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Herbal extract mediated green synthesis of nanoparticles, its characterization and study of its property

Tejas Joshi and Sunetra Chaudhari*

Dept. Of Chemistry, KishinchandChellaram College, 123, DinshawVacha Road, Vidyasagar Prin, K. M Kundnani Chowk, Churchgate, Mumbai, Maharashtra, 400020

ABSTRACT

Green synthesis of nanoparticles is ecofriendly also more effective in action of their properties. In the present study, Aconitum heterophyllum was used in the synthesis of nanoparticles. Characterization of nanoparticles was done by UV spectrophotometer, nano-tracking analysis and XRD. Nanoparticles were successfully prepared and size was found to be around 35nm and XRD confirmed the FCC structure of silver crystal. Antimicrobial activity was established by Ditch-plate method *against Staphylococcus aureus, B. Subtilis, E. coli, P. aeruginosa.* There was considerable inhibition against tested *Staphylococcus aureus, B. subtilis, E. coli, P. aeruginosa* but lower compared to the synthetically prepared nanoparticles.

KEYWORDS: Green synthesis, A*conitum*, antibacterial activity, silver nanoparticles.

***Correspondence author**

Dr. Sunetra Chaudhari

Associate Professor, Department of Chemistry

Kishinchand Chellaram College. Churchgate, Mumbai, 400020

Maharashtra, India

Email: Sunetrach@gmail.com, daremee007@gmail.com

Contact:+91-9987662407; +91-7303330904

INTRODUCTION:

The environment we live in has become extremely damaged by the tremendous production of toxic chemicals and gases. This damage caused by the boom in industrialization and urbanization shows no sign of slowing down, and hence we need to turn to nature itself to find out how to create and proliferate the use of green products. This has led us to the discovery and subsequent advancements in the synthesis of nanoparticles, its characterization and also to study its antimicrobial activities. Amongst their varied properties, nanoparticles have some peculiar characteristics which make them highly suitable for biological use. The biological molecules undergo rigidly controlled operations which make them suitable for synthesis of stable and environment friendly metallic nanoparticles[1]. Using plant extracts is one of the most commonly used methods of synthesizing nanoparticles. This has a huge advantage as plants are not only available everywhere, but they are safer to use and also contain several metabolites and other useful chemicals which need not be processed separately for carrying out the synthesis. This not only enhances the efficiency and eco-friendly character of the synthesis but also cuts down on the time and money spent on other operations[2]. For all the various kinds of nanoparticles in use today, metallic nanoparticles show the most potential. They contain a significantly large surface area to volume ratio which gives them their notable antibacterial properties. This trait is in particular demand by researchers as there is a rise in the microbial resistance against antibiotics, metal ions and most importantly, the increase in the number of resistant strains[3]. The traditional methods of nanoparticles synthesis require a copious volume of harmful and expensive chemicals which in turn produce a huge amount of dangerous and toxic byproducts. This compounds the need for green synthesis and other biological processes which are eco-friendlier. Plant extracts as starting materials are more advantageous over others like microbial cultures, as they are simple to obtain andincorporate in the modified procedures. Metal nanoparticles have garnered immense interest in the recent years owing to their unique characteristics' good conductivity, chemical stability and catalytic activity. They also show antibacterial and antifungal properties which is why they are used in superconducting materials, electric components, cosmetic industries etc[4,5].There is a growing interest in nanoparticles of Gold and Argentum as they present highly beneficial materials. Silver nanoparticles have a bigger surface area and are thus able to provide amazing catalytic activity, biochemical reactivity and atomic behavior when compared to bigger particles having the same chemical composition[6].Many types of organic as well as inorganic reducing agents in aqueous or non-aqueous solvents are used to reduce Ag ions. For example, poly-ethylene glycol block copolymers, sodium citrate, Tollen's reagent, Ascorbate, essential hydrogen, N,N-dimethyl formamide [DMF], and sodium borohydride [NaBH4][7,8,9]. A type of component called capping agents are used for stabilizing the size and properties of the nanoparticles. One advantage of this method is that a huge amount of nanoparticles can be synthesized with as little waste as possible. Development of nanoparticles by this procedure involves production of byproducts that are significantly less toxic than the ones produced by the old methods and thus, are eco-friendlier and greener in nature. This is why production of Silver Nanoparticles using plant extracts and other similar biomass raw materials is being touted as a key branch of nanotechnology. Therefore, the use of natural products as raw materials is a better alternative and more preferred than the toxic chemicals that are traditionally used and the byproducts which pose a potential threat to our physiology[10].Atvish [Aconitum Heterophyllum] is an Ayurvedic herb and is considered very effective for the human body, especially the digestive system. The roots are tuberous, paired and create a broad spectrum of alkaloids. Puri has worked extensively on A. heterophyllum and notes that the plant is used internally as well as externally for ailments. Its stem and underground parts i.e. roots are most extensively used for clinical use is the stem and the underground roots[11].

