

Antibacterial Study and Green Preparation of Silver Nanoparticles through Some Plants

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Abstract

The present study was aimed to synthesize silver nanoparticles from stem extracts of *Caralluma fimbriata* and analyse its antibacterial activity. Generally, nanoparticles have had plenty of commercial usages. Normally, plants' stem extracts can reduce aqueous silver ions so as generating water soluble remarkably stable nanoparticles. The FT-IR analysis was used to study the product chemically. The zone of inhibition was also observed by the silver nanoparticles that have been synthesized in a green method against isolated gram negative and gram-positive bacteria.

Keywords: Silver Nanoparticle; *Caralluma fimbriata*; Antibacterial Activity; FT-IR

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1. Introduction

Today, the nanotechnology is an important research field of modern material science and technology and nanoparticles are being known as basic constituents of nanobiotechnology. The great ratio of surface area to volume is a major and recognizable feature of nanoparticles (Arangasamy Leela *et al.*, 2008). While nanoparticles are commonly synthesized in physical and chemical ways but even the economically possible toxic compounds restrict their [1]. Noble metal nanoparticles have been synthesized by a variety of biological, chemical and physical methods with specific shape and size and for varying applications, but the synthesis of the remaining is expensive requiring hazardous chemicals [2].

Another important research field in the area of nanoparticles is their synthesis and characterization to choose desired shape and size so that an efficient monitoring of many chemical and physical properties [3]. Some biological materials that are used in the process of synthesizing silver nanoparticles include enzymes [4], fungi [5], bacteria [6] and plant leaf extracts [7]. It can be pointed to some advantages of green

synthesis that is ecofriendly and compatible with pharmaceutical and some other biomedical applications since no toxic chemicals have been used in the synthesis process. As well, it is environmentally friendly, cost-effective having easily scaled up routes for large-scale synthesis. In addition, this method does not require high energy, pressure, and temperature and the use of toxic chemicals both in the case of physical and chemical method.

Taxonomical Classification of *Caralluma fimbriata*

Kingdom	Plantae
Order	Gentianales
Family	Apocynaceae
Sub Family	Asclepiadoideae
Genus Species	<i>Caralluma fimbriata</i>
Traditional Name	Kallimulayan

This study has been performed to synthesize silver nanoparticles in the aqueous stem extracts of *Caralluma fimbriata* and analyse the antibacterial efficacy.

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2. Materials and Methods

2.1 Plant collecting

Viralimalai, Pudukkottai district, Tamilnadu, India is the place where the young fresh plant of *Caralluma fimbriata* was collected to be characterized in Rapinat herbarium, St Josephs College, Tiruchirappalli. The plant collected were prepared to separate stem in order to make fine powder for further analysis by pulverizing it after air drying for 15 days in shade.

2.2 The stem extracts

A mixture of 10 gm of powdered plant and 100 ml water was placed in a shaker for the extraction for 24 hours. The What'sMann filter paper (no.1) was then used to filter the solution into an Erlenmeyer flask as the extract to be used for the synthesis of silver nanoparticles.

2.3 Silver nitrate solution

About 0.0169gm silver nitrate was mixed with 100 ml of double distilled water to prepare 1Mm solution.

2.4 Metal plant extract interaction

By adding 10 ml of the fresh and boiled stem extracts to 90 ml of silver nitrate solution in an Erlenmeyer flask the colour of the silver nitrate solution was found to change from colourless to dark brown. The flask was then incubated for 72 hours at room light.

2.5 Phyto nanoparticles

The colour change has been monitored after 72 hours' incubation confirming the formation of silver nanoparticles in the form of aqueous solution from the stem. The solution was then centrifuged at 10,000 rpm for 20 min. For rapid drying, the sediment was mixed with petroleum ether and then collected in a micro centrifuge tube with ethanol for antimicrobial activity tests.

