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GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM FRUIT RIND EXTRACT OF *GARCINIA MANGOSTANA* L. AND EVALUATION OF ANTIBACTERIAL PROPERTIES

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ABSTRACT

Nanotechnology is an emerging area of science that involves the engineering of nano sized particles of various materials. Among the various nanoparticles, the silver nanoparticles are used in various applications due to their unique characters. The production of silver nanoparticles using a chemical method is harmful and produces toxic substances as by-products, so the aim of our study was to green synthesis silver nanoparticles using the fruit rind of *Garcinia mangostana L.* which is being discarded as a waste material. Aqueous rind extract of *G. mangostana* was prepared and synthesis of silver nanoparticles was analysed by UV-Vis spectrophotometer by optimizing various parameters like pH, time and concentration of extract. The nanoparticles were characterized using UV-Vis spectroscopy and the peak was obtained between the wave length of 410 - 433 nm in various treatments. The antibacterial activities of synthesized silver nanoparticles were tested against both gram negative (*Pseudomonas*) and gram positive (*Staphylococcus*) bacteria using the well diffusion method. The aqueous extract shows the remarkable zone of inhibition against *Pseudomonas* and *Staphylococcus*.

***Keywords*:** Green synthesis, Silver nanoparticles, *Garcinia mangostana,* antibacterial activity

INTRODUCTION

Metal nanoparticles have been used widely in recent studies due to their unique electronic, optical, mechanical, magnetic and chemical properties. Silver nanoparticles have attracted much attention due to their unique characteristics for utilizing various applications including pharmaceuticals, agriculture, water detoxification, air filtration, textile industries and as a catalyst in oxidant reactions (Pirtarighat *et al*., 2019). More recently, the field of nanotechnology is gaining an increased interest in plant science, especially for the application of nanomaterials as vehicles of agrochemicals or biomolecules in plants, and the great potential to enhance crop productivity (Sanzari *et al*., 2019).

Nanoparticles can be synthesized using various approaches including chemical, physical and biological processes. Chemicals used for the nanoparticles synthesis and stabilization are toxic and can lead to non-eco-friendly by products (Krithiga *et al*., 2015). There is a growing need to develop an eco-friendly process for the synthesis of nanoparticles that does not employ toxic chemicals. Nowadays green chemistry procedure using various biological systems such as yeast, fungi, bacteria and plant extract for the synthesis of nanoparticles are commonly used.

Green synthesis of nanoparticles is an important methodology that has been used in the synthesis of metallic nanoparticles, using different parts of some selected plants (Sarkar and Kotteeswaran, 2018). Plants provide a better platform for nanoparticle synthesis as they are free from toxic chemicals and provide natural capping agent (Krithiga *et al*., 2015). Although the exact mechanisms of NPs biosynthesis by various plant extracts is ambiguous, it has been revealed that the biomolecules in plant extract such as protein, phenol and flavonoid play a significant role in the reductions of metal ions and capping the biosynthesized nanoparticle (Pirtarighat *et al*., 2019).

Garcinia mangostana L. belongs to the family Guttiferae. This fruit has many properties such as anti-fungal, anti-tumour, cosmetic, medicinal uses as well as oral and pharmacological uses (Karthika, 2017). The Fruit rind of the *G. mangostana* contains 7-13% of tannin and xanthones contributing to anti-bacterial and anti-oxidant properties (Rajakannu *et al*.,2015). Secondary metabolite known as xanthones, have been isolated from the pericarp of *G. mangostana* and are attribute to the medicinal properties of the fruit (Gutierrez-Orozco and Failla, 2013). The present study synthesised silver nanoparticles using aqueous extracts of fruit rind of *G. mangostana* and evaluated the antimicrobial activity against the growth of gram-positive and Gram-negative bacteria.

