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Research Paper

Comparison of Antibacterial Activity of Aqueous Extracts *Trillium govanianum* Rhizome and Biogenic Silver Nanoparticles

Shabeena Yaseen*[1], Zahoor A. Kaloo[2] and Iram Ashraf Qazi[3]

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ABSTRACT

The importance of nanotechnology has been increasingly contemplated in the field of medicine. For instance, the limitations of exploiting the antibacterial effect of silver ions, due to its toxicity, can be overcome with the use of silver nanoparticles (AgNPs). This is because the lower surface area allows higher efficiency of AgNPs at a much lower concentration than required to cause toxicity. Moreover, with the green approach for synthesis of nanoparticles, it is now possible to prevent the harmful effects of chemically synthesized AgNPs. In the current study, the biogenic AgNPs were synthesized using aqueous extracts of *Trillium govanianum* rhizomes and its antibacterial activity was observed. The formation of AgNPs was observed when 1 mM $AgNO_3$ solution was mixed with aqueous rhizome extract in the ratio of 1:9. On comparison, it was found that the AgNPs showed higher zones of inhibition than the aqueous extracts of *Trillium govanianum* rhizomes. Hence, it can be concluded that *Trillium govanianum* contains active bio-reducing agents for green, cost effective and efficient production of AgNPs.

***Key words*:** Nanotechnology, Silver nanoparticles, *Trillium govanianum*, Rhizomes, Biogenic

Nanotechnology has emerged as a promising field in pharmaceutical and medical sectors. It deals with the production and synthesis of particles of the size range between 10–100nm. These particles have unique and more stable properties as compared to original metal compounds depending on their size. It is observed that the stability of nanoparticles (AgNPs) increases with a decrease in size [1]. Although various techniques are available for the synthesis of AgNPs, most of them include the use of concentrated chemicals and harsh reaction conditions. Thus, these protocols have limited the applications of AgNPs to a great extent due to the possibility of their bioaccumulation and toxicity. To overcome the limitations of this technology, 'green synthesis' approaches are gaining much attention in current research and development programmes [2]. Compared to chemical methods, they are eco-friendly, cost-effective, and energy-efficient. Green synthesis of metallic AgNPs can be accomplished using various biological materials (e.g., bacteria, fungi, algae and plants). Among these methods, the use of plant extracts is a relatively simple process to produce AgNPs on a large scale [3].

During green synthesis of AgNPs, the phytochemicals like ketones, aldehydes, flavones, amides, terpenoids, carboxylic acids, phenols, ascorbic acid and others act as reducing agents to convert metal salts into metal nanoparticles [4]. Like any other biological process, the green synthesis of nanoparticles is also dependent on factors such as solvent, temperature, pressure, and pH conditions [5]. Collectively, the end products obtained on reduction of metallic particles by biological materials are known as biogenic nanoparticles. These agents have shown promising applications in the field of biomedical diagnostics, molecular sensing, optical imaging, and labelling of biological systems [6]. They also act as antimicrobial agents and catalytic enzymes [7, 8]. In addition, they show promising role in the food packaging system by preventing the growth of fouling contaminants and improving the shelf life of food [9]. Studies have also shown cytoprotective activity of biogenic AgNPs against HIV-1 infected cells [10].

AgNPs have strong antimicrobial activities and can attack a broad range of microorganisms. The mechanism of action of AgNPs includes disruption of proteins with thiol groups, cell walls, and cell membranes. Silver ions are responsible for uncoupling the respiratory chain from the process of oxidative phosphorylation. This results in collapse of the proton motive force across the bacterial cytoplasmic membrane and death of pathogenic micro-organisms [11]. The use of silver, as potent antibacterial agent, is severely limited due to the toxicity exhibited by silver ions to humans. However, with the help of nanotechnology, the efficacy of silver ions can be exploited to its full potential. Minimizing the size of particles and thus increasing the surface area to volume ratios significantly enhances the efficacy of bactericidal action of AgNPs at a very low concentration [12]. In the present study, AgNPs were synthesized using aqueous extracts of *Trillium govanianum* rhizomes and the antibacterial activity of the same was compared to various solvent extracts of *T. govanianum* rhizomes.