2.6 Microscopic studies

The fresh and boiled extracts of *Caralluma fimbriata* were then observed under Phase Contrast Microscope following centrifugation.

2.7 IR analysis

As the facts of FTIR analysis, most of the molecules absorb light in the infra-red region of the electromagnetic spectrum specifically corresponding to the bonds present in the molecule. The frequency ranges are usually measured as wave numbers over the range 4000-600 cm^{-1} . The Shimadzu IR affinity I instrument was used to analyse relevant samples.

2.8 Silver nanoparticles' antimicrobial activity

Silver nanoparticles were then characterized in terms of antibacterial activity by paper disc method. The bacteria were collected from Department of Microbiology lab, Jamal Mohamed

College, Trichy. The sample organisms used for this examination were *Staphylococcus epidermidis*, *Staphylococcus aureus*, *E.coli*, *Proteus Sps.*, *Bacillus Sps.*, and *Klebsiella Sps.* The zone of inhibition was the scale of antibacterial activity measurement for the synthesized silver nanoparticles.

3. Results and Discussion

The healthcare of human is mainly controlled by medicinal plants. Traditional medicine mostly obtained from plant material has been used by almost 80% of the global population, especially in developing countries due to their fewer side effects and more effectiveness in specific disorders in accordance with the public experience for the use of plant materials against ordinary diseases [8]. Typically, green synthesized silver nanoparticles made from plant extracts have had yellowish brown to brown solutions due to the surface Plasmon excitation of silver nanoparticles [9]. The current research has observed yellowish to brown solutions after incubation for 48 hours (Figure 1, 2, 3 and 4). The fresh and boiled aqueous plant extracts exhibit different phases in the phase contrast microscopic studies (Figure 5 and 6).



Figure 1. The colour changes observed on synthesis of silver nanoparticles on 0 hrs incubation of fresh and boiled stem extracts of *Caralluma fimbriata*



Figure 2. The colour changes observed on synthesis of silver nanoparticles on 12 hrs incubation of fresh and boiled stem extracts of *Caralluma fimbriata*

Table 1. Infra-red spectrum analysis by *Caralluma fimbriyata* boiled stem extracts

SI NO	PEAK VALUE	STRETCHING	INTERPRETATION
1	528.50	C-Br stretching	Halogen
2	572.86	C-Br stretching	Halogen
3	651.94	C-Cl stretching	Halogen
4	673.16	C-Cl stretching	Halogen
5	839.03	C-Cl stretching	Halogen
6	1022.27	C-F stretching	Halogen
7	1076.28	C-F stretching	Halogen
8	1151.50	C-F stretching	Halogen
9	1384.89	C-O stretching	Alcohol
10	1463.97	C-H stretching	Alkenes
11	1527.62	N=O stretching	Nitrocompounds
12	1583.56	N=O stretching	Nitrocompounds
13	1656.85	C-H stretching	Aldehydes
14	1705.07	C=O stretching	Esters
15	1737.86	C=O stretching	Lactones
16	1782.23	C=O stretching	Acid halides
17	1855.52	C=O stretching	Acid Halides
18	1876.74	C=O stretching	Aminoacids
19	2376.30	N-H stretching	Carboxylic acids
20	2848.68	O-H stretching	Carboxylic acids
21	2918.30	O-H stretching	Amides
22	3371.57	N-H stretching	Amides
23	3631.96	O-H stretching	Amides
24	3697.54	O-H stretching	Amides
25	3782.41	O-H stretching	Amides

Table 2. Infra-red spectrum analysis by *Caralluma fimbriyata* fresh stem extracts

