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Research Article

GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM STEM EXTRACTS OF *CARALLUMA FIMBRIYATA* AND ITS ANTIBACTERIAL ACTIVITY

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Abstract

The aim of this study was to synthesis of silver nanoparticles in the aqueous stem extracts of *Caralluma fimbriyata* and investigate its antibacterial activity. Nanoparticles are being used in many commercial applications. It was found that aqueous silver ions can be reduced by aqueous stem extracts of plant parts to generate to extremely stable silver nanoparticles in water. The chemical groups studied using FT-IR analysis. Green synthesized silver nanoparticles showed zone of inhibition against isolated gram positive and gram negative bacteria.

Keywords: Silver nanoparticle; FT-IR; Antibacterial activity; *Caralluma fimbriyata*.

Introduction

The field of nanotechnology is one of the most active research nowadays in modern material science and technology. Nanoparticles are fundamental building blocks of nanobiotechnology. The most important and distinct property of nanoparticles is their exhibit larger surface area to volume ratio (Arangasamy Leela *et al*., 2008). Physical and chemical methods are more popular for nanoparticles synthesis but the use of toxic compounds limit their application economically feasible one (Hasna Abdul *et al*., (2012).An array of physical, chemical and biological methods have been used for synthesis of noble metal nanoparticles of particular shape and size for various applications, but they remain expensive and involve the use of hazardous chemicals (Balagurunathan *et al*., 2011).

Synthesis and characterization of nanoparticles is an important area of research as selection of size and shape of nanoparticles provide an efficient control over many of the physical and chemical properties (Steven *et al*., 1998). Biological materials like plant leaf extracts (Parashar *et al*., 2009), bacteria (Saifuddin, 2009), fungi (Bhainsa and Souza, 2006), and enzymes (Willener and Basnar, 2007) are used for the green synthesis of silver nanoparticles. Green synthesis process offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Green synthesis is cost effective, environment friendly, easily scaled up for large scale synthesis in this method there is no need to use high pressure, energy, temperature and toxic chemicals as in case of chemical and physical method.

Taxonomical Classification of *Caralluma fimbriyata:*

Kingdom	Planate
Order	Gentianales
Family	Apocynaceae
Sub Family	Asclepiadoidiae
Genus	*Caralluma*
Species	*fimbriyata*
Tradtional Name	Kallimulayan

The present study aims at the synthesis of silver nanoparticles from the aqueous stem extracts of *Caralluma fimbriyata.* The present study aims to attempt to test the antibacterial efficacy of silver nanoparticles produced by using the stem extracts of *Caralluma fimbriyata*.

Materials and Methods

Collection of plant sample

Fresh young plant of *Caralluma fimbriyata* were collected from Viralimalai, Pudukkottai district, Tamilnadu, India. The plants were identified in Rapinat herbarium, St Josephs College, Tiruchirappalli. The stem was separated from the collected plant. Then it was air dried in shade for 15 days and then pulverized to fine powder for further analysis

Preparation of the stem extracts

10 gm of plant powdered was weighed and it is mixed with 100 ml water. The extraction was carried out in a shaker for

24 hours. The solution was filtered through What'sMann no.1 filter paper. The filtered samples were collected in a conical flask. The obtained extracts were used for the synthesis of silver nanoparticles.

Preparation of silver nitrate solution

1Mm silver nitrate solution was prepared by the concentration of 0.0169gm in 100 ml double distilled water and stored.

Metal plant extract interaction

90 ml of silver nitrate solution was taken in conical flask. To this add 10 ml of the fresh and boiled stem extract. The color change of the silver nitrate solution was found from colorless to dark brown. Incubate the conical flask at room light for 72 hours.

Concentration of phyto nanoparticles

After 72 hours incubation, the color change was observed. It indicates that the silver nanoparticles were synthesized from stem with the help of aqueous solution. Then this solution was taken in centrifuge tube and it was centrifuged at 10,000 rpm for 20 min. The pellet were taken after centrifugation and mixed with petroleum ether for rapid drying, dried pellets were collected in a micro centrifuge tube with ethanol and the pellets were used for testing antimicrobial activity.

Microscopic Observation

After cenrtrifugation, the fresh and boiled extracts of *Caralluma fimbriyata* observed under Phase Contrast Microscope.

IR spectrum analysis

FTIR relies on the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule. The frequency ranges are measured as wave numbers typically over the range 4000-600 cm-1. The compounds were analyzed using shimaze IR affinity I instrument.

Antimicrobial activity of silver nanoparticles

Then antibacterial activity of isolated plant with silver nitrate based nano particle pellets were tested by paper disc method. The bacteria were collected from Department of Microbiology lab, Jamal Mohamed College, Trichy. The test organisms used for assay are *Staphylococcus aureus, Staphylococcus epidrmidis, Proteus Sps., E.coli, Bacillus Sps.,and Klebsiella Sps,* The antibacterial activity of the synthesized silver nano particles was evaluated by measuring the zone of inhibition.

Results and Discussion

Medicinal plants play a key role in human health care. About 80% of the world population, mainly in developing countries relies on the use of traditional medicine which is predominantly based on plant material. They have lesser side effects and are effective in certain disorders are based on experience in the use of plant products against common diseases (Inder and Sulochana, 2010).

