Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(1): 2453-2457
Received: 09-11-2017
Accepted: 10-12-2017

Alen Godfrey R Jose
Department of Zoology, Presidency College, (Autonomous), Chennai, Tamil Nadu, India

Abirami T
Department of Zoology, Presidency College, (Autonomous), Chennai, Tamil Nadu, India

Kavitha V
Department of Zoology, Presidency College, (Autonomous), Chennai, Tamil Nadu, India

Sellakilli R
Department of Zoology, Presidency College, (Autonomous), Chennai, Tamil Nadu, India

Karthikeyan J
Department of Zoology, Presidency College, (Autonomous), Chennai, Tamil Nadu, India

Correspondence
Alen Godfrey R Jose
Department of Zoology, Presidency College, (Autonomous), Chennai, Tamil Nadu, India

Green synthesis of silver nanoparticles using *Asystasia gangetica* leaf extract and its antibacterial activity against gram-positive and gram-negative bacteria

Alen Godfrey R Jose, Abirami T, Kavitha V, Sellakilli R and Karthikeyan J

Abstract
Plant mediated synthesis of silver nanoparticles was important due to their medicinal applications. In the present study, silver nanoparticles were synthesized using a aqueous leaf extract of *Asystasia gangetica* and the production of AgNP's was confirmed by the absorption spectrum of λ max at 430nm. The particles were characterized by scanning electron microscopy. The FTIR studies show the presence of various functional groups such as NH_2, OH, C=O groups, which are responsible for the reduction process. Disc diffusion method was followed to observe the bactericidal activity. The methanolic leaf extract of *Asystasia gangetica* shows moderate antibacterial activity against gram- negative bacteria, while the green synthesized silver nanoparticles shows a potential bactericidal activity against both gram-positive and gram-negative bacteria studied in the present investigation. The standard antibiotic amoxycillin was used as positive control and 1% DMSO were used as negative control. The bioactive compounds such as tannins and phenolic compounds present in the methanol extract and the nanoparticles capped with the bioactive compounds of plant material are responsible for the bactericidal activity. Further studies on characterization of specific compound responsible for the killing of bacteria, resulted in the invention of new compound to control the drug resistance organisms.

Keywords: silver nanoparticles, *Asystasia gangetica*, antibacterial, SEM, FTIR

1. Introduction
Infection with gram negative and gram positive bacteria in surgical wound is an increasing trend in recent times. Development of multidrug resistant microorganism is a major concern around the world, continuous administration and concentration of the drug plays the major role in the emergence of drug resistant organism [1]. The administration of synthetic antibiotics resulted in hypersensitivity [2] and oxidative damages [3]. To overcome the drug resistant organism it is required to find the bactericidal agent from natural resource such as medicinal plants.

Nanoparticles exhibit potential antibacterial activity against infectious organisms. The synthetic nanoparticles were consists of toxic chemicals which adversely affect the human health and environment, Hence it is necessary to synthesis the nanoparticles with environmental friendly. Medicinal plants were used to synthesis silver nanoparticles [4, 5].

Plant-mediated synthesis of silver nanoparticles gains interest due to their medicinal application and eco friendly nature. *Phyllanthus amarus* mediated synthesized, silver nanoparticles exhibit anti-bacterial activity [6] *Asystasia gangetica* commonly known as Chinese violet is belongs to the family Acanthaceae is a rapidly growing herb [7]. *A.gangetica* contain alkaloid, tannin, saponin, flavonoids and terpenoids [8]. Presence of minerals were also reported in the leaf extract [9]. Crude extracts *Asystasia gangetica* was used to treat rheumatism [10]. Root paste acts against skin allergies [11]. Leaf extracts possesses anthelmintic properties [12]. The objective of the present study was to study the antibacterial efficacy of the crude leaf extract of *Asystasia gangetica* and the green synthesized silver nanoparticles against gram-negative and gram-positive bacteria with phytochemical analysis.

2. Materials and Methods
Chemicals
All the reagents, chemicals and media used in the present investigation were procured from Himedia Laboratory (Mumbai). Solvents were procured from Merck India. The bacterial strains were collected from King Institute of Preventive Medicine, Guindy, India.