SI NO	PEAK VALUE	STRETCHING	INTERPRETATION
1	675.09	C-Cl stretching	Halogen
2	8.9640	C-H stretching	Aromatic compounds
3	1020.34	C-F stretching	Halogen
4	1078.21	C-F stretching	Halogen
5	1157.29	C-F stretching	Halogen
6	1327.03	C-F stretching	Halogen
7	1382.96	C-F stretching	Halogen
8	1465.90	C-F stretching	Halogen
9	1527.62	N-H stretching	Amides
10	1585.49	C=O stretching	Carboxylic acids
11	1660.71	C=O stretching	Ketones
12	1720.50	C=O stretching	Ketones
13	1739.79	C=O stretching	Ketones
14	1811.16	C=O stretching	Acid Anhydrides
15	1853.59	C=O stretching	Ketones
16	1876.74	C=O stretching	Carboxylic acids
17	1903.74	C=O stretching	Alkynes
18	2270.22	C=O stretching	Esters
19	2850.79	C-H stretching	Alkanes
20	2920.23	C-H stretching	Alkynes
21	3410.15	N-H stretching	Amides
22	3695.61	O-H stretching	Alcohol
23	3780.48	O-H stretching	Alcohol

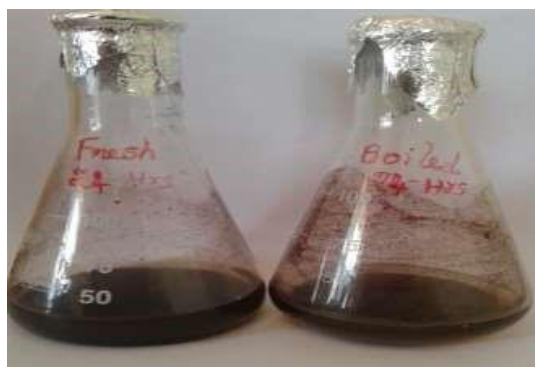


Figure 3. The colour changes observed on synthesis of silver nanoparticles on 24 hrs incubation of fresh and boiled stem extracts of *Caralluma fimbriyata*



Figure 4. The colour changes observed on synthesis of silver nanoparticles on 48 hrs incubation of fresh and boiled stem extracts of *Caralluma fimbriyata*

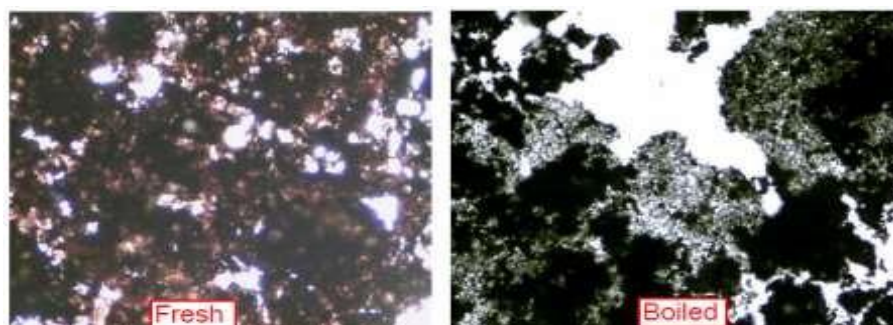


Figure 5. The Phase Contrast Microscopic Observation viewed on 20X Magnification of fresh and boiled stem extract of *Caralluma fimbriyata*.

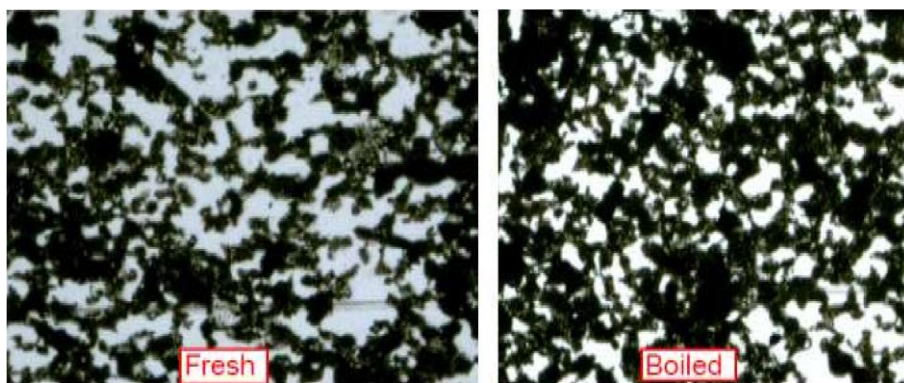


Figure 6. The Phase Contrast Microscopic Observation viewed on 40x Magnification of fresh and boiled stem extract of *Caralluma fimbriyata*