Using cotton pellets induced granuloma in rats7, Verma et al[12]. demonstrated the anti-inflammatory activity of ethanaloicroot extract of A. heterophyllum [225, 450 and 900 mg/kg p.o.]. The diminish of weight of the cotton pellet is an evidence of reduction in inflammation. The results manifested that the effects and the anti-inflammatory properties of the extract were analogous to diclofenac sodium, a standard non-steroidal, anti-inflammatory drug.

A. heterophyllum is an effective diuretic and a very impactful aphrodisiac. It is a potent remedy for gastroenteric fevers, particularly amongst infants and children[13]. A. heterophyllum has also been recorded to have anti-fungal, cytotoxic, antiviral and immune-stimulant properties [14-17].

Phytochemical constituents:

A. heterophyllum has been found to possess phytochemicals that have medicinal values and are used for providing relief from various ailments. Chromatographic separation techniques were used to isolate and characterize composites of A. heterophyllum such as alkaloids, amide alkaloids, flavonoids, flavanol glycosides, diterpenoid and norditerpenoid compounds. Their structures were explained using Nuclear Magnetic Resonance techniques. These composites were the principal focus of medicinal researchers as they show duality in nature, medicinal as well as toxic. A thorough examination of the basal constituents of the roots of A. heterophyllum has led to the isolation of seven new diterpene alkaloids. The weak base fraction produced Heteratisine and three more alkaloids labelled as

Heterophyllisine, Heterophylline and Heterophyllidine. These are lactone alkaloids and have relative of Heteratisine. The strong base fraction led to the production of two new alkaloids [Atidine and F-dihydroatisine]. The very strong base fraction yielded alkaloids that were designated as hetidine and hetisinone. The latter had been discovered previously as a chemically derivative product of hetisine.[18]

MATERIALS AND METHODS:

Chemicals and reagents:

Biological sample: roots of*Aconitum heterophyllum,*

Microbial cultures: *Staphylococcus aureus, B. Subtilis, E. coli, P. aeruginosa.*

Chemicals: Silver Nitrate solution (Merck), nutrient agar [Hi-media], DPPH[Hi media], Ampicillin[Hi-media], Ascorbic Acid, Methanol

Green synthesis of silver nanoparticles using A. heterophyllum

The plant sample is washed and rinsed with distilled water to remove external dust and dried in a muslin cloth for a period of 15 days to remove moisture. A dry powder is made by grinding. The dry powder is boiled in distilled powder until the solvent is reduced to half. The sample is subjected to vacuum filtration to obtain the supernatant. 6.5ml of the supernatant was taken and treated with 50ml of 1mM AgNo3 solution. It was then heated in a microwave for 1 minutes. The formation of yellow colored solution indicates the presence or formation of AgNPs. The solution is the subjected to centrifugation at 10000 rpm for 10minutes. The supernatant is discarded and the pellet obtained is washed several times with distilled water. The pellet now obtained is brownish red in color.

Characterization of plant mediated AgNPs

NanoparticleTracking Analysis: is done for visualization and analysis of particles.

UV Spectrometry analysis: The sample obtained was subjected to UV spectrometry analysis to characterize the nanoparticles synthesized. A spectrum scan was run from 300-800nm to determine the absorption maxima.

XRD Analysis: The obtained nanoparticles were characterized using X-ray diffraction,performed using a Rigaku Smart Lab equipment, operating at45 kV and 200 mA, Cu Kα radiation (1.54059 Å), parallel beam configuration(2θ/θ scan mode), from 30 to 90 2θ°;

Evaluation of properties and activities of the silver nanoparticles

Antimicrobial activity:

Bacterial maintenance: S*taphylococcus aureus, B. Subtilis, E. coli, P. aeruginosa* were maintained on Nutrient agar slants and incubated at 37ºC.

Ditch plate technique: Antimicrobial susceptibility of the organisms towards the crude extract was observed by using the ditch plate technique. 50% [v/v] of the sample mixed with molten agar was poured into ditch created of 1.5cm in width and loopful of bacteria was streaked across. The plates were left overnight to incubate at 37ºC. The Zone of Inhibition was measured

Antioxidant activity: Antioxidant activity of plant extract was assessed based on its, scavenging effect against 0.002% DPPH. Ascorbic acid was used as a standard. Aliquots of sample were mixed in the ratio of 1:1 and incubated in the dark for 30 minutes. The mixture was read at 517nm[19].

