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Green synthesis and characterization of silver nanoparticles using *Sphaeranthus indicus* with its phytochemical study

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ABSTRACT

Synthesis of silver nanoparticles using aqueous Sphaeranthus indicus L. Leaf extract at different conditions showed the nano silver formation in 15 minutes, whereas a 1:1 ratio of silver nitrate and plant extract formed silver nanoparticles in 25 minutes. The TEM results reveals that average size of silver nanoparticles synthesized from Green, Pink flower, leaf extract was found as spherical shape and 16-20 nm in size. The phytochemical screening of the methanol, Ethyl acetate, Pet. ether extract of Sphaeranthus indicus L. revealed the presence of phytochemicals like Alkaloids, Flavonoids, saponins, terpenoids, sterols, Tannins, Anthraquinones, and proteins.

KEY WORDS: Sphaeranthus indicus, Synthesis, silver nanoparticles, phytochemical screening.

1. INTRODUCTION

Natural products provide opportunities for new drug discoveries which can be used for disease treatment (Chin, 2006). The medicinal plants products can be used to inhibit the development of various microorganisms (Ranjitham, 2013) which is very economical (He, 2013; Niraimathi, 2013). We have reported the biosynthesis of metal nanoparticles by using various plant extracts (Madhukar Rao Kudle, 2013; Rama Koyyati, 2014; Karunakar Rao Kudle, 2013; 2014; 2015). In continuation of our studies systematic investigation was undertaken to screen a local *Sphaeranthus Indicus L plant*. The presence of various photochemical study of aqueous Green, Pink flower and leaf extract of *Sphaeranthus Indicus L.* Leaf extract. Further, the ethanol extract of *Sphaeranthus Indicus*. Leaf extract was used for the biosynthesis of silver nanoparticles. In view of the importance health remedy, the present study was designed to evaluate various phytochemicals and fluorescent characteristics of *Sphaeranthus indicus L* leaf and seed extracts

2. MATERIALS AND METHODS

All reagents used were of analytical grade. Double distilled Millipore water was used in study. The leaves,flower,root from *Sphaeranthus Indicus L.* were procured from a local plant in botanical garden, Osmania University, Hyderbad. Silver nitrate solution (1mM) was used throughout the study Chemicals all reagents used in the study were of analytical grade. Silver nitrate ($AgNO_3$) was obtained from Sigma Aldrich.

Synthesis of Silver Nanoparticles: Silver nanoparticles were synthesised from silver nitrate using aqueous Green, Pink flower and leaf extract of *Sphaeranthus indicus L.* as reducing agent. UV-VIS Spectra Analysis, FTIR Analysis and TEM analysis was performed as described in our earlier papers **(**Madhukar Rao Kudle, 2013; Rama Koyyati, 2014; Karunakar Rao Kudle, 2013; 2014; 2015).

Preliminary Investigation of Phytochemicals:

Collection and processing of plant samples: Fresh Green, Pink Flower and leaf Extract of *Sphaeranthus indicus L.* were collected in the month of February- 2014 from Botanical Garden, Osmania University, Hyderabad, India.

Phytochemical Screening: The samples were crushed into fine powder and dissolved separately in 100ml of solvent. The solution was kept at room temperature for seven days to allow the extraction of compounds from flower and Leaf. The solution of each sample was stirred after every 24hrs using sterile glass rod. After 7 days the Solution was filtered through what man filter paper No-1 and a greenish filtrate was obtained. The solvent was evaporated and sticky substances obtain that was stored in the refrigerator.

Antibacterial activity from the crude extract of the leaf, flower of the plant Sphaeranthus Indicus L. was evaluated. *Sphaeranthus Indicus* L. is medicinally important plant, used in Ayurveda and unani system of medicine. Methonal, Pet ether and Ethyl Acetate extracts of the Green, Pink flower and leaf of Sphaeranthus *Indicus* L. were investigated for the presence of phytochemicals viz. Tannins, Saponins, Flavanoids (alkaline reagents test), Terpenoids (Salkowski test), Cardiac Glycisides (Kellar-Killiani test), Alkaloids, Anthraquinone by following standard biochemical methods (Chandrashekar, 2014).

