhibition of four different bacterial strains (*Bacillus subtilis, Klebseille pneumoniae, Salmonella paratyphi and Staphylococcus aureus*) treated with various concentration of AgNPs. The aqueous extract of AgNPs of *C. sparsiflorus* at 20µg/disc showed no inhibitory activity against all bacterial species. However, AgNPs at 40, 80 and 100 µg/disc showed an increase in the inhibition of bacterial species. The inhibition zone for the bacterial spp, tested ranged from 3.00 to 16.00 nm. *(B.subtilis* 5.60-11.60mm*, K. pneumoniae* 5.50-16.00mm, *S.paratyphi* 4.00-14.00mm and *S.aureus* 3.00-12.00mm). Tectracyclin (30mcg/disc) was used as the comparative standard. AgNPs exhibited broad spectrum antibacterial activity towards four different bacterial strains. The maximum zone of inhibition was found against *K.pneumoniae* (16.00mm) and followed by *S. paratyphi* (14.00mm). The antibacterial activity of the silver nanoparticles may be centered on permeability of bacterial cells due to cell wall layers or its changes [28, 29]. AgNPs had a considerably minimal bacterial activity against gram-positive bacteria compared to gram-negative one. This is because of high lipopolysaccharide and thick peptidoglycan layer of the gram-negative bacteria. The negatively charged AgNPs can bring to gram-negative cell wall better on the contrary. The gram-positive bacteria are made up of rigid peptidoglycan layer and thus are more stable with minimal binding sites for AgNPs[30]. The nanoparticles rather attack the respiratory chain cell division finally leading to cell death. The nanoparticles release silver ions in the bacterical cells, which enhance their bactericidal activity[31]. Several studies recommend that AgNPs may connect to the surface of the cell membrane agitating permeability and respiration functions of the cell[31]. It is also possible that AgNPs not only interact with the surface of membrane, but can also potential inside the bacteria[32]. However, further studies are still required to know the exact mechanisms of antibacterial activity of the AgNPs.

**Table 3: Inhibition activity of AgNPs from *C.spansiflorus* against the tested bacterial spp**

| Zone of inhibition in mm | | | | | | |
|---|---|---|---|---|---|---|
| Organisms | Tetracyclin 30mcg/disc | *C. Sparsiflorus* Aqueous extract (100µg) | AgNPs in different concentration | | | |
| | | | 20µg | 40µg | 80µg | 100µg |
| *Bacillus subtilis* | 21.00 | 2.00 | - | 5.60 | 8.50 | 11.60 |
| *Staphylococcus aureus* | 18.00 | 3.00 | - | 3.00 | 7.00 | 12.00 |
| *Klebsiella pneumoniae* | 19.00 | 2.00 | - | 5.50 | 8.00 | 16.00 |
| *Salmonella paratyphi* | 20.00 | 4.00 | - | 4.00 | 9.00 | 14.00 |

## CONCLUSION

In conclusion, environmentally benign and low cost synthesis of silver nanoparticles can be achieved using the whole plant extract of *C. sparsiflorus*. The synthesized silver nanoparticles good antibacterial activity against gram negative pathogens *K. pneumoniae* and *S. paratyphi*. Therefore nanoparticles of silver in combination with commercial available antibiotics could be used as a antibacterial agent after further trails on experimental animals.

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## REFERENCES

1. Mohanpuria P, Rana NK, Yadav SK. Biosynthesis of nanoparticles, technological concepts and future applications. Journal of Nanoparticle Research. 2007; 10: 507-517.
2. Thakkar KN, Mahatra SS, Parikh RK. Biological synthesis of metallic nanoparticles. Nanomadicine and Nanotechnology. 2010; 6:257-262.
3. Sharma VK, Yngard RA, Lim, Y. Silver nanoparticles green synthesis and their antimicrobial activities. Advances Colloid Interface Science. 2008; 145:83-96.
4. Dipankar C, Murugan S. The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from Iresineherbstii leaf aqueous extracts. Colloid surf B: Biointes 2012; 98:112-119.
5. Sivalingam P, Antony JJ, Siva D, Achiraman S, Ambarasam K. Mangrove Streptomyces sp. BDUKASIO as nanofacting for fabrication of bactericidal silver nanoparticles. Colloid Sur B: Biometes 2012; 98:12-17.
6. Kalimuthu K, Suresh Babu R, Venkataraman D, Bilal, M, Gurunatham, S. Bio synthesis of silver nanoparticles by *Bacillus licheniformis*. Colloid Surf B: Biointutace 2008; 65:150-153.
7. Ingle A, Gade A, Pierrat S, Sonnichsen C, Rai M. Mycosynthesis of silver nanoparticles using the fungus *Fusarium acuminatum* and its activity against some human pathogenic bacteria. Current Nanosis 2008; 4:141-144.
8. Meng X, Wang Z, Hui LV. Constituents and bacterio static activity of volatile matter from four power plant species. Indian Journal of Agriculture Research. 2010; 44:157-167.
9. Brindha P, Sasikala B, Purushothaman KK. Pharmacognostic studies on *Merugan kizhangu*. Bullutin Medicine. Ethno Botanical Research. 1989; 3:84-96.
10. Anonymous Indian Pharmacopoeia, vol. I & II, Government of India. Ministry of Health and family welfare. The controller of publication. Civil lines, Delhi-110054. 1996.
11. Lala PK Lab Manuals of Pharmacognosy. 5th Edition, CSI Publishers and distributors, Calcutta, 1993.
12. Hulther E, Fendler JH. Explostation of localized surface plasmon resonance. Advances Materials. 2004; 16:1688-1706.
13. Sun S, Murray C, Weller D, Folks L, Mosar. Monodisperse Fe pt nanoparticles and ferromagnetic Fe pt nanocrystal superlattices. Science. 2000; 267:1989-1992.
14. Vilchis-Nestor AR, Sanchez–Mendieta V, Camacho–Lopaz MA, Comez–Espinosa RM, Camacho-Lopez MA, Arenas–Alatorre JA. Solventless synthesis and opticle properties of Au & Ag nanoparticles using *Camellia sinensis* extrac.t Materials Letters. 2008; 67:3103-3105.
15. Zhang W, Qiao X, Chem J, Wany H. Preparation of silver nanoparticles in waher in oil AOT reverse micelles. Journal of Colloidal Interface Science. 2006; 302:170-173.
16. Chimentao RJ, Kirm I, Medina F, Rodriguez X, Cesteros Y, Salagre P, Sueiras JE. Different morpholopies of silver nanoparticles as catalysts for the selective oxidation of styrene in the gas phase. Chemical Communication. 2004; 4:846-847.
17. Muthukrishnan S, Bhaky S, Senthilkumar T, Rao. MV. Biosynthesis, Characterization and antibacterial effect of plant