MATERIALS AND METHODS

Chemicals, collection and preparation of extract

All the reagents purchased were of analytical grade and used without any further purification. Silver nitrate ($AgNO_3$) was purchased from Sigma-Aldrich with a ≥99.5% purity. *G. mangostana* fruits were collected from Thrikkur Panchayath of Thrissur District, Kerala, India and were identified by the Faculty of the Department of Botany, Vimala College (Autonomous) Thrissur. Fruit rind was washed with distilled water to remove dust and particles, followed by shade-drying at room temperature. The rind was then ground to make fine powder using a blender. The powdered mangosteen rind were weighed and transferred into 500 ml beaker containing distilled water, mixed well and boiled for 25 min. The extract obtained was filtered through Whatman No.1 filter paper and the filtrate was collected in a 250 ml Erlenmeyer flask and stored in refrigerator for further use (Veerasamy *et al*., 2011). The extract was dissolved in distilled water and made to a concentration of 1mg/ml and used in further studies.

Synthesis of Silver nanoparticles

Different Molar aqueous solution of Silver nitrate (0.05 M, 0.01 M and 1 M) is prepared by dissolving $AgNO_3$ in distilled water. The rind extract of varying concentrations were added to the aqueous solution of $AgNO_3$ of different molarities. $AgNO_3$ solutions were mixed with the aqueous extract of *G. mangostana* fruit rind at a ratio of 5:2. The flask was wrapped with an aluminium foil and added 2 drops of NaOH. Reduction of silver nitrate to silver ions was confirmed by the colour change from colourless, dark yellow to deep brown. The formation of silver nanoparticles was also confirmed by UV-Vis spectrophotometric determination.

UV–Vis spectra analysis

UV–Vis spectral analysis was done by using UV-2600 230V UV–Vis spectrophotometer Shimadzu with a resolution of 1 nm between 100 and 900 nm possessing a scanning speed 300 nm/min was used. The reduction of pure Ag+ ions was monitored by measuring the UV–Vis spectrum of the reaction medium after diluting a small aliquot of the sample with distilled water. 1 ml of the sample was pipetted into a test tube and diluted with 2 ml of distilled water and subsequently analysed at room temperature.

Fixation of different parameters

Time

Following the same procedure, optimization of the time required for the completion of reaction was calculated. The reaction was monitored from 0 to 24 hrs. Silver nanoparticles formed are studied for absorbance in varying time 15 min, 30 min, 45 min, 1 h, 5 h and 24 h. The absorbance of the solutions was measured using spectrophotometer.

pH

The above procedure was followed to optimize pH, where the reaction pH was maintained at 4, 5, 6, 7 and 9 respectively. The pH was adjusted by using 0.1 N HCl and 0.1 N NaOH. The absorbance of the resulting solutions was measured spectrophotometrically.

Concentration ratio of silver nitrate and rind extract

Optimization of the ratio of silver nitrate to the varying quantity of extract was carried out using similar method. The different ratios were E0-5:0.5, E1-5:1, E2-5:2, E3-5:3, E4-5:4. The absorbance of the resulting solutions was measured spectrophotometrically.

Antibacterial activity

The antibacterial activity of synthesized silver nanoparticle was compared with Silver nitrate, Plant extract, Standard Amoxicillin (positive control), Water (negative control). The samples were tested for antibacterial activity against Staphylococcus and Pseudomonas. Antibacterial activity was evaluated using the well diffusion method on Mueller-Hinton agar (MHA) (Qais *et al*.,2018). The inhibition zones were reported in millimetre (mm). Agar wells (8°mm) were punched with the help of sterilized micropipette tips. The plates were then incubated for 24 h at 37 °C, and diameters of zone of inhibition were recorded in millimetre (mm). All tests were performed in triplicate.

RESULT AND DISCUSSION

The current study exposed a simple approach for the synthesis of silver nanoparticles by using aqueous extract of the rind of *G.mangostana* as the reducing agent. Many solvents, including methanol, ethanol, acetone, and water, have been used for extracting bioactive compounds from the plant material (Truong *et al*., 2019). In general, chemical approach that uses sodium borohydride, formamide, sodium citrate and ascorbic acid as reducing agents for NPs production is accompanied with toxicity issue (Lee *et al*., 2019). Liquids that use water solvents are much safer for health and the environment than using chemical solvents. Therefore, in this study, we used water as a solvent in plant extracts (Aritonang *et al*., 2019).

