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Green synthesis of silver nanoparticles and its physico, phytochemical and antimicrobial properties of *Cissus quadrangularis* and *Gymnema sylvestre* from South India.

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Abstract: The emerging branch of Bio-Nanotechnology combines biological principles with physical and chemical approaches to produce nano sized particles with specific functions. Silver nanoparticles has been widely studied due to the excellent physicochemical properties, high chemical stability, catalytic activity and antibacterial activity, eco-friendly nature and cost-effectiveness. The main objective of this work is to green synthesis of silver nanoparticles with *Cissus quadrangularis* and *Gymnema sylvestre* and to assess their antibacterial properties. The observed results suggested that using *C.quadrangularis* and *G.sylvestre,* synthesised silver nanoparticles and exhibited significant antimicrobial properties. The results demonstrate that the Ag-NPs reported in this work are capable of eradicating pathogenic resistant bacteria in an infection *in vivo*. Obtained results suggest the potential use of Ag-NPs, synthesized by green chemistry methods, as therapeutic agents against infections caused by resistant and non-resistant strains.

Key words: Green synthesis, nanoparticles, *Cissus quadrangularis* and *Gymnema sylvestre*

Introduction

Nanoparticles are of great interest due to their extremely small size and large surface to volume ratio, which lead to both chemical and physical differences. Nanoparticle is a ultrafine unit with unique size, distribution and morphology, usually ranging from 1 to 100 nanometres (nm). Studies of nanoparticles (NPs) are of great importance nowadays for their widespread applications as antimicrobial and catalytic agents. The development of green synthesis of silver nanoparticles is evolving in the field of nanotechnology. Silver nanoparticles are the most common in medicine and pharmacy applications in the field of nanobiotechnology.

Antimicrobial properties of silver nanoparticles caused the use of these nanometals in different fields of medicine, various industries, animal husbandry, packaging, accessories, cosmetics, health and military. Silver ions are highly toxic for microorganisms and, therefore, have multiple roles in the medical field [S. Galdiero, *et al.,* 2011]. Silver nanoparticles show potential antimicrobial effects against infectious organisms are well known as inhibitory and antibacterial materials [Dwivedi, *et al.,* 2012].

The biological synthesis of gold and silver nanoparticles using plants has received more attention as a suitable alternative to chemical procedures and physical methods. Among various known synthesis methods, plant extract mediated silver nanoparticle synthesis is widely preferred as its cost-effective, environmentally friendly without use of harsh, toxic, expensive chemicals and safe for human therapeutic uses (Kumar and Yadav 2009). Synthesis of nanoparticles using plant extracts is very cost effective, and therefore can be used as an economic and valuable alternative for the large-scale production of metal nanoparticles. Extracts from plants may act both as reducing and capping agents in nanoparticle synthesis. Plant-mediated green synthesis of silver nanoparticle is considered a widely acceptable technology for rapid production of silver nanoparticles for successful. Biosynthesis of nanoparticles from green synthesis is advantageous over chemical and physical methods as it is a cost effective and environmentally useful method and it is not necessary to use high pressure, energy, temperature and toxic chemicals. Plants provide a better platform for nanoparticle synthesis as they are free from toxic chemicals as well as provide natural capping agents. Moreover, use of plant extracts also reduces the cost of microorganism isolation and culture media enhancing the cost competitive feasibility over nanoparticle synthesis by microorganisms. The use of plants for synthesis of nanoparticles is rapid, low cost, eco-friendly, and a single-step method for biosynthesis process. Hence our study is focussed to prepare silver nanoparticles with *Cissus quadrangularis* and *Gymnema*

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sylvestre collected from South India and evaluation of its physico, phytochemical and antimicrobial properties.

Solvents and chemicals:
All chemicals were purchased from Qualigens fine Chemicals, Mumbai and S.D. fine chemicals, Mumbai. All culture media components and antibiotics used in this study were procured from Hi Media, Mumbai, India. Silver nitrate GR used as such (purchased from Merck, India).

