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Synthesis of Antimicrobial Silver Nanoparticles by Using Flower of Calotropis Gigantea

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ABSTRACT

In this report, green synthesis of silver nanoparticles was achieved by Calotropis gigantea flower extract which act as reducing and capping agent. The synthesized silver nanoparticles were characterized by UV-Visible spectroscopy, SEM-EDX.UV Visible spectra shows the maximum absorbance peak at 430nm which confirms the formation of silver nanoparticles. SEM-EDX analysis reveals the spherical shape of silver nanoparticles and size of about 30-35nm. The EDX spectra reveals the presence of elemental silver as 70%. The antibacterial activity of Calotropis gigantea flower intervened silver nanoparticles against Shigella and Vibrio cholerae shows enhanced activity. The silver nanoparticles also shows potential antioxidant activity as the concentration increases and the absorbance is noted at 517nm.

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BACKGROUND

Nanomaterials are getting incredibly well known as natural, remedial, clinical, antimicrobial agents, fluorescent labels and furthermore as transfection vehicles [1]. Silver nanoparticles with great properties have become new promising region of research because of their size and large surface area [2]. Since old occasions, people have generally utilized plant based natural medications against different maladies. Present day medications are mostly derived from herbs based on traditional information and practices. Almost, 25% of the significant pharmaceutical compounds accessible today are mostly gained from natural resources [3,4]. Silver nanoparticles has gained more interests due to their enormous properties such as chemical stability, good conductivity and most importantly it possess antibacterial, anti-viral, antifungal and anti-inflammatory properties. The benefits and uses of silver nanoparticles has also got attention in industries such as cosmetic industry, food industry, electronic components making industries [5,6].

Calotropis gigantea Linn (Apocynaceae) is a small shrub or tree commonly called as milkweed. The flowers of Calotropis are spotted in white or lavender colour. The Calotropis plant has milky stem, oval and light green leaves. The flower of the plant contains cardiacglycosides, calotopin, uscharin, calotoxin, calactin, uscharidin, and gigantin, protease calotropin DI and DII and calotropin FIand FII [7,8]. In conventional physical and chemical methods, the developed nanoparticles pass on a risk of lethality to the environment and life of human beings. The green intervention method is a simple, cost effective stable method wherein nanoparticles with biocompatibility are made that is more safer for human beings and for the environment [9]. In this study, we synthesize silver nanoparticles using Calotropis gigantea flower aqueous extract which act as a harmlesss reducing and capping agent. The aim of the study is to reveal the antibacterial and antioxidant properties of the Calotropis gigantea flower intervened silver nanoparticles.

MATERIAL AND METHODS

Silver nitrate, DPPH, ascorbic acid were purchased from Sigma Aldrich, India. Mueller Hinton Agar were purchased from Hi media Pvt.Ltd, India. The flowers of Calotropis gigantea plant were collected from Arcot, Vellore, Tamilnadu and India. The bacterial cultures such as Shigella and Vibrio cholera were isolated and collected from Saveetha Dental College & Hospital, SIMATS, Poonamallee, Chennai.

Preparation of flower extract

The collected fresh flowers of Calotropis gigantea was washed thoroughly under tap water and with Milli-Q water. 25gm of crushed flowers of Calotropis gigantea was added to 100ml double distilled water. The mixture was then heated using a heating mantle at 70°C for 15 minutes. By this method, all phytochemical compounds present in the Calotropis gigantea flower gets diffused in the aqueous solution. The aqueous mixture was filtered using Whatmann No.1 filter paper and the filtered aqueous extract was stored at low temperature for further use.

Fig 1: Calotropis gigantea

Synthesis of silver nanoparticles:

A deliberate amount of 10 ml of the Calotropis gigantea flower extract was added to 90 ml of 1mM of silver nitrate. The reaction mixture was kept in the magnetic stirrer at room temperature (RT) for 48hours. The reaction mixture was observed and noted for a colour change and synthesis of nanoparticles was preliminarily confirmed by using UV-Visible double beam spectrophotometer in the wavelength range of 360-500nm. Biosynthesized silver nanoparticles was collected by centrifugation method at 8000rpm for 10

minutes. The obtained silver nanoparticle pellet was washed with double distilled water for 3-4 times and then calcined in hot air oven at 70°C for 2 hours. The powdered silver nanoparticles were stored in air tight vials for further studies.

Characterization

The maximum absorbance of Calotropis gigantea flower mediated silver nanoparticles were measured by using double beam UV-vis spectrophotometer(uv-2450,Shimadzu) in the wavelength range of 360-500nm.The synthesized silver nanoparticles was subjected to test the elemental analysis using Energy dispersive X-ray detector (EDX) attached to the SEM machine.

