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Dr. A Leema Rose
PG and Research Department of Chemistry, Holy cross college (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

F Janeeta Priya
PG and Research Department of Chemistry, Holy cross college (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

Dr. S Vidhya
PG and Research Department of Chemistry, Holy cross college (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

Preeja P Thattil
PG and Research Department of Chemistry, Holy cross college (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

Correspondence
F Janeeta Priya
PG and Research Department of Chemistry, Holy cross college (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

Antifungal and antioxidant activity of the microwave assisted green synthesis of silver nanoparticles using aloin derivative

Dr. A Leema Rose, F Janeeta Priya, Dr. S Vidhya and Preeja P Thattil

Abstract
Nanoparticles are considered to be the pre-eminent component of the rapidly advancing field of nanotechnology. In recent years, the green synthesis of silver nanoparticles using various plant extracts has attracted great attention. The use of nanoparticles in the field of medicine is increasing in the present world of nanotechnology. Green synthesis of nanoparticles has attracted scientist as a safe, easy and cheap mode of nanoparticles ion in the developing and also in developed countries. In the present study, the silver nanoparticles were synthesized using the Aloin derivative extracted from *Aloe barbadensis*, a medicinal plant. The plant extract act as a reducing agent as well as a capping agent. The bio reduced silver nanoparticles were characterized using UV-Visible spectroscopy, SEM. A broad SPR band was observed at 440nm in the UV-Visible spectra. X-ray diffraction revealed their crystalline nature. Scanning electron microscope showed spherical shaped and monodispersed nanoparticles. The antifungal and antioxidant activity of the synthesized silver nanoparticles were also examined.

Keywords: silver nanoparticles, green synthesis, UV-visible spectroscopy, SEM, antifungal activity and antioxidant activity

Introduction
Nanotechnology is presently one of the most dynamic disciplines of research in contemporary material science whereby plants and different plant products are finding an imperative use in the synthesis of nanoparticles. In general, particles with a size of less than 100 nm are referred to as nanoparticles. Entirely novel and enhanced characteristics such as size, distribution and morphology have been revealed by the particles in comparison to the larger particles of the mass material [1]. Various approaches are available for the synthesis of silver nanoparticles include chemical [2], electrochemical [3], radiation [4], photochemical methods [5] and biological techniques [6]. Although chemical and physical methods may successfully produce pure well defined nanoparticles [7] compared to biological synthesis, these methods are quite expensive and dangerous to the environment [8]. Use of biological methods, plant extract or plant biomass could be an alternative to chemical and physical methods for the production of nanoparticles in an eco-friendly and cost effective manner [9]. Most remarkable applications of silver nanoparticles include magnetic and optical polarizibility [10], electrical conductivity [11], catalysis [10], antimicrobial activity [12, 13], DNA sequencing [14] and Surface-enhanced Raman Scattering [15].
In the present study, we established that the gel extract of *Aloe barbadensis* were used in the reduction of Ag(I) and in the formation of stable silver nanoparticles and tested the effect
Antifungal and antioxidant activity. *Aloe Vera* has been used for medicinal purposes in several countries [16]. By the early 1800s, Aloe Vera was in use as a laxative in the United States, but in the mid-1930s, a turning point occurred when it was successfully used to treat chronic and severe radiation dermatitis [17]. The method used here for the synthesis of silver nanoparticles is the microwave assisted simple Bio-reduction technique which cost effective, energy efficient and time saving process.

Materials and Methods
Preparation of plant extract
Fresh leaves of *Aloe barbadenis* were collected from the Manachanallur village in Trichy. The leaves were washed with distilled water. After washing, the leaves were cut to collect the

yellow gel. Then the yellow gel was distillated. After distillation, the liquid was collected and stored in refrigerator for further analysis.

Synthesis of Silver Nanoparticles

50 µl of plant extract solution and 1mm silver nitrate solution have been employed for the synthesis of silver nanoparticles (AgNPs). A colour change of reaction mixture from transparent yellow to dark reddish brown is observed. This colour change indicates the formation of silver nanoparticles (Ag^0). Further reduction of the Ag^+ ions was monitored over time by UV-Visible spectral analysis.

Characterization of synthesized silver nanoparticle

The reaction of silver nitrate solution with Aloin derivative extracted from *Aloe barbadensis* as the capping and reducing agent was optically measured using shimadzu UV-Visible spectrophotometer. Shape and size of the synthesized silver nanoparticles were studied by using scanning electron microscope.