Medicinal plants
Both plant materials (**Table 1**) selected by using previous work done, folklore reports and the plant materials were collected from South Indian region and they were identified with the help of Gamble and based on the information available from literature (Warrier, *et al.,* 1994-1996; Pullaiah, 2002) and through field observations were collected for the present study.

Table 1: List of medicinal plants with common name and family chosen for study

Plant species	Common Name	Family	Part studied	Collected from	Coordinates
Cissus quadrangularis	Asthisamharaka	Vitaceae	Aerial Parts	Aruku Valley, Visakhapatnam Dist.	18.3273° N, 82.8775° E
Gymnema sylvestre	Podapatri	Apocynaceae	Aerial Parts	Chintapalli, Visakhapatnam Dist.	17.8713° N, 82.3533° E

Both plants were collected during the flowering periods of these plants have been grown in Visakhapatnam District, Andhra Pradesh. The plant was then identified and was confirmed by a taxonomist working at Andhra University. All the fresh and healthy plant parts of leaves collected locally and were washed with running tap water and double distilled water respectively for several times to remove all the dust and unwanted visible particles, cut into small pieces and dried at room temperature and then shade dried for 3–5 days, powdered by using mechanical grinder and then stored for further investigations.

Preparation of plant extract
Organic solvents of varying polarities are generally selected in modern methods of extraction to exploit the various solubilities of plant constituents. Soxhlet extraction is widely used for both initial and bulk extraction. Its main advantage is that the material is extracted continuously, i.e., the solvent saturated in solubilized metabolites empties into the flask, fresh re-condensed solvent then re-extracts the material in the thimble. This method is less time- and solvent-consuming than maceration or percolation.

100 g of ground weighed material of fine coarse powder was successively extracted by different solvents of hexane, chloroform and methanol, in a specific sequence based on increasing polarity. The soxhlet hot extraction procedure for each of the above solvents was run for about 6 hours, until a colorless solvent was seen in the siphon tube, which indicated complete extraction. The solvents were removed under reduced pressure and controlled temperature by rotary evaporator. The extracts were dried and stored in a clean glass bottle and kept at 4-6^{0}C for further antimicrobial screenings.

Plant aerial parts aqueous extract was used to prepare silver nanoparticles based on cost effectiveness, ease of availability and its medicinal property. About 10 gm of finely incised leaves of each plant type were weighed separately and transferred into 250 mL beakers containing 100 mL distilled water and boiled for about 30 min. The extracts were then filtered thrice through Whatman No. 1 filter paper to remove particulate matter and to get clear solutions which were then refrigerated (4°C) in 250 mL Erlenmeyer flasks for further experiments. In each step of the experiment, sterility conditions were maintained for the effectiveness and accuracy in results without contamination.

Preparation of 1 mM $AgNO_3$ solutions
One millimolar solution of $AgNO_3$ (0.017 gms) was prepared by dissolving in 100ml deionized water (DIW) and stored in amber coloured bottle in cool and dry place.

Optimization Parameters
Effect of concentration
Silver nitrate was taken in different concentrations (1.0 mM, 1.5 mM and 2 mM) for the synthesis of AgNP. The UV-Visible absorption spectrum for the above-mentioned concentrations of silver nitrate were observed.

Silver nanoparticle (Ag NP) synthesis
Silver nanoparticles (Ag NPs) were synthesized using oxidation – reduction method. Silver nanoparticles were fabricated using plant extracts as reducers. In the present study

synthesis is commenced with 1mM aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 2 ml of plant extracts added to 8 ml of aqueous solution of 1mM silver nitrate for reduction into Ag+ ions and kept at room temperature. The reaction was performed in dark at room temperature overnight to minimize photoactivation of silver nitrate until the color changed from colorless to yellow.