Also, different kind of flavonoids, benzophenones, and anthocyanins present in the plant may be involved closely in the reduction of nanoparticles (Lee *et al*., 2016). Phenol compounds present in plant extracts might have great role in the green synthesis of nanoparticles due to their high antioxidant activity. High amounts of phenolic compounds were evaluated in the aqueous extract of rind of *G. mangostana* which enhances the IP synthesis of nanoparticles (Burlacu *et al*., 2019). Different experiments were tried for the synthesis of silver nanoparticles using rind extracts of *G. mangostana*. However, different controlling parameters, such as metal salt concentration, mixing ratio of biological extract and metal salt, pH value, temperature, incubation period and aeration, require optimization for producing homogenous nanoparticles of a similar size and shape (Khandel *et al*., 2018). In a study, based on the UV-Vis results an increased in the amount of *G. mangostana* peel powder and concentration of $AgNO_3$ increased the peak intensity of UV-Vis spectra. This result suggests that higher amount of *G. mangostana* peels powder and concentration of AgNO3 speed up the formation of AgNPs (Lee *et al*., 2019). As the temperature was increased a blue shift occurs due to the reduction on the size of the particle from large sized to small size (Ndikau *et al*., 2017).

To the combination of extract and silver nitrate (5:2), NaOH was added. The addition of NaOH in the mixture resulted in the colour change and formation of nanoparticles in the three Molar concentrations of silver nitrate studied. But the better results were shown by 0.01 M concentrationat 413 nm (Figure 1). The UV-Vis spectroscopic studies show there

is a tendency for production of AgNPs in heated samples at 0.01 M concentration of silver nitrate at 429 nm (Figure 2).

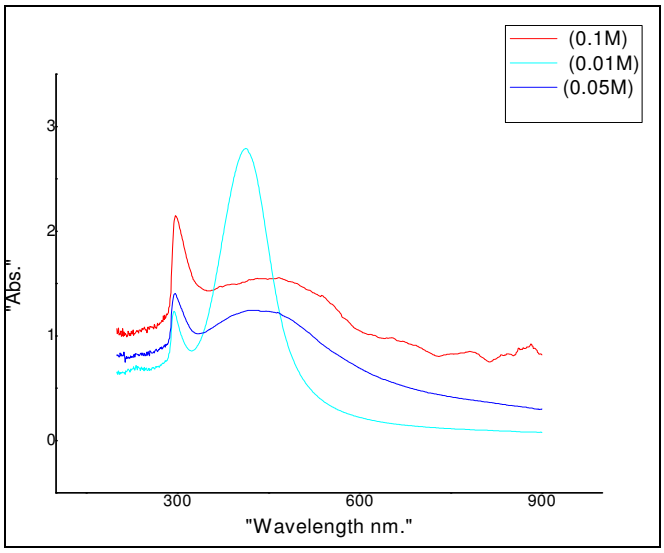

Fig. 1 : UV-Visible spectrum of synthesised silver nanoparticles with the addition of NaOH.

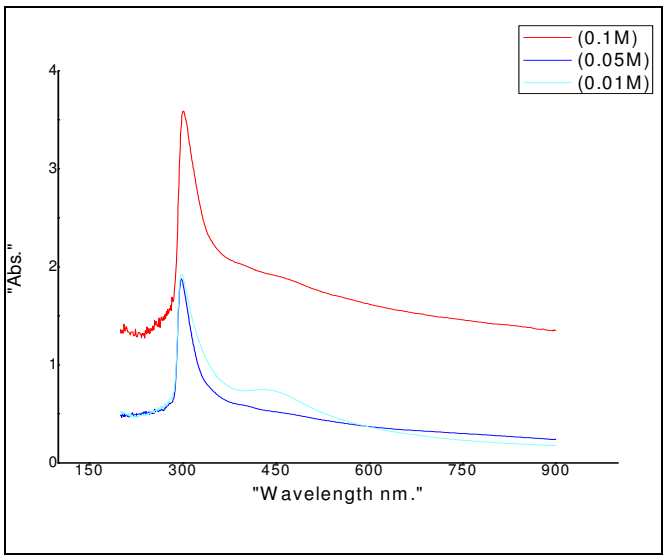

Fig. 2 : UV-Visible spectrum of synthesised silver nanoparticles at different molarities.