Plant extract

The collected plant leaves were separated, cleaned and washed thoroughly to remove the debris and allowed to shade dried for two weeks. The dried leaf was grinded into coarse powder using mixer blender. The grinded powder was further macerated using different solvent at 37˚C for 72 hrs. The extracts were filtrating primarily with cloth followed by whatman filter paper and the extracts were concentrated using rotary vacuum evaporator. The concentrated extracts were refrigerated until further use.

Synthesis of silver nanoparticles

Silver nanoparticles were synthesized following the previous method of [13] Karthikeyan *et al*. 5grams of fresh leaves were boiled at 80˚C for 15 minutes and 50 ml of the boiled extract suspended with 950 ml of 1mM silver nitrate solution and mixture was kept in dark for further reaction. The color change was observed periodically to confirm the reduction of silver ions. The brown color solution was analyzed by spectrophotometer absorption for the confirmation of formation of silver nanoparticles. The synthesized silver nanoparticles were separated by centrifugation by which the unreacted phytochemicals were removed. The pellets were resuspended with deionized water and further centrifuged at 15000 rpm for 30 minutes. The suspension was dried and stored at 4˚C for further characterization.

Characterization of silver nanoparticles

The process of reduction of silver ions was monitored by observing the UV-Vis spectroscopy. The spectrum observed around 400-440nm indicates the formation of silver nanoparticles. The reduction process and formation of NPs were analyzed by measuring the spectrum from 300nm to 900nm using Thermo UV-10 - UV Vis spectrophotometer. Scanning electron microscopic studies was carried out to observe the particle size.

FTIR analysis

Fourier Transform Infrared Spectroscopy (FTIR) was performed to analyze the presence of various functional groups. The methanolic leaf extract and the green synthesized silver nanoparticles were analyzed with Perkin-Elmer-FTIR spectrophotometer.

Antibacterial assay

Antibacterial efficacy of the plant extract and green synthesized silver nanoparticles were tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Escherichia coli.* Agar disc diffusion method was followed to observe the bactericidal activity. The standard antibiotic amoxicillin was used as positive control, and 1% DMSO was used as negative control. The zone of inhibition in mm was recorded as antibacterial activity.

3. Result

Qualitative phytochemical analysis of different extracts of *Asystasia gangetica* was presented in Table 1 and it reveals the presence of steroids, tannin, alkaloids, flavonoids, cardiac glycosides in methanol leaf extract of the plant. Aqueous leaf extract mediated synthesized AgNPs were primarily confirmed by the color change from light yellow to brownish yellow color. The color change is due to the surface plasmon resonance of the AgNPs. The absorbance peak was observed at 430 nm suggests the bioreduction of silver by the plant extract into nanoparticles (Fig 1). The synthesized silver nanoparticles were characterized by scanning electron microscopy and reveal the presence of spherical nanoparticles with the size range of 40 to 60nm(Fig 2).

FTIR analysis reveals the presence of various functional group in the AgNP's (Fig 3). The peak at 718.5 cm^{-1} and 1395.0 represents the presence of alkyl halides. Aromatic amine and primary amine were evident with the peak at 1458 cm^{-1}, 1611 cm^{-1} and 3319 cm^{-1}. Alcohol and phenols represented by OH group was observed at 3168.4 cm^{-1} and 3208.6 cm^{-1}. In addition the presence of nitride represents C-N bond, Carboxyl group represents C=O bond at 2684.3 cm^{-1} were also observed in the present study. The methanol leaf extract of *Asystasia gangetica* (Fig 4) reveals the presence of various functional groups at 3334.4 cm^{-1}, 2133.7 cm^{-1}, 1718.6 cm^{-1}, 1609.5 cm^{-1}, 1490.7 cm^{-1}, 1036. 1 cm^{-1}, 932.8 cm^{-1} and 896.9 cm^{-1} representing primary amines, alkyne, esters, amines, alkane, alkyl halide, alkene and alkyl halide respectively.