RESULTS:

Nanoparticle tracking analysis

The size of the nanoparticle was found to be 34nm ± 21nm with a drift velocity of 1931nm/s. The total concentration to be 1.30E8 particles/ml. [Fig 1]

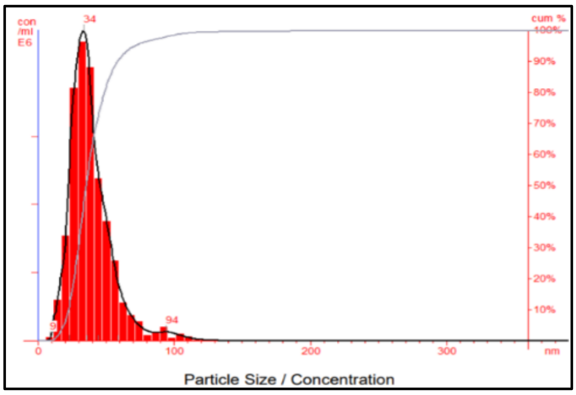

Fig1: Graphical representation of NTA

UV spectrometry analysis:

The absorption maxima of the green synthesis of AgNPs was found to be at 412 which is nearer to absorption maxima (synthesis with the help plant leaves) of AgNPs at 420nm [20].

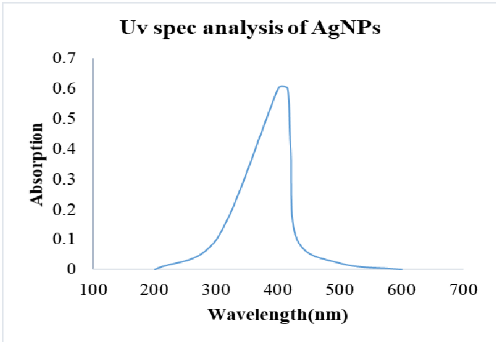

Fig 2. Graphical representation UV spec analysis

X-ray Diffraction:

The diffraction spectrum of green synthesized AgNps have shown Bragg Peaks (angle 2θ) at 29.5º, 34º, 38.2º, 44.38º, 54.98º, 59º, 64.44º, 77.46º which corresponds to 210, 122, 111, 200, 142, 241, 220 and 331 miller indices that suggest FCC structure.

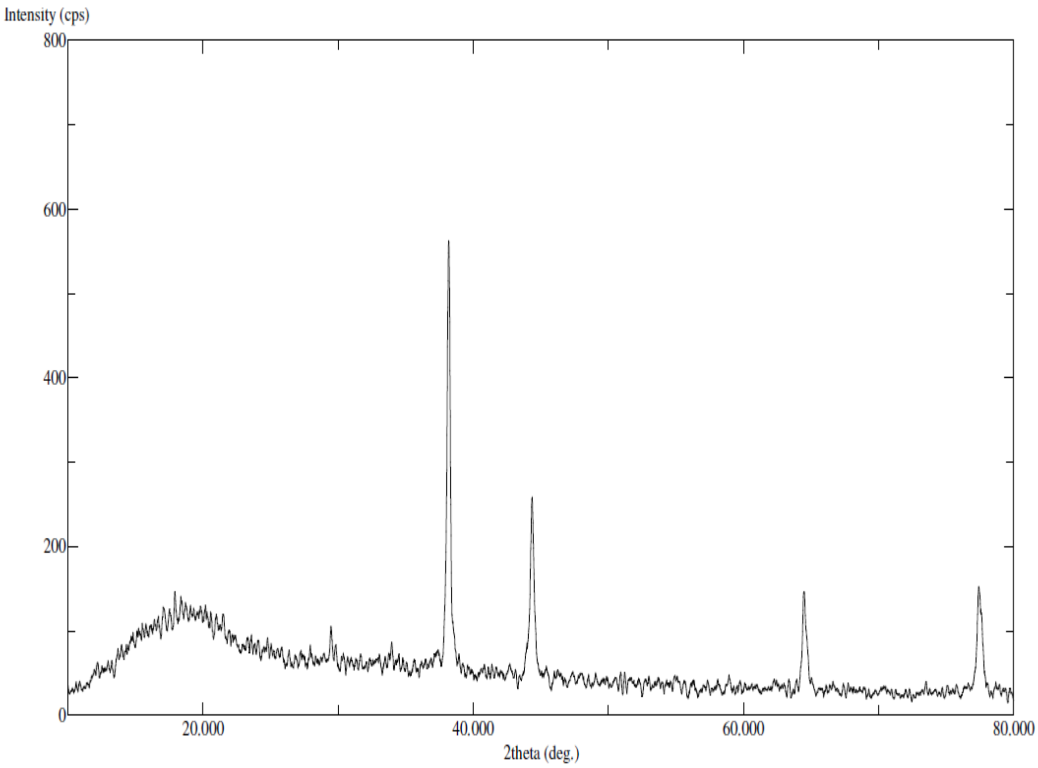

Fig3. Graphical representation XRD spectrum

Antimicrobial Susceptibility testing:

Green nanoparticles showed weaker inhibition in comparison to synthetically prepared nanoparticles. The aconitum nanoparticles showed strong inhibition against *S. aureus* and *B. subtilis* however weaker inhibition against *E. coli* and *Pseudomonas aeruginosa. Ampicillin* was used as a control.