Antimicrobial activity

The 10µL of fresh bacterial cultures such as Shigella and Vibrio cholerae were inoculated in sterile Hi-veg broth medium and incubated for 18 hours, in an orbital shaker at 120-150rpm. Mueller Hinton agar was prepared and 5mm wells were made using a sterile polystyrene tip. Different concentrations of biosynthesized silver nanoparticles such as 25µL, 50µL, 100µL were added along with the positive control amoxyrite 10µg/mL. The plates were incubated at 37°C for 24 hours and the zone of inhibition were measured [10].

Antioxidant activity

The DPPH (1,1-diphenyl-2-picryl-hydrazil) free radical looking through development of Calotropis gigantea flower mediated silver nanoparticles was settled by the procedure revealed in [12].Different fixations (2-10 µg/ml) of calotropis gigantea flower intervened silver nanoparticles was mixed in with 1 ml of 0.1 mM DPPH in methanol and 450 µl of 50 mM Tris HCl buffer (pH 7.4) and incubated for 30 minutes. After incubation, the decline in the amount of DPPH free radicals was assessed based on the absorbance at 517 nm. BHT was used as control. The rate restriction was resolved from the following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

RESULT AND DISCUSSION

Visual Observation

The flower of Calotropis gigantea contains the cardiac glycosides, calotopin, uscharin, calotoxin, calactin, uscharidin and gigantin, protease calotropin DI and DII and calotropin FI and FII [13]. The colour change in the reaction mixture was noted carefully, which preliminarily determines the bioreduction capacity of the plant extract [14]. The formation of light brown colour indicates the formation of silver nanoparticles. The formation of silver nanoparticles was further confirmed by UV-Visible spectroscopy, EDX analysis.

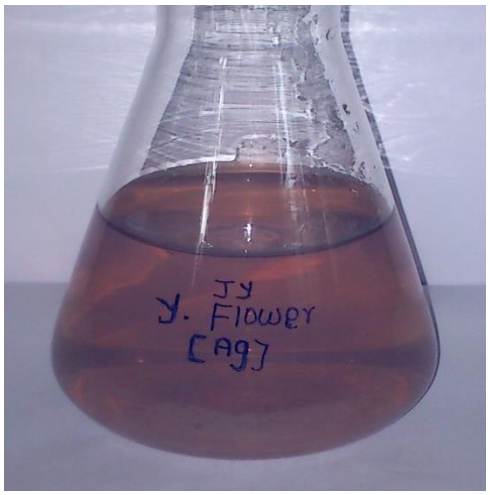

Fig 2: Formation of silver nanoparticles using Calotropis gigantea flower extract and its colour change.

Characterization of silver nanoparticles:
UV-Visible spectroscopy:

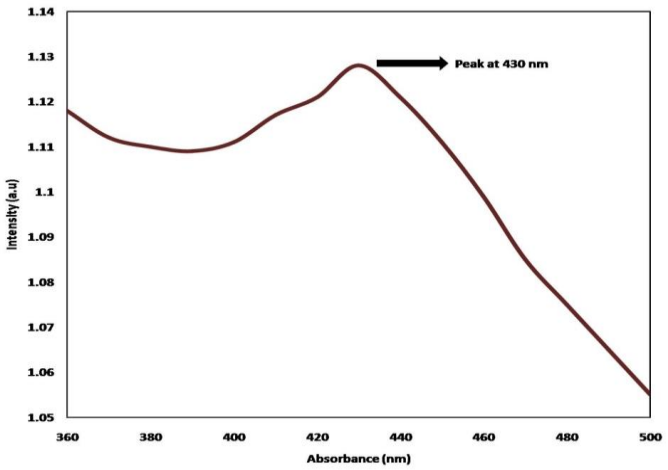

Fig 3: UV-Visible spectra of biosynthesized silver nanoparticles.

The UV-Visible spectra of Calotropis gigantea flower extract mediated silver nanoparticles is depicted in fig 3. The maximum absorption peak at 430nm indicates the formation of silver nanoparticles and it affirms the ability of the flower extract to reduce silver nitrate into silver nanoparticles.

SEM-EDX analysis

The surface morphology of silver nanoparticles were analysed by using SEM. The SEM image of Calotropis gigantea flower extract intervened silver nanoparticles are depicted in fig 4. The SEM image clearly reveals the formation of silver nanoparticles in spherical shape and demonstrates average size of silver nanoparticles was 30-35nm.It also confirms the reducing and capping ability of the phyto constituents present in the flower extract.