Antifungal activity

By disc diffusion method, the antifungal activity of the synthesized silver nanoparticles was studied. Potato dextrose agar media for fungi is used, sterilized and solidified. The antifungal strain (*Penicillium chrysogenum*) was swabbed on the plates. The dried silver nanoparticle sample were weighed (10mg/10ml) and dissolved in sterile distilled water to prepare appropriate dilution to get required concentrations of about 50µl (50µg), 100µl (100µg) and 150µl (150µg). Control used as de ionized water. They were kept for incubation at 37^0c for 24 hours. Zone of inhibition for control and silver nanoparticles were measured and the mean values of zone of inhibition were presented.

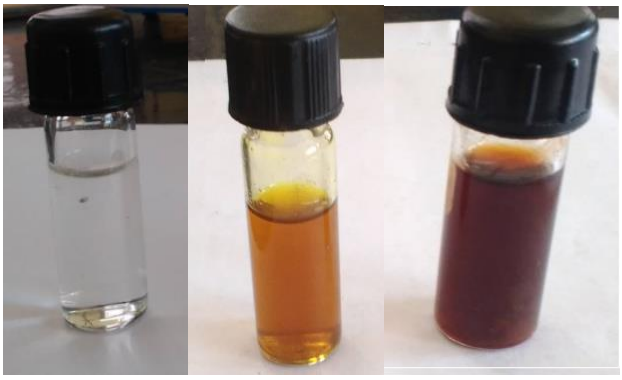
Antioxidant activity

Various concentrations of samples (10µg, 50µg and 100 µg) were taken in a series of test tubes. To this added 1.9 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 min. and allowed to cool. The absorbance of the aqueous solution of each was measured at 695 nm against a blank. Antioxidant capacities are expressed as equivalents of ascorbic acid. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. Butylated hydroxy anisole (BHA) was used as reference standard.

Results and Discussion

Visual observations

1mM aqueous solution of silver nitrate was used for the synthesis of silver nanoparticles. For the synthesis of silver nanoparticles, 1mM silver nitrate was added to the Aloin derivative extracted from *Aloe barbadensis* to make up the final solution. These were kept at room temperature for 30 minutes. The colour change of extract from yellow to dark reddish brown was checked periodically. The reaction mixture was centrifuged at 18,000 rpm for 25 minutes in order to obtain the pellet which was used for further study. Supernant was discarded and the pellet was dissolved in Deionized water. The pellets were stored at 4°C for further use.



(A) **(B)** **(C)**

Fig 1: (A-Silver nitrate solution, **B-**Plant extract, **C-** Color Change indicates the Formation of silver nanoparticles)

UV-Visible absorbance spectroscopy analysis

The bio-reduction of nanoparticle was monitored periodically by UV-Vis spectroscopy.

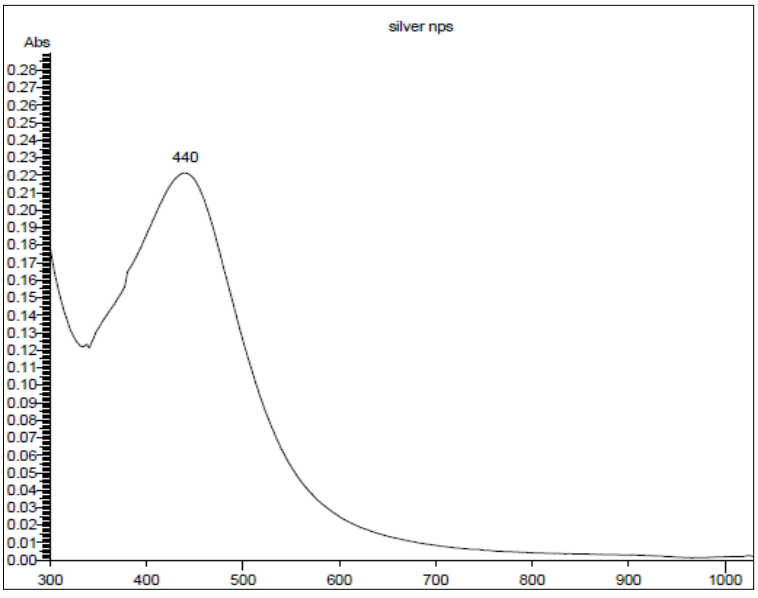


Fig 2: UV-Visible spectroscopy for silver nanoparticles.

The absorption band of silver nanoparticles has been reported be in the range of 400-500 nm [18, 19]. The samples used were diluted with 2 ml Deionized water and subsequently measured by the UV-Vis spectrum at regular different time intervals UV-Vis spectrograph of the colloidal solution of silver nanoparticles was recorded as a function of time by using a quartz cuvette with water as reference. The UV-Vis spectrometric readings were recorded at a scanning speed of 200 to 800 nm [20].