The green synthesis of silver nano particles through plant extracts were carried out. It is well known that silver nano particles exhibit yellowish brown to brown color in aqueous solution due to excitation of surface Plasmon vibration in silver nanoparticles (Thirumurugan *et al*., 2010). In the present study the appearance of yellowish to brown color in the reaction vessels after incubation of 48 hrs (Fig.1, 2, 3 & 4). In the phase contrast microscopic observation different phases are seen in the aqueous fresh and boiled plant extracts (Fig. 5 & 6).

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

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

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

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

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

S.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=0 stretching	Nitrocompounds
12	1583.56	N=0 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	Carboxilic acids
20	2848.68	O-H stretching	Carboxilic 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

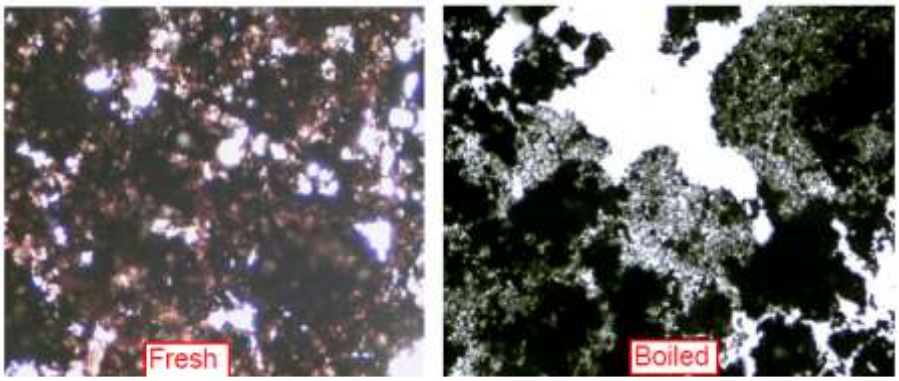

Fig. 5: The Phase contrast Microscopic Observation viewed on 20X Magnification of fresh and boiled stem extract of *Caralluma fimbriyata*\

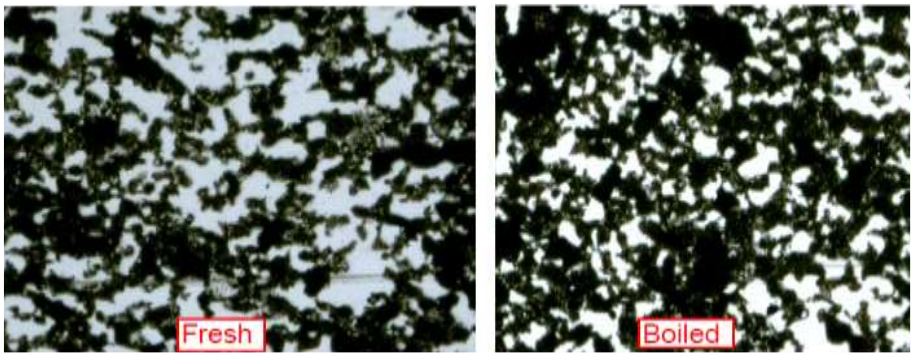

Fig. 6: The Phase contrast Microscopic Observation viewed on 40x Magnification of fresh and boiled stem extract of *Caralluma fimbriyata*

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

S.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

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

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

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

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

Sample		Bacterial strains	Zone of inhibition in Diameter (mm)		$X^2=\sum[0-E)^2]/E$
			Standard value	Observed value (Boiled stem)	
1.	*Caralluma fimbriyata*	*S. aureus*	20	17	0.45
2.		*B. subtilis*	20	16	0.8
3.		*Proteus sp.*	20	16	0.8
4.		*Klebsiella sp.*	20	16	0.8
6.		*E. coli*	20	17	0.45

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

The FTIR spectrum was used to identify the functional groups of the active components based on the peak value in the region of infrared radiation. In the present study reported presence of Halogen, alcohol, esters, carboxylic acids and amides present in both two samples. Aromatic compounds, Ketones, alkynes, alkanes and Acid anhydrides are present only in fresh stem extracts. In boiled stem extracts Alkenes, nitro compounds, aldehydes, lactones, aminoacids, and Acid halides are present. (Table 1 & 2).

In the earlier study green synthesized silver nano particles of *Azhardirachta indica* showed higher activity against *Klebsiella* species. In the present study the antibacterial activity of the aqueous fresh and boiled stem extract of *Caralluma fimbriyata* was assayed *in vitro* by disc diffusion method against 6 bacterial strains. The gram positive bacterial strains revealed the maximum zone of inhibition for the fresh extracts of *S. aureus* (16mm), *Bacillus* (15mm) and gram negative bacterial strains of *E. coli* (17mm), *Klebsiella* (16mm) and the gram positive bacterial strains maximum activity showed the boiled stem extracts of *S.epidermidis* (18mm), *S. aureus* (17mm) and gram negative bacterial strain of *E. coli* (17 mm). The comparision of fresh and boiled stem extracts of *Caralluma fimbriyata* the boiled extracts showed the highest activity (Table 3 & 4).

Conclusion

The study included the synthesis of silver nano particles from the stem of plant *Caralluma fimbriyata* its antibacterial activity. The bioreduced silver nano particles where characterized using FTIR Analysis. In present study found that stem can be also a good source for synthesis of silver nanoparticles and its activity against various pathogenic organisms. Thus it can be concluded that *Caralluma fimbriyata* possess more activity against pathogenic microorganisms and further studies would reveal its other activities**.**

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