Rapid synthesis of AgNPs was observed after 5 hrs, incubation through visual observation of colour change from pale yellow to dark brown in the reaction mixture. The fully reduced solution could cool down to room temperature, then centrifuged at 10000 rpm for 30 minutes in high speed centrifuge. The process was repeated by dispersion of pellets in water, to obtain colored supernatant solutions. The sample was then stored at -4°C for further use. The supernatant liquid was then decanted, and the precipitate was washed with Millipore water and then with ethanol and then dried in hot air oven. This paste was then collected in a ceramic crucible and heated in an air heated furnace at 400 degree Celsius for 2 hours. A light-yellow coloured powder was obtained and this was carefully collected and packed for characterization. This was repeated thrice to ensure better separation of the AgNPs and was used for characterization studies.

Mechanism of reduction of silver to AgNP's

Formation of AgNP's was noted over a time period of 30 minutes to three hours for the various concentrations. The actual mechanism involve for the reduction is based on the presence of different phytocompounds like flavonoids, steroids etc.

Characterization of silver nanoparticles

Synthesis of silver nanoparticles was confirmed by taking the absorbance in UV-Vis spectra at a range 200-800 nm at a resolution of 1 nm. The formation of silver nanoparticles was observed by the colour change and monitored by UV-visible Double beam spectrophotometer.

UV-VIS Spectra Analysis

The bio reduction of pure silver ions (Ag+) in solutions to Ag nanoparticles were monitored by measurement of the UV-Visible spectral data of the reduction medium at different time intervals taking 1 mL of the samples, versus 1 mL of deionized water as blank. UV/Vis data resolution has been accomplished by using a UV/Vis spectrophotometer operated at 1 nm intervals ranging from 200 to 800 nm. In this relation, the absorption signals in a wavelength range of 420-450 nm were considered, which are identical to the characteristic UV/Vis spectrum of metallic Ag representing the successful synthesis of silver nanoparticles. The shifting of SPR peaks represents the variation in particles size. The shift toward lower wavelengths side indicates the decreasing particle size and increasing state of segregation. Moreover, the decrease in line width of SPR peak can be due to the shape isotropy of the silver nanoparticles.

Samples (1mL) of the suspension were collected periodically to monitor the completion of bio reduction of Ag+ in aqueous solution, followed by dilution of the samples with 2 ml of deionized water and subsequent scan in UV-visible (vis) spectra, between wave lengths of 200 to 700nm in a spectrophotometer, having a resolution of 1 nm. UV-vis spectra were recorded at intervals of 0 min, 15 min, 30 min, 45 min, 60 min and 24 h.

Physicochemical parameters

The physicochemical parameters were used to analyse the purity and quality of the extract. The extracts were evaluated for parameters like loss on drying, total ash and extractive values according to the methods recommended by the World Health Organization (WHO, 1998). Ash value is useful in determining authenticity and purity of drugs and these values are important for quantitative. Studied plant materials were evaluated by following physicochemical parameters.

Loss on Drying (LOD):

Loss on drying is the loss in weight in percent w/w resulting from loss of water and volatile matter of any kind that can be driven off under specific conditions. 5 gm of air-dried plant material (extract) was placed in a crucible of silica. The extract was spread in a thin uniform layer. The crucible was then placed in the oven at 105^0C for 4 hours and cooled in a desiccator to room temperature and weight of the cooled crucible plus powder was noted.

Extractive values:

Extractive values determine number of active constituents present in given plant material in given solvent. Extracts were prepared with various solvents by standard methods. Percentage of dry extract was calculated in terms of air-dried plant powder. Accurately weighed 5 gm of extract placed in the glass stoppered

conical flask and macerated with 25 ml of methanol (95%) for 6 hours with frequent shaking, mixture allowed to stand for 18 hours. After completion of 18 hours, filtered rapidly taking care not to lose any solvent. Transferred the filtrate in tared flat bottom porcelain dish. Filtrate was evaporated to dryness on water bath, dried at 105^0C for 6 hours cooled in desiccator for 30 min and weighed. Calculated the content of extractable matter in milligrams per gram of air-dried material.

$$\% \text{ of extractive value} = \left(\frac{\text{Extract obtained}}{\text{Weight of powdered sample}} \right) \times 100$$

Ash values:

Ash values are indicative to some extent of care taken in collection and preparation of extract of study and for foreign matter content of extract. The object of ashing is to remove all traces of organic material interfering in an analysis of inorganic elements.