The antibacterial efficacy of the methanolic leaf extract and green synthesized AgNP's were evaluated against, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Escherichia coli* with different concentrations of extracts. The zone of inhibition was measured to assess the bacterial growth and the results were presented in Table – 2. A dose dependent increase in the antibacterial activity was observed with reference to the treatment with crude extract as well as silver nano particles. Gram negative bacteria viz. *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Escherichia coli* were more sensitive when compared with standard antibiotics. However, the methanol extract does not show any impact on *Staphylococcus aureus* a gram positive bacteria. The zone of inhibition varies from 8mm to 11mm with reference to all the tested organisms. The silver nanoparticles shows a increasing trend with reference dosage in the antibacterial efficacy against both gram- positive, gram-negative bacteria and the zone of inhibition ranges from 8mm to 14mm. *Proteus vulgaris* and *Klebsiella pneumoniae* were more sensitive when compared with other organisms tested in the present study.

Table 1: Phytochemical analysis of different leaf extract of *Asystasia gangetica*

S. No	Bioactive compound	Aqueous extract	Methanol extract
1	Steroids	++	+++
2	Terpenoids	-	+++
3	Tannins	++	++
4	Saponins	-	-
5	Coumarins	-	++
6	Phenols	-	-
7	Alkaloids	++	++
8	Flavonoids	++	++
9	Cardiac glycosides	+	+++
10	Carbohydrates	+	+++
11	Proteins	-	-

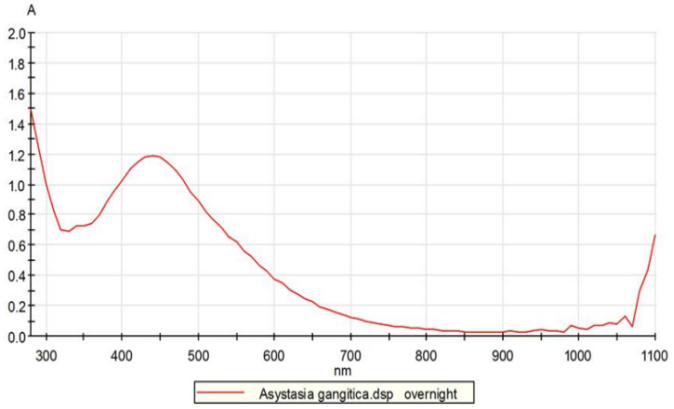

Fig 1: UV-Vis spectrum of silver nanoparticles (*Asystasia gangetica*)

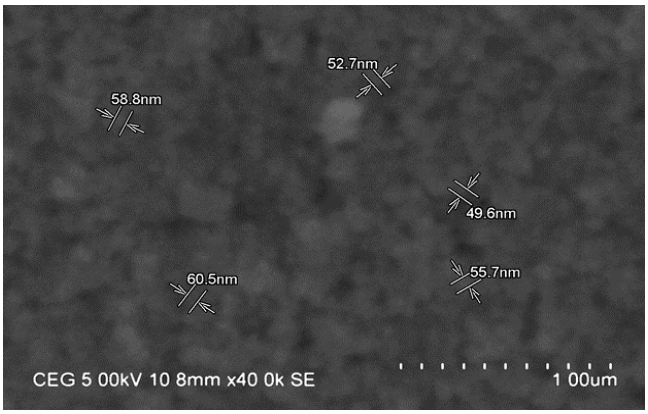

Fig 2: Scanning Electron Microscopic Images of Synthesized Nanoparticles using Aqueous Leaf Extract of *Asystasia gangetica*

Table 2: Antibacterial activity (Zone of inhibition) of methanol leaf extract of *Asystasia gangetica*

Organism	Zone of inhibition (mm)				
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	Amoxicillin
Staphylococcus aureus	9	9	9	10	10
Pseudomonas aeruginosa	10	8	9	10	6
Proteus vulgaris	9	9	10	10	11
Klebsiella pneumoniae	8	8	10	11	6
Escherichia coli	9	10	10	11	7

Table 3: Antibacterial activity (Zone of inhibition) of Green synthesized Silver nanoparticles