Biosynthesis of silver nanoparticles from the green, pink flower solvent leaf extract of sphaeranthus indicus l: 2 ml of the Methanol, Pet. Ether and Ethyl Acetate extract was added into 30 ml of aqueous solution of 1 mM silver nitrate for reduction of Ag+ions and stirred at room temperature for 12 hours.

The amount of protein was estimated by the method (Lowry, 1951). A standard plot was prepared using Bovine Serum Albumin (BSA) as the standard.

Fluorescent Study Of Seed And Leaf Powder: 0.2gms of seed powder and dried leaf powder were taken into clean and dried test tubes. To each tube 5ml of different organic solvents like 5% NaOH, 10% NaOH, 5% KOH, 50 % HNO_3, Conc. HNO_3, Conc. Sulphuric acid were added separately. Then, all the tubes were shaken and they were allowed to stand for about 20-25 min. The solutions obtained were observed under the visible light and UV light for their characteristic colourreaction and were compared with a standard colour chart and colours were recorded (Rama Swamy Nanna, 2013; Rashmi Yadav, 2011; Gayathri, 2015).

3. RESULTS AND DISCUSSION

The results of the qualitative screening of the silver nano particles, phytochemical and fluorescence study from *Sphaeranthus indicus* L plant green, pink color flower and seed extracts of the plant species.

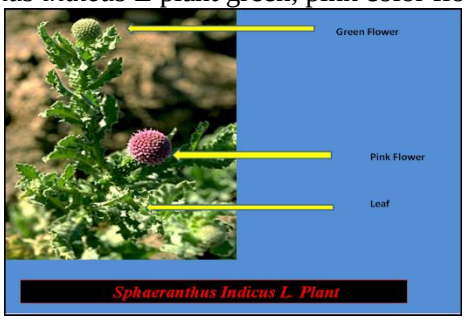

Figure.1. Sphaeranthus Indicus L. Plant Green, Pink flower and Leafs

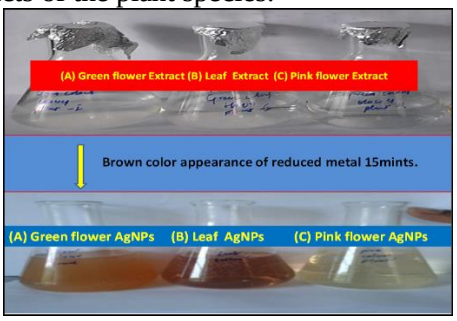

Figure.2. Sphaeranthus indicus L. plant green flower AgNPs (A), Leaf AgNPs (B), Pink Flower AgNPs

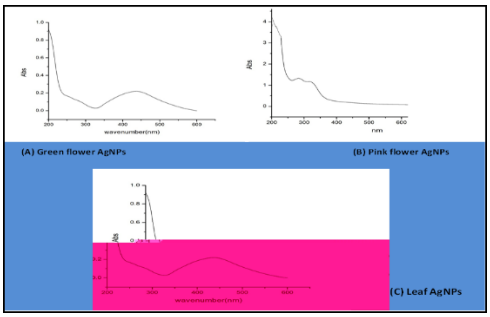

Figure.3. Change in color after addition of Green flower, Pink flower and Leaf extracts in AgNPs UV-Spectra's

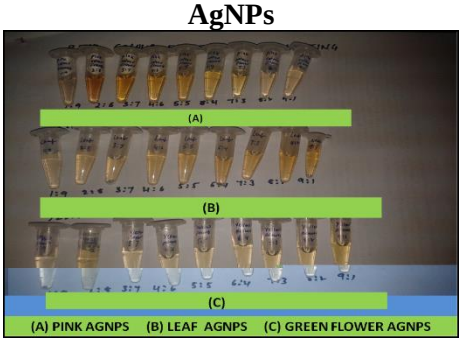

Figure.4. Sphaeranthus Indicus L. Plant Pink flower AgNPs (A), Leaf solutions AgNPs (B), Green flower AgNPs (C) with different Con.c of silver nitrate (1% to 0.062%) there was a change in coloure of leaf solution ofter addition of different concentration of $AgNO_3$