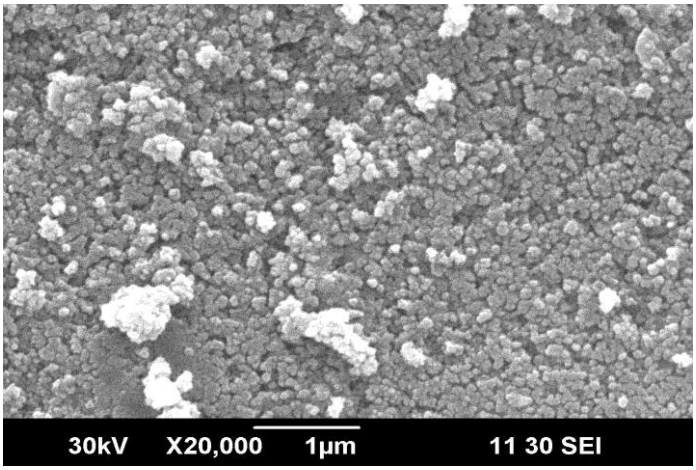

Fig 4: SEM image of biosynthesized silver nanoparticles.

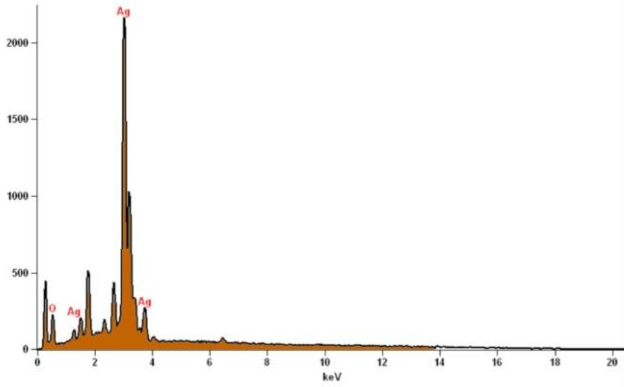

Fig 5: EDX analysis of Calotropis gigantea flower mediated silver nanoparticles.

The elemental analysis of Calotropis gigantea flower mediated silver nanoparticles were depicted in fig 5. The EDX spectra clearly reveals that the silver nanoparticles reduced by Calotropis gigantea flower extract have the weight percentage of silver as 60%.

Antimicrobial activity

Fig 6: Antimicrobial activity of synthesized silver nanoparticles.

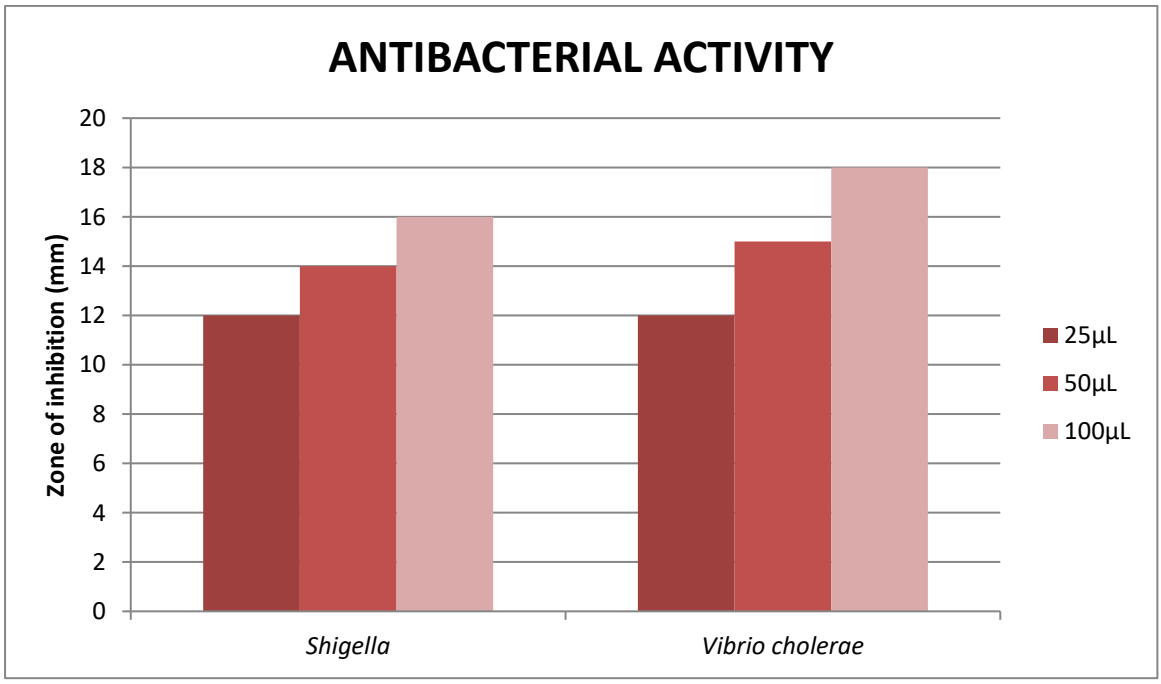

Fig 7: Histogram of Calotropis flower intervened silver nanoparticles.