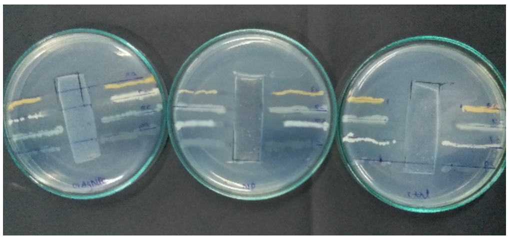

Fig4. AST plates [left to right] of Green AgNPs, synthetic NPs, and control [Ampicillin]

		Zone of Inhibition								
Sr. no	**Organism**	**Control**			**Synthetic Nanoparticles**			**Green Nanoparticles**		
		[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]
1	*S. aureus*	21	24	23	28	27	25	22	24	24
2	*B. subtilis*	21	22	21	15	18	17	25	23	27
3	*E. coli*	18	16	17	25	23	27	21	21	19
4	*P. aeruginosa*	17	16	18	14	15	15	19	20	21

Table: zone of inhibition exhibited by the samples on the selected bacteria

Antioxidant activity:

The natural antioxidants of the Aconitum contribute to its antioxidant activity. In comparison the activity is slightly lesser than ascorbic acid.

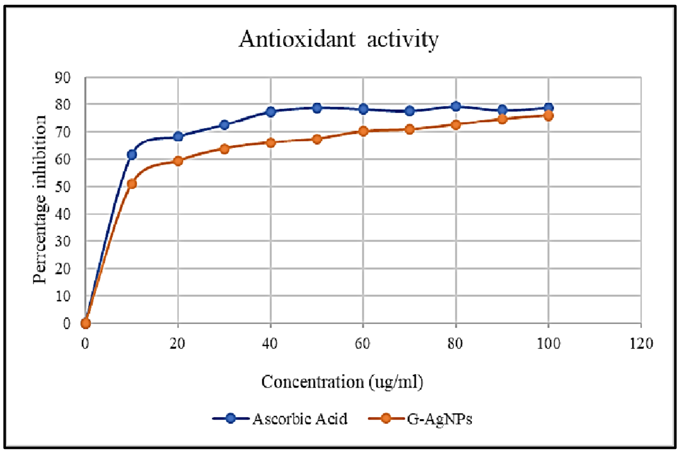

Fig5: Graphical representation of antioxidant assay performed by DPPH assay

DISCUSSION:

Atvish has been commonly associated with the treatment of intestinal and digestive ailments. Traditionally, in wester Himalayan region it has often been used in Ayurvedic for relieving flatulence and also advised for a regular bowel regimen. Traditional Chinese practitioners also use aconite for pain relief caused by trigeminal and intercostal neuralgia. Aconite is also used as a mild diaphoretic and to slow pulse rate by its effect on brainstem centers. Due to its association with several clinical applications, Aconitum and its phytoconstituents have been studied for its properties and extraction. Nanoparticles have been known to enhance the biological activities of the plants. Therefore, silver nanoparticles were made to study its antimicrobial activity especially towards enteric bacteria. The silver nanoparticles were synthesized chemically as well as by green synthesis to compare the activity and efficiency. It can be noted that while the antimicrobial activity of G-AgNPsappears to be lesser, by statistically analysis by T test at 0.05 significance level, the activities of chemical synthesis and green synthesis is comparable. Since green synthesis is also economically and ecologically feasible, it is a better option.

CONCLUSION:

The green synthesis of silver nanoparticles was carried out by using *Aconitum heterophyllum.* The physical characteristics of the nanoparticles were determined. The size of the synthesized AgNPs was determined using a nanoparticle tracking system, the mean size was found to be 34nm. The chemically and biologically synthesized nanoparticles were studied for their antimicrobial activity. The activities of nanoparticles by both syntheses were found to be statistically comparable. They both showed good activity towards *S. aureus* and *B. subtilis* and lesser activity towards *E. coli* and *Pseudomonas aeruginosa.* We can hence conclude that Aconitum can be used as a good candidate to treat enteric infections and be explored in clinical research and since nanoparticles increase catalytic activity and efficacy, using nanoparticles as a drug delivery system could work out well.

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