Organism	Zone of inhibition (mm)					
	2 µg/ml	4 µg/ml	6 µg/ml	8 µg/ml	10 µg/ml	Amoxicillin
Staphylococcus aureus	9	10	10	10	10	10
Pseudomonas aeruginosa	8	8	8	8	9	6
Proteus vulgaris	10	10	11	13	14	6
Klebsiella pneumoniae	8	8	9	9	11	6
Escherichia coli	9	9	9	9	10	7

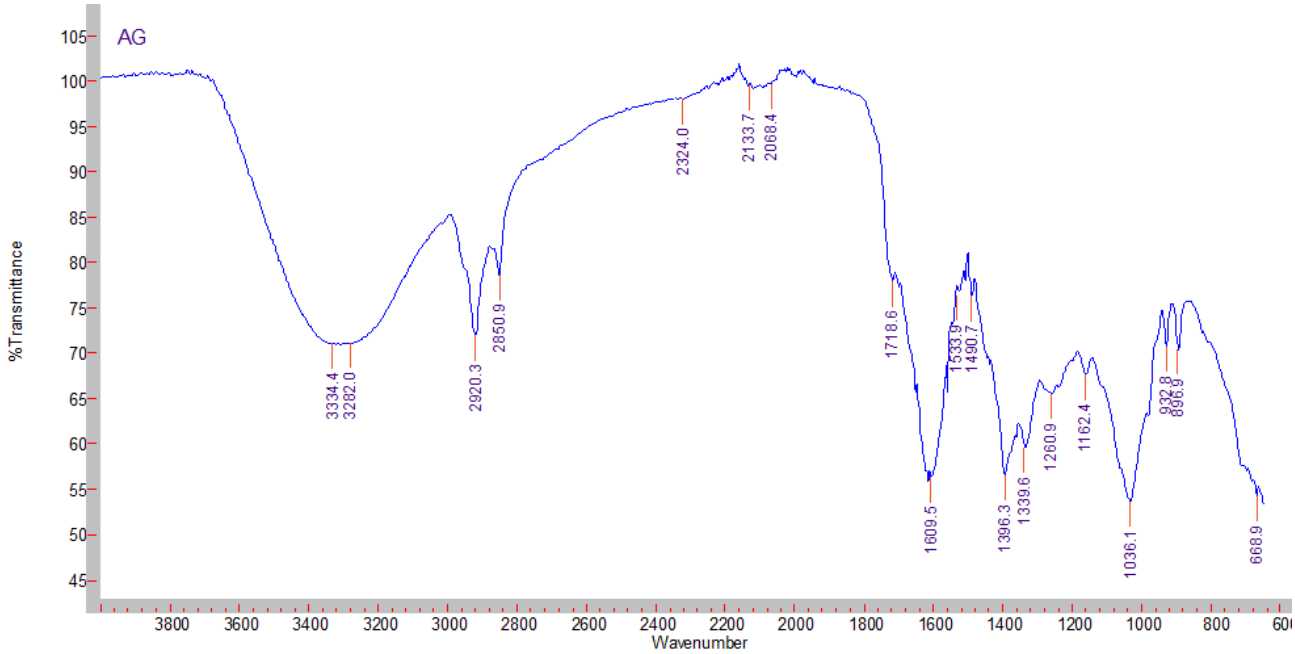

Fig 3: FTIR analysis of Methanol crude leaf extact of *Asystasia gangetica*

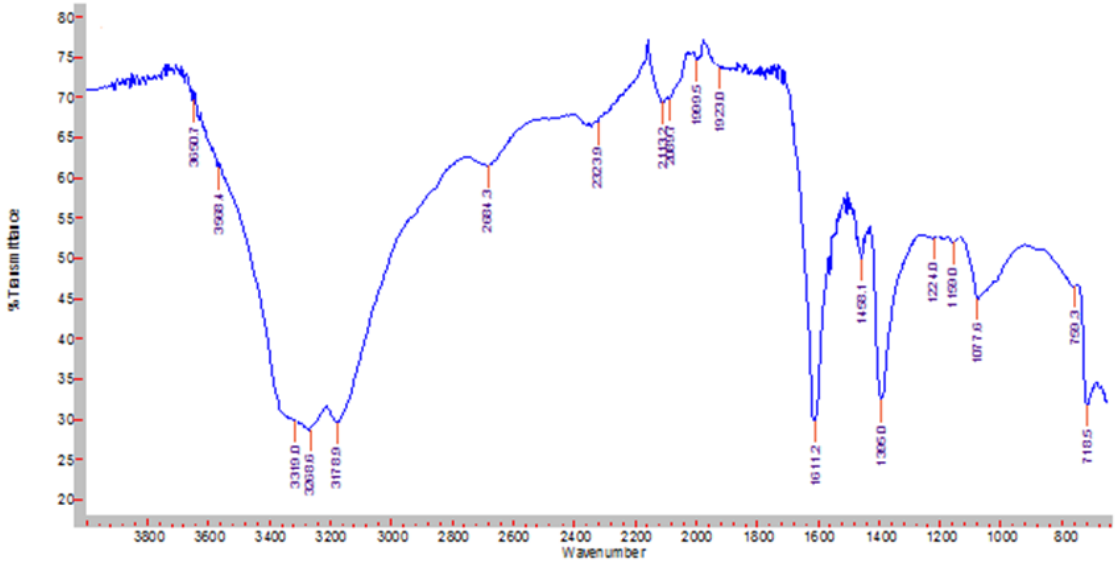

Fig 4: FTIR analysis of Green synthesized Silver Nanoparticles of *Asystasia gangetica*

4. Discussion

World Health Organization estimated that around 80% of the world population using plant extracts as folk medicine. The antibacterial activity of the plant extract various depends on the presence (or) absence of bioactive compounds. The methanolic leaf extract of *Asystasia gangetica* shows potential antibacterial activity against gram negative bacteria. However, the extract is inactive against gram positive bacteria. The study also reveals the presence of various phyto-constituents such as terpenoids, tannins, coumarins, alkaloids, flavonoids, cardiac glycosides, in the methanol leaf extract. The presence of tannins and phenolic compounds have been shown to possess antibacterial activity against microorganisms [14]. The present results are in agreement with the above finding has the methanol extract possesses high amount of tannins and phenolic compounds. The plant mediated silver nanoparticles were more effective against the tested organisms when compared with methanol extract. AgNPs were capable of adhere and interact with cell membrane of gram-negative bacteria [15] the particles alters the membrane permeability, leading