Total Ash value:

This method is designed to measure total amount of material remaining after ignition. 5 gm of extract was taken in tarred platinum crucible. Extract was spread in fine even layer at bottom of the platinum crucible. This platinum crucible with drug material was kept in muffle furnace for ignition at high temperature. Temperature of furnace increased gradually up to 450^0C. The material was kept at this temperature for 6 hours till complete ignition of extract occurred, that is till complete white colored ash was obtained, intermittent weighing was also done. Crucible was then taken out from furnace, cooled and weighed. The total ash was calculated by subtracting the weight of crucible with ash of extract after ignition from weight of crucible with drug powder before ignition. Percentage of total ash was calculated with reference to air-dried drug.

$$\% \text{ of Total ash value} = \left(\frac{\text{Weight of total ash}}{\text{Weight of crude drug taken}} \right) \times 100$$

Preliminary phytochemical investigation

The extracts of phytochemical analysis for identification of bioactive chemical constituents were carried out by using standard methods of Sofowora, Trease & Evans, Kokate and Harbone (Sofowora, 1993; Trease and Evans, 1989; Kokate, 2005).

Test for Terpenoids:

To 1-2 ml of all the extracts 1% HCl was added and allowed to stand for 5-6 hours. Later, these extracts were treated with 1ml of Trim-Hill reagent (a solution of 10 ml of acetic acid, 1 ml of 0.2% copper sulphate in water and 0.5 ml of concentrated hydrochloric acid) and heated in a boiling water bath for 5-10 minutes. Formation of bluish green color indicates the presence of terpenoids.

Salkowski Test:

To the 1ml of plant extract, 2ml of chloroform was added. Then 3ml of conc. H_2SO_4 was added carefully to form a layer. A reddish-brown coloration of the interface indicates the presence of terpenoids.

Liebermann-Burchard test:

This test indicates the presence of sterols and terpenoids. Either the compound or the plant extract was dissolved in chloroform and added two to three drops of acetic anhydride and a drop of concentrated sulphuric acid through the test tube wall. A brown ring or pink color indicates the presence of terpenoids and play of colors (Pink-blue-green) shows the presence of sterols.

Test for Quinones:

The extracts were treated separately with Alc. KOH solution. Appearance of colors ranging from red to blue indicates the presence of Quinones.

Test for Coumarins:

1-2 ml of all the extracts were taken in separate tubes and covered with a piece of paper soaked in NaOH and heated. When these tubes yield a yellow fluorescence under UV light indicates the presence of coumarins.

Test for glycosides:

Molish's test:

2-3 drops of molish reagent was added to the extracts and mixed well. To this, few drops of conc. H2SO4 was added carefully. Formation of reddish-purple colored ring at the junction of two layers indicates the presence of glycosides.

Identification alkaloids by precipitation method:

Mayor's reagent: (Potassium mercuric iodide solution)

Dissolve 1.35gms of $HgCl_2$ in 60ml of distilled water. Dissolve 5gm of potassium iodide in 10ml of distilled water and make it up to 100ml with distilled water. Add 5ml of extract with mayor's reagent cream or pale-yellow precipitate is obtained.

Wagner's reagent: (Iodide and potassium iodide solution)

2mgs of KI and 1.25 gms of iodide are dissolved in 100ml of distilled water. When the alkaloid extract is treated with Wagner's reagent, brown or reddish-brown color precipitate obtained.

Dragendroff's reagent: (Potassium bismuth iodine solution)
Solution A: 1.7gms of basic bismuth nitrate and 20gms of tartaric acid are dissolved in 80ml of distilled water.
Solution B: 16gms of KI is dissolved in 100ml of distilled water.
Stock solution: 1:1 (v/v- mixture of A and B solution as prepared). This solution may be stored in the refrigerator. This reagent (5ml) can be diluted by adding 50ml of 20% tartaric acid. When all alkaloid extract is treated with Wagner's reagent-brown or reddish-brown color precipitate is obtained.