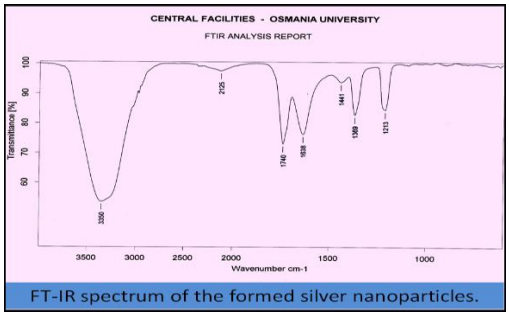

Figure.5. Change in color after addition of Pink flower, Green flower and Leaf AgNPs in FTIR Spectra functional group Identification

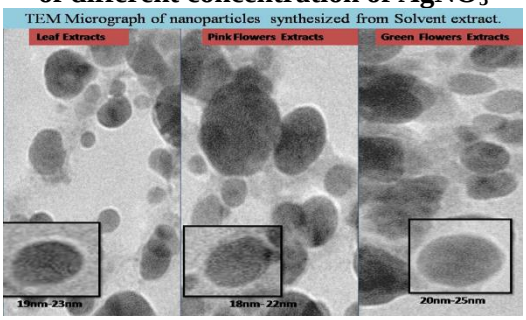

Figure.6. TEM micrograph of size of AgNPs synthesized from leaf Extract from Sphaeranthus indicus L.

Table.1. Fluorescent analysis of Pick, Green flower and Leaf Powder of Sphaeranthus Indicus L

Organic solvents	Pick flower powder		Leaf powder		Green Flower powder	
	Visible light	Fluorescence UV light	Visible light	Fluorescence UV light	Visible light	Fluorescence UV light
5% NaOH	Light Brown	Brown	Brownish	black	brown	Light green
10% NaOH	Brown	Reddish	Brown	black	Dark brown	Light green
Conc. H_2SO_4	Light Brown	Brown	Brown	Light green	Brown	Dark green
50 % HNO_3	Light Brown	Brown	Light yellow	purple	Light Yellow	Dark green
Conc.HNO_3	Light Brown	Brown	Light brown	Dark green	Light Brown	yellow
5% KOH	Light Brown	yellow	Brown	Dark green	Brown	Light yellow

Table.2. Phytochemical analysis of Green Leaf Extracts of *Sphaeranthus Indicus L.* +=Presents; -= Absent

S.No	Leaf Extracts			
		Methanol	Pet. Ether	Ethyl acetate
1	Alkaloids	-	-	-
2	Flavonoids	-	-	-
3	Anthraquinones	-	-	-
4	Saponins	-	-	-
5	Steroids	-	-	-
6	Glycosides	+	-	-
7	Terpenoids	-	+	+
8	Tannins	-	-	-

Table.3. Phytochemical analysis of pink flower Extracts of Sphaeranthus *Indicus L,* +=Presents; -= Absent

S.No	Pink Flowers Extracts			
		Methanol	Pet. Ether	Ethyl acetate
1	Alkaloids	+	_	_
2	Flavonoids	+	_	_
3	Anthraquinones	+	_	_
4	Saponins	-	_	_
5	Steroids	-	+	_
6	Glycosides	_	_	+
7	Terpenoids	+	_	_
8	Tannins	_	_	_

Table.4. Phytochemical analysis of Green flower Extracts of *Sphaeranthus Indicus L.* +=Presents; -= Absent

S.No	Green Flowers Extracts			
		Methanol	Pet. Ether	Ethyl acetate
1	Alkaloids	_	_	_
2	Flavonoids	+	_	_
3	Anthraquinones	+	_	_
4	Saponins	-	_	_
5	Steroids	-	_	_
6	Glycosides	_	_	+
7	Terpenoids	_	_	_
8	Tannins	+	_	_

Table.5. Estimation of protein from different solvent Pink flower extracts by Lowry method