to irreversible damages to bacterial cells [16]. AgNPs cause irreversible damages in the cell membrane of *Staphylococcus aureus* gram positive bacteria [17]. Silver has been used against disease causing organisms as a antiseptic and antibacterial agent, against gram-positive and gram negative bacteria [18]. The silver nanoparticles capped with plant material were more potential bactericidal agent due to its larger surface area to volume ratio. The present study reveals that the silver nanoparticles potentially damages the membrane architecture of the bacteria by adhering to it, suggesting the role of plant mediated silver nanoparticles to be a better alternative to synthetic antibiotics as bactericidal agent.

5. Conclusion

Antibacterial efficacy of methanol leaf extract of *Asystasia gangetica* and its mediated synthesized silver nanoparticles were studies in the present investigations. The methanolic extract shows moderate activity against gram-negative organisms. While the AgNP's show a potential antibacterial efficacy against gram-positive and gram-negative bacteria. The present investigation supports the applications of medicinal plant extracts, and the silver nanoparticles against the tested organisms. However, further studies are required to evaluate the specific bioactive compounds responsible for the bactericidal activity and the compounds capped with the silver nanoparticles for its stability and potential bactericidal efficacy.

6. Acknowledgement

The author gratefully acknowledges the University Grants Commission, New Delhi, India, for funding the Major Research Project (41-125/2012(SR)) to carry out this research.