Hager's reagent: (Saturated solution of picric acid)
When the alkaloid extract is treated with Hager's reagent, yellow precipitate is obtained.

Identification of alkaloids by colour reagents:
Purine, Xanthin group of alkaloids (e.g. Caffeine) do not respond to the precipitation method tests. Colour reagent like potassium chloride with caffeine, mineral acids for colchicines, or p-distinct amino-benzaldehyde with indole alkaloids produce distinct colours.

Detection of Flavonoids
Ferric chloride Test:
Test solution when treated with few drops of $FeCl_3$ would result in the formation of blackish red color indicating the presence of flavonoids.

Alkaline Reagent Test:
Plant extracts were treated with few drops of NaOH solution. Formulation of intense yellow color, which becomes color less on addition of dilute acid indicates the presence of flavonoids.

Lead Acetate Test:
Plant extracts were treated with few drops of lead acetate solution. Formation of a yellow precipitate indicates the presence of flavonoids.

Shinoda's test:
The presence of flavonols, flavones, and their glycosides can be confirmed by testing the methanolic or alcoholic plant extract or compound treated with magnesium and hydrochloric acid. The reaction mixture develops pink, scarlet or crimson red color, indicates the presence of flavonoids.

Wilson's boric and citric acid test:
This test confirms the presence of 5-hydroxyl group in flavonoid nucleus. And yellow color appears with Wilson's boric and citric acid reagent indicates the presence of hydroxyl group in flavonoids.

Labet test:
Several flavones and isoflavones contain methylene dioxy group, are detected by the production of green color when treated with gallic acid and sulphuric acid.

Detection of Steroids
2ml of acetic anhydride was added to 0.5g ethanolic extract of each sample with 2ml of H_2SO_4. The color changes from violet to blue or green indicates the presence of steroids.

Detection of Tannins
To the 1ml of plant extract, few drops of 1% $FeCl_3$ solution were added. The appearance of blue, black, green or blue green precipitate indicates the presence of tannins.

Detection of Phenols
Ferric Chloride Test: To the 1ml of plant extracts, 3ml of distilled H_2O was added. To this few drops of neutral 5% $FeCl_3$ solution were added. A dark green color indicates the presence of phenols.

Detection of Saponins
Foam Test: About 2ml of distilled H_2O and 1ml of plant extract were mixed and shaken vigorously. A stable persistent froth indicates the presence of Saponins.
Froth Test: Plant extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam, which is stable for 15 minutes indicates the presence of Saponins.

Detection of Cardiac Glycosides
Kella – Killani Test:
Plant extract was dissolved in glacial acetic acid containing traces of $FeCl_3$. Then the tube was held at an angle of 45°, 1ml of conc. H_2SO_4 was added down the side purple ring at the interface indicates cardiac glycosides.

Fehling's test:
Fehling's solution:
Fehling's solution is divided into two parts A and B.

a. Fehling's solution A is copper sulphate solution: 10gms of solid $CuSO_45H_2O$ crystals are dissolved in 100ml of water.

b. Fehling's solution B is solution of sodium potassium tartrate (Rochelle salt) in water. 10 gms of Rochelle salt is dissolved in 100ml of water.

Mix solutions A and B and the deep blue solution is Fehling's solution.

The presence of aldehydes can be confirmed by testing the methanolic plant extract or compound treated with Fehling's solution the reaction mixture develops red precipitate indicating the presence of aldehyde. If there is no colour precipitation it indicates the presence of ketone.

Antimicrobial Studies

The antibacterial activity of the crude extracts and silver nanoparticles was tested against both Gram + ve and Gram – ve bacteria as well as Fungal organisms (Table 2).