***Shabeena Yaseen**
shahshabeena@rediffmail.com

[1-3]Department of Botany, University of Kashmir, Hazratbal - 190 006, Srinagar, Jammu and Kashmir

MATERIALS AND METHODS

Preparation of aqueous extract of T. govanianum rhizomes

The dried rhizome powder (30 g) was mixed with 90 mL of water and heated at 60°C for 20 mins to extract the biochemical constituents of *T. govanianum*. After extraction, it was filtered through Whatman filter paper no.1 and the filtrate was used as an aqueous extract of *T. govanianum* rhizomes.

Synthesis of silver nanoparticles using T. govanianum extracts

The above filtrate was also used as an agent for bio-reduction of silver nitrate to prepare AgNPs. The silver nitrate solution (0.001M) was prepared using deionised water. For preparation of the solution, 0.1699 g of analytical grade $AgNO_3$ (99.5% purity) was weighed using an analytical weighing balance and then transferred into a 1000 mL volumetric flask containing 400 mL of distilled water. The mixture was stirred continuously to ensure that all the solid particles of $AgNO_3$ are dissolved. After complete dissolution of salts, the volume was made up to 1000 mL with distilled water. To determine the optimal ratio of reactants (by volume), different ratios were used. The ratio of the reactants i.e., 1 mM $AgNO_3$ to plant extract used in our study were 1 : 1, 1 : 3, 1 : 6, 1 : 9 and 1 : 12. The production of AgNPs was confirmed by observing the color change from light-yellowish to brown after 35 minutes. The conical flask containing the sample was then removed from the water-bath and allowed to cool to room temperature (25°C) [13].

Characterization of silver nanoparticles

The formation of the reduced AgNPs in colloidal solution was monitored by using a UV–Vis spectrophotometer (Lambda 25, PerkinElmer, precisely, UK). The absorption spectra of the supernatants were taken between 300 and 700 nm, using a UV–Vis spectrophotometer. The deionized water was used as the blank.

Test isolates used in the study

The antibacterial activity of *T. govanianum* extracts were tested against four bacterial strains i.e., *Staphylococcus aureus* 6538p, *Escherichia coli*, *Bacillus subtilis and Salmonella typhi*.

Antimicrobial activity of silver nanoparticles

The antibacterial activity of AgNPs prepared using *T. govanianum* extracts was evaluated based on the agar cup method [14]. For carrying out the agar cup method, the Mueller Hinton agar petri-plates containing 15mL volumes of the media were used. The media was autoclaved, cooled to ~40°C and then seeded with 24h old cultures of test isolates, before preparation of above plates. After solidification of the media, wells were made in the agar plates using a metal borer and different volumes of extract (i.e., 20µL -100µL) of different solvent extracts were loaded in these wells. The plates with bacterial isolates were incubated at 37°C and those with fungal isolates were incubated at room temperature (~25°C) for 24h. All experiments were carried out in triplicates and results were represented as mean.