Shigella, a gram negative pathogen that causes deadly infections such as dysentery more predominantly among children below 5 years [15]. In this study, Shigella is used as a model organism to test the antibacterial activity of synthesized silver nanoparticles. Shigella shows its maximum zone of inhibition with zone diameter of 16mm at 100µL concentration.

Vibrio cholerae, a gram negative pathogen that causes enteric diarrhoeal disease. The infection begins with the ingestion of contaminated water and the organism pass the stomach acid barrier and colonizes in the epithelial cells of the small intestine and release its virulence factor to cause cholera disease in humans [16]. In this study, Vibrio cholerae shows its maximum zone of inhibition with zone diameter of 18mm at 100µL concentration. Thus, both gram negative pathogens Shigella and Vibrio cholerae is sensitive to the Calotropis gigantea flower mediated silver nanoparticles.

Antioxidant activity

The free radical scavenging activity was investigated by DPPH method. The DPPH act as a free radical and Calotropis gigantea flower intervened silver nanoparticles act as radical scavenger.

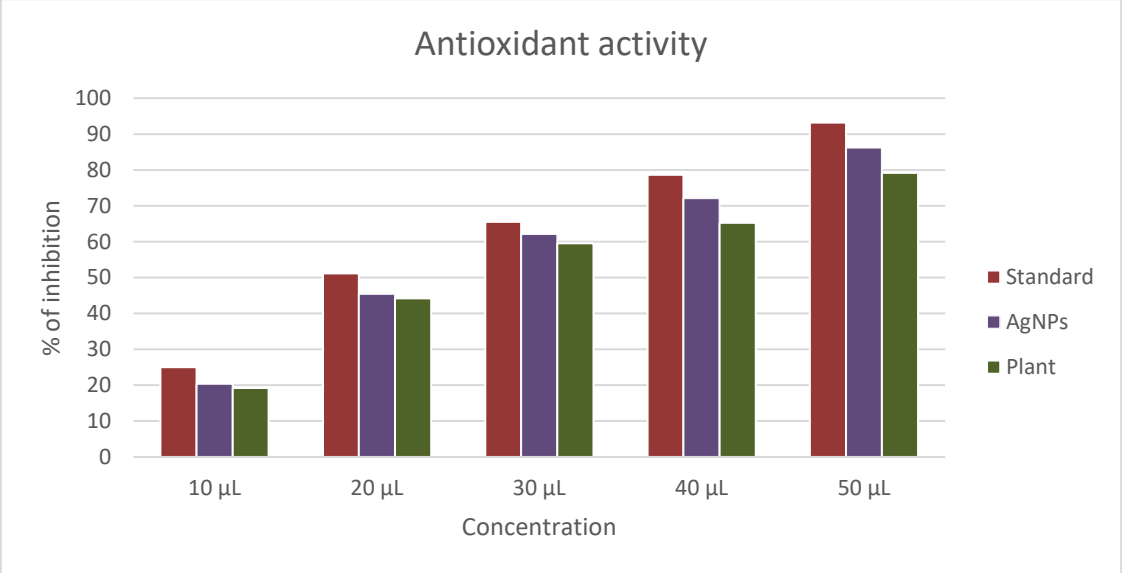

Fig 8: Antioxidant activity of biosynthesized silver nanoparticles.

Fig 8 shows the histogram of antioxidant activity of the synthesized silver nanoparticles. It depicts as when concentration increases the absorbance at 517nm decreases. Calotropis gigantea flower mediated silver nanoparticles shows higher antioxidant activity than the flower extract. And the biosynthesized silver nanoparticles has equal antioxidant potential when compared with the standard values (Ascorbic acid).

CONCLUSION

In this research work, silver nanoparticles have been synthesized using Calotropis gigantea flower extract and the synthesized silver nanoparticles showed remarkable stability. The results of silver nanoparticles prepared characterization were shown this plant can be used as a reducing and stabilizing agent and also, the prepared nanoparticles were used as an effective antibacterial and antioxidant material. The UV–Vis spectroscopy, EDX- SEM analysis confirm the existence of elemental silver and its spherical form and size of about 30-35nm. The synthesized silver nanoparticles by Calotropis gigantea flower have been confirmed to show enhanced activity against pathogenic bacteria. The present research is a simple, cost effective, eco-friendly and nontoxic method for the synthesis of silver nanoparticles. Therefore, it can be concluded that the synthesis of nanoparticles using plants is an efficient method.

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