Table 2: Microorganisms used in the study

S.No.	Microorganisms	Type of Strain	MTCC No.	Clinical Significance
1.	*Bacillus subtilis*	Gram-positive	MTCC 441	Food poisoning
2.	*Staphylococcus aureus*	Gram-positive	MTCC 737	Soft tissue infections
3.	*Enterococcus faecalis*	Gram-positive	MTCC 439	Urinary tract infections
4.	*Escherichia coli*	Gram-negative	MTCC 443	Diarrhoea
5.	*Proteus vulgaris*	Gram-negative	MTCC 426	Nosocomial infections
6.	*Pseudomonas aeruginosa*	Gram-negative	MTCC 1688	Urinary tract infections
7.	*Candida albicans*	Fungus	MTCC 227	Urinary yeast infection
8.	*Epidermophyton floccosum*	Fungus	MTCC 613	Skin and nail infections
9.	*Trichophyton mentagrophytes*	Dermatophyte	MTCC 7687	Skin Diseases

The antimicrobial activity was carried out by employing 24h cultures with given compounds by using Agar-Well diffusion method. The medium was sterilized by autoclaving at 120°C (15 lb/in2). About 20 ml of the Nutrient Agar Medium/ Potato Dextrose Agar seeded with the respective strains Bacteria/Fungal were transferred aseptically into each sterilized Petri plate. The Plates were left at room temperature for solidification. Each plate, a single well of 6mm diameter was made using a sterile borer. The test compounds were freshly reconstituted with suitable solvents (DMSO) and tested at various concentrations (500mg, 250mg, 100mg). The samples and the control along with standard (Ciprofloxacin/ Clotrimazole) were placed in 6-mm diameter well. In Antimicrobial assays fungal plates were incubated at 28± 2°C where as 37± 2°C for bacteria. Standard with 5μg/ml was used as a positive control for antibacterial activity. Activity diameter of the zone of inhibition was measured using Himedia antibiotic zone scale. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in triplicates. Observations and results were represented in Table.

Results

The yield of the crude extracts of various polarities of hexane, chloroform and methanol are presented in Table 3. Methanol was an extremely efficient extractant with an average of 23.61 ± 1.024 % extracted. The maximum yield was obtained for the crude extracts of *Cissus quadrangularis* (23.61 % mg/g dried plant material).

Table 3: The percentage yield of the crude extracts and various fractions (mg/g dried plant material)

S.No.	Plant Name	Hexane	Chloroform	Methanol
1.	*Cissus quadrangularis*	3.01	3.26	17.82
2.	*Gymnema sylvestre*	1.10	1.48	11.84

Physicochemical parameters

Physicochemical parameters were following those mentioned in Ayurvedic Pharmacopoeia of India. The percentage of loss of weight on drying, total ash, acid insoluble-ash, water-soluble ash and sulphated ash were obtained by employing standard methods of analysis. Plant products including phenolic, quinines, saponins, Flavonoids, coumarins, tannin, steroids and Alkaloids are found in the sample. Fats and oil were found in a small amount but anthraquinones and protein were detected. So, the presences of these phytochemical

constituents promote rapid healing and the formation of new tissues as discussed below.

Table 4: Percent of Loss on Drying of medicinal plant

S.No.	Plant Name	% of Loss on Drying
1.	*Cissus quadrangularis*	5.21
2.	*Gymnema sylvestre*	6.15

The loss of weight was calculated as the content in mg per g of air-dried material. The percent loss on drying was then calculated for each plant powder. The results obtained were reported in table 2. Percentage of loss on drying was found in *Cissus quadrangularis* (5.21) and *Gymnema sylvestre* (6.15).

Extractive Values

Plant extractives were analysed to estimate the percentage yield of individual extracts and found that, the yield was abundant in methanol rather than chloroform and hexane. Due to the high polarity of methanol most of the chemical constituents of extracts would be dissolved in it and thus percentage yield was increased tremendously than other solvents. Due to higher yield in methanol, methanolic extracts were selected for further studies.

Table 5: Extractive values of Medicinal Plants

S.No.	Plant Name	% of Extractive Value
1.	*Cissus quadrangularis*	22.42
2.	*Gymnema sylvestre*	20.25

The percentage yields of extracts in methanol are high in *Cissus quadrangularis* (22.42) *Gymnema sylvestre* (20.25).