7. References

1. Rodriguez Barreiro R, Abendroth C, Vilanova C, Moya A, Porcar M. Towards a Microbial Thermoelectric Cell. PLoS, ONE. 2013; 8(2):1-11.
2. Legendre DP, Muzny CA, Marshall GD, Swiatlo E. Antibiotic hypersensitivity reactions and approaches to desensitization. Clin Infect Dis. 2014; 58(8):1140-8.
3. Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins JJ. A common mechanism of cellular death induced by bactericidal antibiotics. Cell. 2007; 130(5):797-810.
4. Beg M, Maji A, Mandal AK, Dal S, Aktara MN, Jha PK, Hossain M. Green synthesis of silver nanoparticles using Pongamia pinnata seed: characterization, antibacterial and spectroscopic investigation of interaction with human serum albumin. J mol Recognition. 2017; 30(1):1-8.
5. Abirami T, Jose AGR, Bavani Govindarajalu, Karthikeyan J. Ecotoxicology of green synthesized silver nanoparticles on fresh water fish *Mystus gulio*. Int J Pharm Pharm Sci. 2017; 9(11):192-198.
6. Chitra AM, Ramesh S. Antibacterial activity of silver nanoparticles synthesized using *Phyllanthus amarus* and *Andrographis paniculata* ethanolic extracts. Ind. J Vet. Anim. Sci Res. 2015; 44(1):12-19.
7. Janakiraman N, Jansi JJ, Johnson M, Jeeva S, Malar RJJT. Phytochemical analysis of Asystasia gangitica (L) T. Anderson J Harm Res Pharm. 2012: 1(1):19-32
8. Hamid AA, Aiyelaagbe OO, Ahmed RN, Usman LA, Adebayo SA. Preliminary phytochemistry, antibacterial and antifungal properties of extracts of *Asystasia gangetica* Linn T. Anderson grown in Nigeria. Adv Appl Sci Res. 2011; 2:219-226.
9. Odhava B, Beekrumb S, Akulaa U, Baijnathc H.

Preliminary assessment of nutritional leafy vegetables in KwaZulu-Natal, South Africa. J Food Comp Anal. 2007; 20:430-435.
10. Shanmugam S, Rajendran K, Suresh K. Traditional uses of medicinal plants among the rural people in Sivagangai district of Tamil Nadu, Southern India. Asian Pacific J Tropical Biomedicine. 2012; S429-S434.
11. Senthilkumar M, Gurumurthi P, Janardhanan K. Some medicinal plants used by Irular, the tribal people of Marudhamalai hills, Coimbatore, Tamil Nadu. Natural Product Radiance. 2006; 382-388.
12. Jiju V, Megha Gorantla, Chamundeswari D. Evaluation of anthelmintic activity of methanolic extract of *Asystasia gangeticum*. Int J Pharm Life Sci. 2013; 4(6):2727-30.
13. Karthikeyan J, Mullai Nila K, Thooyavan G, Vimalkumar E. Larvicidal and antibacterial efficacy of green synthesized silver nanoparticles using *Melia dubia*. Int J Pharm Sci. 2014; 6(7):395-399.
14. Bhalodia NR, Shukla VJ. Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* l.: An ethnomedicinal plant. J Adv Pharm Technol Res. 2011; 2(2):104-9.
15. Franci G, Falanga A, Galdiero S, Palomba L, Rai M, Morelli G, *et al*. Silver Nanoparticles as potential antibacterial agents. Molecules. 2015; 20(5):8856-74.
16. Tamayo LA, Zapata PA, Vejar ND, Azocar MI, Gulppi MA, Zhou X, *et al*. Release of silver and copper nanoparticles from polyethylene nanocomposites and their penetration into Listeria monocytogenes. Mater Sci Eng C Mater Biol Appl. 2014; 40:24-31.
17. Wei D, Sun W, Qian W, Ye Y, Ma X. The synthesis of chitosan-based silver nanoparticles and their antibacterial activity. Carbohydr Res. 2009; 344(17):2375-82.
18. Lazar V, Chifiriuc MC. Medical significance and new therapeutical strategies for biofilm associated infections. Rom Arch Microbiol Immunol. 2010; 69(3):125-38.