SEM analysis

Each of the colloidal solution containing AgNPs were centrifuged at 4,000 rpm for 15 min, and the pellets were discarded and the supernatants were again centrifuged at 25,900 rpm for 20 min. This time, the supernatants were discarded and the final pellets were dissolved in 0.1 mL of Deionized water. The pellet was mixed properly and carefully placed on Aluminium foil followed by air-drying.

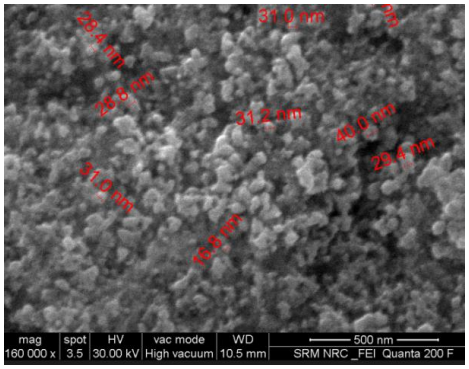


Fig 3: SEM images of silver nanoparticles.

The Aluminium foil itself was used during scanning electron microscopy (SEM) analysis. The images of silver nanoparticles were obtained in a scanning electron microscope the details regarding applied voltage, magnification used and size of the contents of the images were implanted on the images

Antifungal activity

The silver nanoparticles present to have good fungicidal activity [19-22]. Based on the zone of inhibition produced, the synthesized silver nanoparticles showed good antifungal activity against the fungi *Penicillium chrysogenum* which is shown in the below figure 4.

Fig 4: Antifungal activity of silver nanoparticles.

Measurement of zone of inhibition

The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the samples were measured using a millimeter scale.

Table 1: Antifungal Activity for *Penicillium chrysogenum*
Antioxidant activity

Sample	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
AgNps	0	3	5	6	8	10	50
	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
	0	0	0	3	5	*	

The Aloin derivative extracted from *Aloe barbadensis* gel was tested in different concentrations against butylated hydroxy anisole to find the radical scavenging activity. The antioxidant activity was found to be the maximum for 100 µg/ml BHA assay. Free radical scavenging activity of the silver nanoparticles on BHA radicals was found to increase with increase in concentration. These findings are supported from the literature survey which reports that silver nanoparticles synthesized from various leaf extracts showed good antioxidant activity [23], [24], [25], [26].

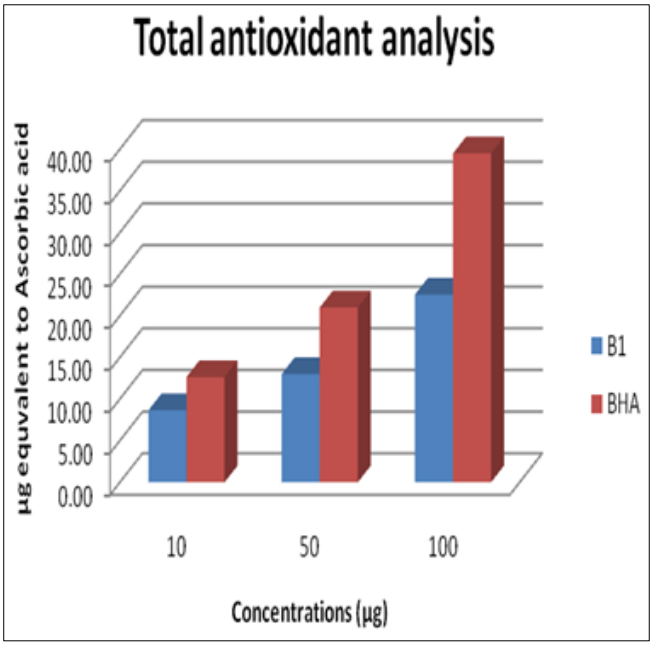


Fig 5: B1 and BHA assa

Table 2: Total anti oxidant activity

Concentration (µg)	µg equivalent to AA B1	µg equivalent to AA BHA
10	8.65	12.65
50	13.00	21.00
100	22.55	39.50

Conclusion

Green synthesis of silver nanoparticles by using Aloin derivative extracted from *Aloe barbadensis* is a very cost effective, safe, non toxic, eco-friendly route of synthesis which can be manufactured at a large scale. The Aloin derivative extracted from *Aloe barbadensis* showed great capability to synthesis AgNPs at optimum temperature conditions. The UV absorbance peak 440nm clearly indicates the synthesis of AgNPs. The SEM study was helpful at their

morphology and distribution. The EDAX analysis confirmed the presence of silver in the synthesized nanoparticles. The AgNPs shows good Anti-Oxidant property. The AgNPs have great Antifungal activity against *Penicillium chrysogenum.*

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