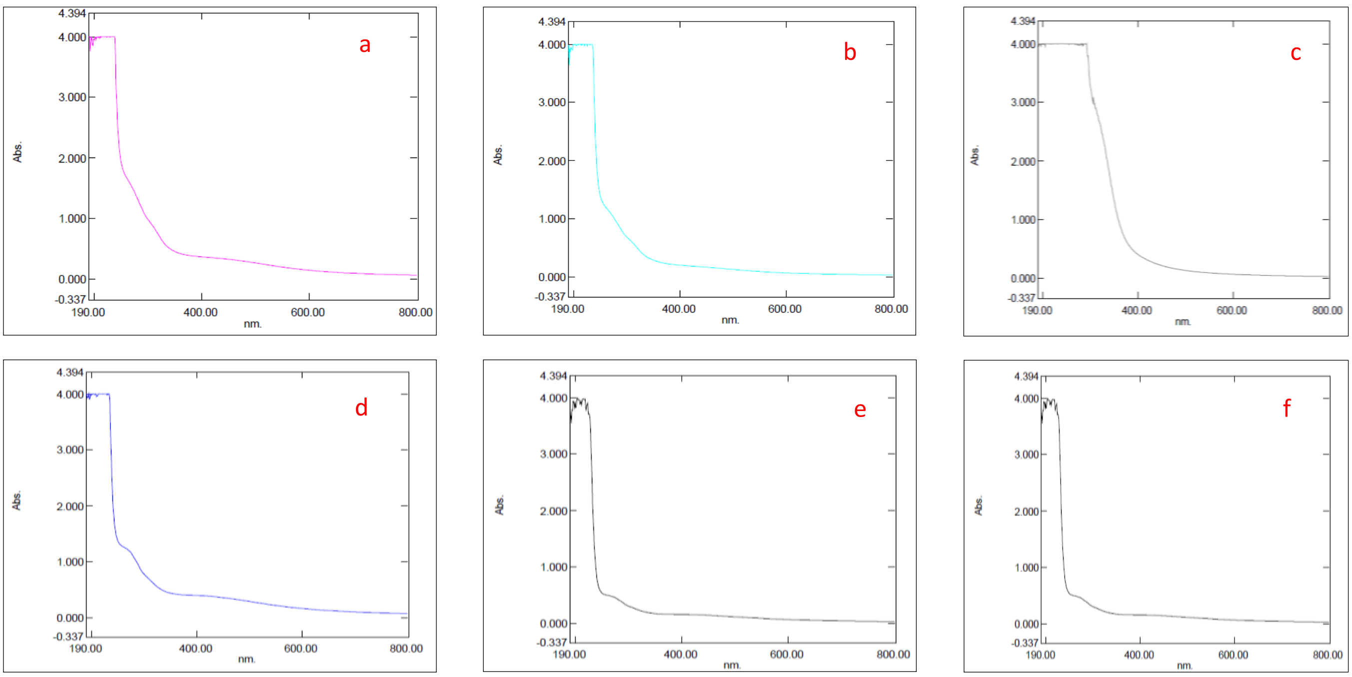
Data analysis

Data were analysed statistically (SPSS version 19) using one way analysis of variance (ANOVA). The significance of differences among means were calculated using Duncan's multiple range test (DMRT) at p=0.05. The results were expressed as mean ± standard error (SE).

RESULTS AND DISCUSSION

Synthesis and characterization of silver nanoparticles prepared using T. govanianum extract

The biosynthesis of AgNPs was confirmed by monitoring the change in color of the silver nitrate solution from colorless to brown. The UV spectrum of AgNPs synthesized using different concentrations of silver nitrate solution is represented in (Fig 1) and the absorption peaks observed are indicated in (Table 1). The nanoparticle synthesis occurred when 1:9 ratio of 1 mM $AgNO_3$ solution to plant extract was used.



UV spectrum of AgNPs synthesized using silver nitrate solution and *T. govanianum* extract at (a) 1:1 (b) 1:3 (c) 1:6 (d) 1:9 (e) 1:12 ratios and (f) blank (deionised water)

Fig 1 UV spectrum of silver nanoparticles

Table 1 Absorption peaks observed at different dilutions of silver nitrate

S. No.	Wavelength 'nm'	Absorbance
Observations at 1:1 dilution of silver nitrate and *T. govanianum* extracts		
1.	230	4.000
2.	229	3.988
Observations at 1:3 dilution of silver nitrate and *T. govanianum* extracts		
3.	233	4.000
4.	219	4.000
5.	225	3.969
6.	215	3.984
Observations at 1:6 dilution of silver nitrate and *T. govanianum* extracts		
7.	289	4.000
8.	258	4.000
9.	238	4.000
10.	224	4.000
11.	283	3.966
12.	249	3.996
13.	231	3.996
14.	212	3.997
Observations at 1:9 dilution of silver nitrate and *T. govanianum* extracts		
15.	226	4.000
16.	217	4.000
17.	221	3.995
Observations at 1:12 dilution of silver nitrate and *T. govanianum* extracts		
18.	215	3.980
Blank		
19.	277	4.000
20.	237	4.000
21.	217	4.000
22.	204	4.000
23.	253	3.980
24.	226	3.962
25.	207	3.990

The green approach is an environment-friendly technique for the synthesis of nanoparticles. Hence, they are increasingly implemented in numerous biological studies. Plant extracts are rich sources of secondary metabolites. Most of the extracts contain several compounds that can easily reduce silver nitrate (AgNO3) to AgNPs. Biological methods for the production of nanoparticles are considered as a safe and environment friendly alternative to the conventional physical and chemical methods as it is cost effective, and the usage of high pressure, energy, temperature and toxic chemicals is completely eliminated [1]. The cost-effective green approach of Ag-NPs synthesis was undertaken in our study by using *T. govanianum* extracts. The characterization of synthesized AgNPs can be done easily by observing the change in surface plasma resonance, band intensities and peak formation. Ideally, the silver nitrate solution shows no absorption in the range of 400-800nm. However, if the silver in $AgNO_3$ solution is reduced on exposure to plant extract, then a peak formation is observed at approximately 400 nm. The peaks observed at a lower range (i.e., 200nm -350nm) indicate the presence of several organic components of the plant which interact with silver ions [12].