Estimation of protein from different solvent Pink flower extracts										
Concentrate (µl)	Methanol Extracts leaf			Pet. Ether				Ethyl acetate		
	Leaf (µg)	Pink flower (µg)	Green flower (µg)	Stem (µg)	Leaf (µg)	Pink flower (µg)	Green flower (µg)	Leaf (µg)	Pink flower(µg)	Green flower(µg)
50	0.86	1.0	1.0	-	0.04	0.7	0	0.10	0.06	0
100	0.89	1.0	1.0	-	0.12	0.14	0	0.27	0.13	0
200	0.97	1.0	1.0	-	0.14	0.28	0	0.45	0.26	0

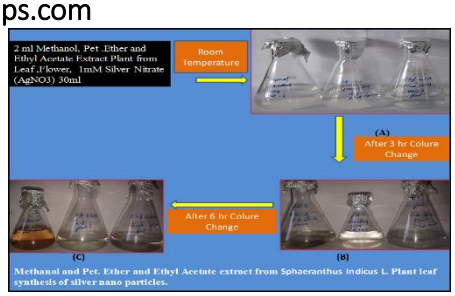

Figure.7. Methanol and Pet. Ether and Ethyl Acetate extract from *Sphaeranthus Indicus L.* Plant leaf synthesis of silver Nano particles

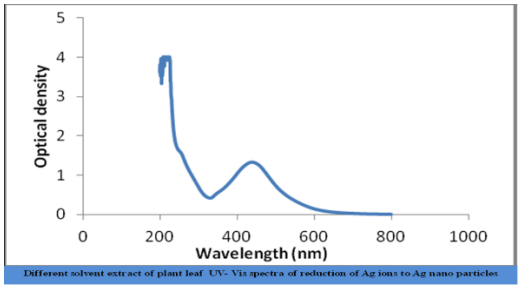

Figure.8. Change in color after addition of *Sphaeranthus Indicus L.* Leaf extracts in silver UV-Spectra's