Ash values

In the evaluation of crude extracts, total ash value is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica. The amount and composition of ash remaining after combustion of plant material varies considerably according to the part of the plant, age, treatment etc. The constituents of ash also vary with time and from organ to organ. Ash usually represents the inorganic part of the plant. The total ash method is designed to measure the total amount of material remaining after ignition.

Table 6: Physicochemical analysis of plants ash

S.No.	Plant Name	% of Total Ash Value
1.	*Cissus quadrangularis*	2.48
2.	*Gymnema sylvestre*	8.42

At present investigation we found that the percentage of total ash content was highest in *Gymnema sylvestre* with value 8.42.

Preliminary phytochemical investigation

Phytochemical screening:

After performing the analysis of bioactive compounds of these two medicinal plant extracts, results obtained are as shown in Table 7.

Table 7: Qualitative phytochemical analysis of methanol extract

Plant Name	Terpenoid	Flavonoid	Alkaloid	Glycoside	Phenol	Cardiac glycoside	Tannins	Coumarins	Quinone	Saponin
C.quadrangularis	+	+	+	+	+	+	-	+	+	+
G.sylvestre	+	_	+	+	+	+	+	+	+	+

(+), presence. (-), Absence

Antimicrobial activity

In the present study, hexane, chloroform and methanol extracts of two medicinal plants were studied against nine clinically important, drug resistant strains. Both plants were studied against Gram + ve and Gram – ve bacteria as well as fungal strains. Standard antibiotics Ciprofloxacin for bacteria and Fluconazole for fungi were used as positive controls. Only methanol extracts exhibited moderate to high inhibition activity against all the microflora screened, Hexane and Chloroform extracts showed only mild to no activity, hence only methanol extracts reports were analyzed. The antimicrobial activities of different extracts against clinically isolated microbial strains were represented in Table 8. In the present study details of the plants along with their family name, common name, part used have been listed in Table - 1. *Cissus quadrangularis,* showed a significant activity against most of the tested organisms at less than 100mg/ml concentration. Among the bacterial strains studied *Staphylococcus aureus* was resistant than other bacteria and *Klebsiella pneumoniae* was susceptible to almost all the medicinal plants tested. Whereas the fungal strains were inhibited by most of the plants tested. Among the fungal strains studied *Epidermophyton floccosum* was susceptible.

Table 8: Activity of different concentrations of plant extracts on different microflora

Plant Name/Organism		B.S	S.A	E.F	E.C	P.A	P.V	T.M	E.F	C.A
C. quadrangularis	100 mg/ml	18 ± 0.19	19 ± 0.08	10 ± 0.10	21 ± 0.10	17± 0.12	12 ± 0.21	15± 0.53	18 ± 0.41	14 ± 0.11
	MIC mg/ml	25 ± 0.50	25 ± 1.05	75 ± 0.44	25 ± 0.50	25 ± 0.50	75 ± 0.50	25 ± 0.50	25 ± 0.50	25 ± 0.50
G. sylvestre	100 mg/ml	8 ± 0.32	12 ± 0.31	09 ± 0.09	18 ± 0.09	12± 0.28	21 ± 0.13	13± 0.69	11 ± 0.23	13 ± 0.42
	MIC mg/ml	100 ± 1.5	75 ± 0.55	100 ± 1.01	50 ± 1.5	50 ± 1.5	25 ± 1.5	50 ± 1.50	75 ± 1.5	50 ± 1.50

*zone of inhibition in mm; 6mm borer size; MIC mg/ml

Silver nanoparticle (AgNP) synthesis

After reduction for 48 hours, plant extract solution color changed from yellow to brown. Formation of brown is due to the surface plasmon resonance property of silver nanoparticles.