The functional groups of active components were also identified by the FTIR spectrum on the basis of peak value. To summarize, the presence of alcohol, carboxylic acids, esters, amides, and halogen was reported in both samples. The fresh stem extracts contained aromatic compounds, acid anhydrides, alkanes, alkynes, and ketones. Nitro compounds, aminoacids, acid halides, alkenes, aldehydes, and lactones were present in boiled stem extracts (Table 1 and 2).

Green synthesized silver nanoparticles from *Azhardirachta indica* has shown higher activity against *Klebsiella* species in previous studies. This study has examined the antibacterial activity of the fresh and boiled aqueous stem extract of *Caralluma fimbriyata* *in vitro* against six different bacterial strains by disc diffusion method. The maximum zone of inhibition was for the fresh extracts belonging to gram-positive bacterial strains of *S. aureus* (16mm), *Bacillus* (15mm) and gram-negative bacterial strains of *E. coli* (17mm), *Klebsiella* (16mm); and the maximum activity was for the boiled stem extracts with gram-positive bacterial strains of *S.epidermidis* (18mm), *S. aureus* (17mm) and gram-negative bacterial strain of *E. coli* (17 mm). Totally, the boiled extracts of *Caralluma fimbriyata* had the highest activity in comparison with fresh stem extracts (Table 3 and 4).

4. Conclusion

In this study, silver nanoparticles have been synthesized using the stem of plant *Caralluma fimbriyata* and its antibacterial activity has been investigated. These biologically reduced silver nanoparticles were also analysed by FTIR methods. Everything considered the stem can be considered as an excellent source for the synthesis of silver nanoparticles having good activity against several pathogenic organisms. Hence as a conclusion, *Caralluma fimbriyata* owes greater activity against pathogenic microorganisms requiring further studies to demonstrate its other features.

Table 3: Zone of inhibition of silver nanoparticles formed by aqueous fresh stem extracts of *Caralluma fimbriyata* against bacterial strains

SI No.	Sample	Bacterial strains	Zone of inhibition in Diameter (mm)		$\chi^2 = \sum [0-E]^2 / E$
			Standard value	Observed value (Fresh stem)	
1	<i>Caralluma fimbriyata</i>	<i>S.aureus</i>	20	16	0.8
2		<i>B.subtilis</i>	20	15	1.25
3		<i>Proteus</i>	20	18	0.2
4		<i>Klebsiella</i>	20	14	1.8
5		<i>S.epidermidis</i>	20	14	1.8
6		<i>E.coli</i>	20	15	1.25

Table value $\chi^2(0.05)=3.841$, Chi-square value significance at 5%level.

Table 4. Zone of inhibition of silver nanoparticles formed by aqueous Boiled stem extracts of *Caralluma fimbriyata* against bacterial strains

SI No.	Sample	Bacterial strains	Zone of inhibition in Diameter (mm)		$\chi^2 = \sum [0-E]^2 / E$
			Standard value	Observed value (Boiled stem)	
1	<i>Caralluma fimbriyata</i>	<i>S. aureus</i>	20	17	0.45
2		<i>B. subtilis</i>	20	16	0.8
3		<i>Proteus sp.</i>	20	16	0.8
4		<i>Klebsiella sp.</i>	20	16	0.8
5		<i>E. coli</i>	20	17	0.45
6		<i>S. aureus</i>	20	17	0.45

Table value $\chi^2(0.05)=3.841$, Chi-square value significance at 5%level

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