Kingdom: *Plantae*, Class: *Magnoliopsida*, Order: *Asterales*, Family: *Asteraceae*, Genus: *Sphaeranthus*, Species: *indicus* (Figure.1). The colour change was noted by virtual observation in Sphaeranthus Indicus L. Leaf, Green and Pink Flower extract incubated with aqueous solution of AgNO3. It started to change colour from watery to yellowish brown due to the reduction of silver ions, this exhibit the formation of silver nanoparticles (Figure.2). Absorption spectrum at different wavelengths ranging from 400-500 nm revealed a peak of λ max at 450 nm (Figure: 3). The Sphaeranthus Indicus L. Leaf, Green and Pink Flower extract without silver nitrate solution did not show any change in colour. The colour intensity increased with duration of time and colour of extract change brown after heating 15mints, of incubation and no significant change after words. *Sphaeranthus indicus* L. Leaf Extract Solution plant and Green, Pink flower solution with different Conc of silver nitrate (1% to 0.062%) (b,c,d,e,f) there was a change in coloure of flower solution ofter addition of different concentration of $AgNO_3$ (figure.4). Change in color after addition of Plant flower and leaf extracts in AgNPs (UV-Spectra's). The FTIR spectra study of functional group and shifted electron regions of leaf AgNPs (Figure: 5). The TEM images clearly reveals that shape and size of silver nanoparticles was 16-20 nm, 19nm-22nm and 20nm-25nm with spherical shaped. It was noticeable that edges of particles were lighter than the centre (Figure: 6). It exhibit that few biomolecules like proteins of extract capped the silver particles. The shape diffraction spots clearly suggest that the particle is of single crystal quality. Fluorescent study of Green, pink flower and leaf powder of *Sphaeranthus indicus* L. using different chemical reagents showed different coloration under visible light and UV light. One of the important features of fluorescence is that UV light induces a fluorescent nature in many natural products (e.g. Alkaloids) where fluorescence is not seen in natural day light. Among various solvents tested, Fluorescent analysis of Plant Green flower powder (a)Pink flower powder (b) Leaf Extract(c) powder with different organic solvents 5% NaOH, 10% NaOH, 5% KOH, 50 % HNO3, Conc HNO_3, Conc Sulphuric acid. These results are supportive with fluorescent studies performed with *Sphaeranthus indicus* L. Leaf powder. Some of substances may be often converted into fluorescent derivatives by using different chemical reagents though they are not fluorescent, hence we can often assess qualitatively some crude drugs using fluorescence as it is the most important parameter of pharmacognostical evaluation (Figure:7,8,9 and Table.1). The phytochemical screening of the plants revealed some differences in the constituents of the Green, Pink Flower and Leaf of plants tested *Sphaeranthus indicus* L. tested positive for all the phytochemicals tested .The results of phytochemical screening are shown in positive tests Alkaloids, Flavonoids, Anthraquinones, Saponons, Steroids,Glycosids, Terpenoids and Tannins (Figure.10,11,12,13,14,15 & Tables.2,3,4). The plant Sphaeranthus Indicus L Methanol extracts bounded protein in leaf contain (0.86µg/50µl 0.89µg/100µl, 0.97µg/200µl), Green Flower (1.0µg/50µl, 1.0µg/100µl, 1.0µg/200µl), and Pink flower (1.0µg/50µl, 1.0µg/100µl. 1.0µg/200µl), Pet. Ether extracts bounded protein in leaf contain (0.04µg/50µl 0.12µg/100µl, 0.14µg/200µl), Green Flower (0µg/50µl, 0µg/100µl, 0µg/200µl), and Pink flower (0.7µg/50µl, 1.4µg/100µl. 0.28µg/200µl) and Ethyl Acetate extracts bounded protein in leaf contain (0.10µg/50µl 0.27µg/100µl, 0.45µg/200µl), Green Flower (0µg/50µl, 0µg/100µl, 0µg/200µl), and Pink flower (0.06µg/50µl, 0.13µg/100µl. 0.26µg/200µl) were the different concentration estimation Lowery Method (Figure:16,17 and 18 and Table.5). 2 ml of the Methanol, Pet ether extract and Ethyl acetate was added into 30 ml of aqueous solution of 1 mM silver nitrate for reduction of Ag+ions and stirred at room temperature for 6 hours. The colour change was noted by virtual observation in *Sphaeranthus indicus* L. Leaf and Green Flower extract incubated with aqueous solution of AgNO3.(figure:19). In the present investigation it was noted that silver nanoparticles synthesized using aqueous extract exhibited reddish brown colour in aqueous solution due to excitation of Surface Plasmon Vibrations. The UV absorption spectra of biosynthesis of nanoparticles in all methods gave absorption maximum at 450cm^{-1}. The Presence of such metabolites are indicative of their role in the reduction of silver nitrate to silver nanoparticles. Synthesis of silver nanoparticles using aqueous Sphaeranthus indicus L. Green, Pink flower and Leaf extract at different conditions shows that method formed nano silver in 15 minutes, whereas a 1:1 ratio of silver nitrate and plant extract formed silver nanoparticles in 25 minutes. The TEM results reveals that average size of silver nanoparticles synthesized from Green, Pink flower, leaf extract was found as spherical shape and 16-20 nm

in size. The phytochemical screening of the methanol, Ethyl acetate, Pet. ether extract of *Sphaeranthus indicus* L. revealed the presence of phytochemicals like Alkaloids, Flavonoids, saponins, terpenoids, sterols, Tannins, Anthraquinones, and proteins. The present work also reveals that the extract from the leaves of Sphaeranthus Indicus L. possesses good potential presumably because of its phytochemical constituents. These findings could also be of commercial interest to both pharmaceutical companies and research institutes in the production of new drugs. The fluorescent analysis of powdered drug play an important role in the determination of quality and purity of the drug. Some of substances may be using different chemical reagents though they are not fluorescent. Hence we can often assess qualitatively some crude drugs using fluorescence.

4. CONCLUSION

Green Synthesis of silver nanoparticles using *Sphaeranthus indicus* L. Leaf extract demonstrated rapid formation of the nanoparticles within 15 minutes, with spherical shape and an average size of 16-20 nm. Phytochemical screening of different extracts revealed the presence of phytochemicals like Alkaloids, Flavonoids, saponins, terpenoids, sterols, Tannins, Anthraquinones and proteins

5. ACKNOWLEDGEMENT

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