Antimicrobial activity of Silver Nanoparticles

The estimated bare Ag and synthesized Ag NPs average crystallite size are given in the Table. With the help of well diffusion method, the antibacterial activity of Ag NPs was studied against various pathogenic microorganisms. Zone of inhibition around the disc for the synthesized AgNPs and obtained values are presented in the Table. It was observed that the synthesized Ag NPs showed better antibacterial activity against all microbes and its value. However, *Staphylococcus aureus* has higher inhibition of zone. This is because of accumulation of the large amount of Ag+ ions on negatively charged cell membrane leads to lose the permeability control and causes cell death. Moreover, AgNPs are reacting with sulfur contain proteins in the core of the cell as well as phosphorous contain compounds such as DNA spirit affect the respiratory chain and cell separation in bacteria at last causing to the death of the cell.

Table 9: Antimicrobial properties of Silver Nanoparticles

Microorganism	Cq-Nps	Antibiotic (Ciprofloxacin) 10 µg/ml	Control (DMSO)
Bacillus subtilis	19± 0.21	34 ± 0.17	0
Escherichia coli	17± 0.56	36 ± 0. 14	0
Staphylococcus aureus	27± 0.03	32 ± 0.40	0
Pseudomonas aeruginosa	19± 0.11	28± 0.09	0
Proteus vulgaris	25± 0.17	37 ± 0.15	0
Candida albicans	19± 0.45	21 ± 0.11	0
Epidermophyton floccosum	17± 0.27	31 ± 0.21	0
Trichophyton mentagrophytes	16± 0.33	33± 0.51	0

Discussion

The number of multi drug resistant micro flora and the appearance of the strains with reduced susceptibility to antibiotics are constantly increasing. This situation provided the drive to the search for new antimicrobial substances from various sources like medicinal plants. It is important to investigate scientifically these plants which have been used in traditional medicines as potential source of novel antimicrobial compounds. The current investigation revealed the presence of several physico, phytochemicals, good antibacterial, antioxidant and anticancer activity. In order to assess the quality and purity of any sample, standardization process is essential. Standardization process includes physicochemical analysis is necessary for identification, authentication, detection of adulteration and compilation of quality control of crude extracts. Extractive values are primarily useful for the determination of exhausted and adulterated drugs. It is also useful to evaluate the chemical constituents present in crude drug and helps in determination of specific constituent's soluble solvents. In the present study, plant extractives were analyzed to estimate the percentage yield of individual extracts and found that, the yield was abundant in methanol rather than chloroform and hexane. Due to the high polarity of methanol most of the chemical constituents of extracts would be dissolved in it and thus percentage yield was increased tremendously than other solvents.

In our study antimicrobial activity could be attributed to the presence of these antimicrobial compounds, which is also proved by other studies (Ahmed *et al.,* 1993; Batista *et al.,* 1994; Tsuchiya *et al.,* 1996; Barre *et al.,* 1997; Amaral *et al.,* 1998). Activities are may be due to the presence of different phytochemical classes. These phytochemicals are responsible for the stabilization of silver nanoparticles. Several

earlier research reports correlated the plant phytochemicals with their bioactive attributes and reported the antibacterial and free radical scavenging activities of the phytochemical constituents. The quantity of phytochemical components may vary with the plant part, leaf and stem (Patra *et al.,* 2011). Phenolic constituents generally observed in plants are stated to have potential antioxidant and antimicrobial activities. Different phenolic compounds such as tannins existing in plant cells are effective inhibitors of hydrolytic enzymes used by plant pathogens. Several studies have been concentrated on the biological activities of phenolic compounds, which are acting as powerful antioxidants and free-radical scavengers (Devi *et al.,* 2011). Phenols are the precise crucial plant components with diverse biological roles including antioxidant activity and their radical scavenging ability is reasoned to their OH groups (Vinayagam and Sudha, 2011).

Conclusion

The present investigation demonstrated that the studied plant species can produce silver nanoparticles extracellularly and silver nanoparticles are quite stable in solution. The biosynthesized silver nanoparticles showed considerable antimicrobial properties. The present study data indicated that green synthesized nanoparticles could be considered alternative medicine for bacterial and fungal treatments. Our investigations in the future will focus mainly on answering specific questions and the knowledge gained will enhance our understanding of the mechanism of the nanoparticles and will integrate the picture for biological and biotechnological applications.

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