Comparison of antibacterial activity of T. govanianum rhizome extract and silver nanoparticles

In the present study, 20µL, 40µL, 60µL, 80µL and 100µL volume of aqueous extracts and AgNPs were added to the wells formed in agar plates containing test isolates to evaluate their antibacterial activity. Table 2 represents the antibacterial activity of plant extracts and AgNPs, observed at different concentrations. The mean zones of inhibition for AgNPs were found to be in range of 10 – 23 mm for tested bacterial isolates. On comparison with the antibacterial activity of the plant extract alone, the AgNPs showed higher zones of inhibition. This observation was more evident in case of *S. typhi*. The plant extracts showed zone of inhibition of 9 and 8.1 respectively at 20µL and 40µL concentrations. At higher concentrations, the observed zones were 14-16.5mm in size. However, the AgNPs showed enhanced activity with zone of inhibition in the range of 19-20mm at different concentrations.

Table 2 Zones of inhibition of the solvent extracts and silver nanoparticles against bacterial isolates

Agent	Isolates	Zone of inhibition in 'mm'				
		20µL	40µL	60µL	80µL	100µL
Rhizome extract	*S. aureus*	11.5	17.28	17.28	19.8	19.40
	E. coli	11.76	14.11	14.11	15.87	15.28
	B. subtilis	-	10.23	10.23	10.46	9.40
	S. typhi	9	8.1	14.11	16.46	14.34
Silver nanoparticles	*S. aureus*	11.7	18	17	20	23
	E. coli	12.5	15	15	20	17
	B. subtilis	10	11	14	19	17
	S. typhi	-	20	20	19	20

The AgNPs synthesized in our study significantly inhibited the bacterial growth and showed increased zone of inhibition, as compared to the plant extracts alone. The antibacterial activity of Ag-NPs produced by using other plants has also been reported in literature. Examples of these plants include *Planta goovata* seed extract, carob leaf extract, *Lepisanthes tetraphylla* leaf extract and *green tea* extract [15-18]. The antimicrobial activity observed in our study may be largely due to the common phyto-constituents in the plant. In general, the genus *Trillium* is rich sources of the three phyto-constituents i.e., flavonoids, sterols and saponins. The anti-oxidant, anti-allergic, anti-inflammatory and antimicrobial activities of flavonoids and sterols of *Trillium govanianum* are well known [19-20]. Saponins are considered as the most potent antibacterial and anti-cancer agent due to their detergent like activity [21]. Similar to our study, the antibacterial activity of *Trillium govanianum* rhizomes against pathogenic isolates of *E. coli, Yersinia pestis* and *S. aureus* are also reported [22].

Although several plants have been investigated for preparation of biogenic nanoparticles, the use of *Trillium* species is rarely reported in literature. In the most recent study by [23], synthesis of AgNPs was attempted using *Trillium govanianum* crude extracts. They reported the involvement of aromatic amines in the bio- fabrication and synthesis of the AgNPs. They were analysed using bio- analytical techniques like by UV-Vis spectrophotometer, X-ray diffractometer, and FTIR spectrophotometer. Based on these techniques they were characterized as distinctive, mono- dispersed and face-centered cubic in nature with an average size of 9.99 nm. It also showed antimicrobial activity against 12 (6 bacterial and 6 fungal) strains. It was also stable to stress induced by high concentrations of NaCl and pH shift. They further reported the ease of reduction of silver from Ag^+ to Ag^0 during synthesis of AgNPs which can be monitored by simply observing the change in color of the solution.

CONCLUSION

The current study describes the green synthesis of silver nanoparticles using *Trillium govanianum* rhizomes. The medicinal benefits of *Trillium govanianum* - an endangered Himalayan plant have been well documented. However, very few studies have reported the bio-reducing property of this plant to synthesize silver nanoparticles. Our study confirms a higher efficiency of silver nanoparticles as compared to plant extracts. Hence, it can be used as an effective agent for synthesis of silver nanoparticles.

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