mediated silver nanoparticles using *Ceropegia thwaitesii* – An endemic species. Indian Crop Production. 2015; 63:119-124.
18. Patil MP, Kim GD. Eco friendly approach for nanoparticles synthesis and mechanism behind antibacterial activity of silver and anticancer activity of gold nanoparticles. Applied Microbiology and Biotechnology. 2017; 101:79-92.
19. Venkatesan J, Kim SK, Shim MS. Antimicrobial, antioxidant and anticancer activities of biosynthesized silver nanoparticles using marine algae *Ecklonic cava* Nanometer. 2017; 6:235.
20. Ahmed S, Ahmed M, Swami BI, Ikram S. A review on plant extract medicated synthesis of silver nanoparticles for antimicrobial applications a green expertise. Journal of Advances Research. 2016; 7:17-28.
21. Kumaraswamy M, Sudipta KM, Jayanta K, Bala Subramanya S. The green synthesis, characterization, and evaluation of the biological activities of silver nanoparticles synthesized from *Leptadenia reticulata* leaf extract. Applied Nanoscience. 2015; 5:73-81.
22. Paoren S, Saising J, Septama AW, VoravuhiKunchai SP. Green synthesis of silver nanoparticles using plants from Myrtaceae family and characterization of their antibacterial activity. Materials Letters. 2017; 209:201-206.
23. Bonde SR, Rathod DP, Ingle AP, Ade RB, Gade AR, Rai MK. *Murraya koenigii* – mediated synthesis of silver nanoparticles and its activity against three human pathogenic bacteria. Nanoscience Methods. 2012; 1:25-26.
24. Chinnappan S, Kandasamy S, Arumugam S, Seralathan KK, Thangaswamy S, Muthusamy G. Biomimetric synthesis of silver nanoparticles using flower extract of *Banhinia purpurea* and its antibacterial activity against clinical pathogens. Environmental Science and Pollution Research. 2017; https://doi.org/10.1007/s11356-017-0841-1.
25. Mallikarjun K, Narsimha G, Dillip G, Praveen B, Shreedhas B, Lakshmi S. Green synthesis of silver nanoparticles using *Ocimum* leaf extracts and their characterization. Digital Journal of Nanometer Biostructure. 2011; 6:181-186.
26. Mishra S, Singh HB. Biosynthesized silver nanoparticles as a narrow weapon against phytopathogens exploring their scope and potential in agriculture. Applied Microbiology and Biotechnology. 2015; 99:1097-1107.
27. Pal S, Tak YK, Song JM. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoaprticle?. A study of the gram negative bacterium *Escherichia coli*. Applied Environmental Microbiolgy. 2007; 73:1712-1720.
28. Yu DG. Formation of colloidal silver nanoparticles stabilized by $Na^+$-poly (r-glutamic acid)-silver nitrate complex via chemical reaction process. Colloid Surf B. 2007; 59:171-178.
29. Chamakura K, Perez-Ballestero R, Cuo Z, Liu BS. Comparison of bactericidal activities of silver nanoparticles with common chemical disinfectants. Colloid Surf B. 2011; 84:88-96.
30. Fayaz, AM, Balaji, K, Girital, M, Yadav, R, Kalichelvan, PT, Venkatesan, R. Biogenic synthesis of silver nanoparticles and their synthesistic effect with antibiotics: a study against gram-postivie and num-negative bacteria. Nanomedicine and Nanotechnology Bio medicine. 2010; 6:103-109.
31. Morones JR, Elechiguerra JL, Camacho A, Ramirez JT. The bactericidal effect of the silver nanoparticles. Nanotechnology. 2005; 16:2346-2353
32. Sondi I, Salopelesondi B. Silver nanoparticles antimicrobial agent: a case study on E.coli as a model for gram negative bacteria. Journal of Colloid Interface. 2007; 275:177-182