Different parameters were optimized like pH, time and concentration of extract. The first factor studied was pH. The possible mechanism of smaller size synthesis of AgNPs under alkaline condition is due to the reduction of silver ions by electrons provided by OH- ions. The size of the particles decreased with increase in alkaline condition may be related to the growth of the silver nanoparticles nucleus, whereas at lower pH, nucleation occurs, resulting in silver nanoparticles synthesis were favoured (Phanjom and Ahmed, 2017). It is seen that at pH 7 the maximum absorbance is obtained at 414 nm (Figure 3).With the increasing of pH, the reaction rate increases and particle size decreases(Marciniak *et al.*, 2020). At acidic medium (pH 4), the formation of nanoparticles is not observed.

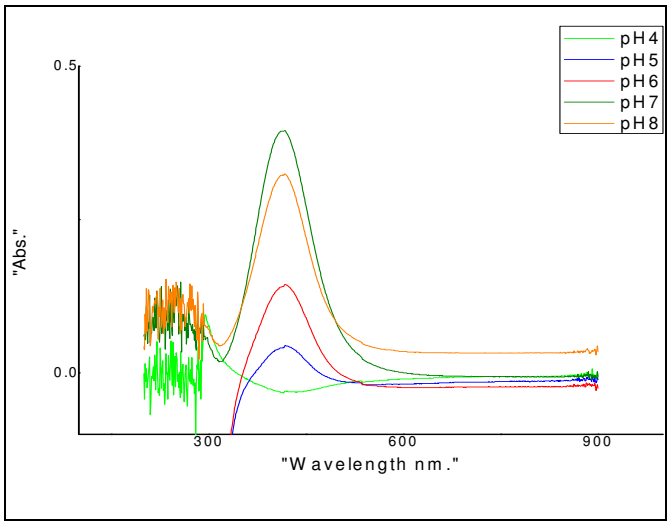

Fig. 3 : Effect of pH variation in synthesis of silver nanoparticles.

The optimum time required for the completion of reaction from our study is 15 minutes. The quality and type of nanoparticle synthesised using green technology are greatly influenced by length of time. A satisfactory result in the synthesis of silver nanoparticle is obtained when the formed nanoparticles sustain for a long period of time without any aggregation. The synthesis of silver nanoparticle was measured in the UV-Vis spectrum at different time intervals from 5 min to 24 hrs. The maximum absorbance of silver nanoparticle was at 15 min at 410 nm (**Figure 4**). The decreased absorbance at 5 hrs and 24 hrs must be due to the aggregation of nanoparticles. The peak was going wide. This shows that within a less time the silver nanoparticles are getting formed. In a study, it is observed that the incubation period of the reaction mixture shows a gradual increase in absorbance spectrum with surface resonance Plasmon resonance band (Balavijayalakshmi and Ramalakshmi, 2017).

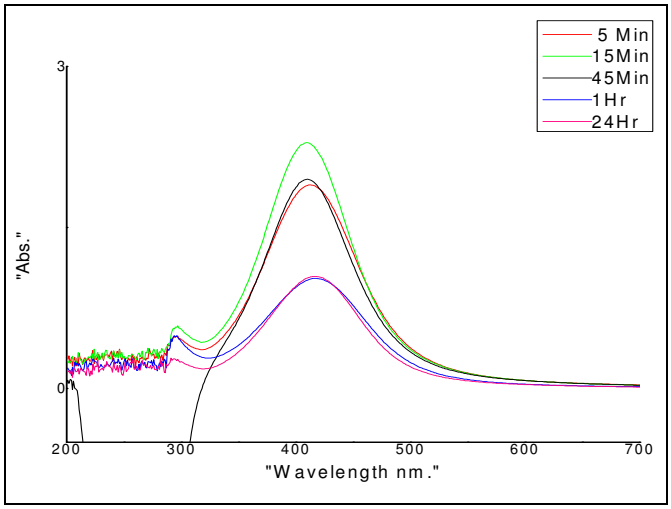

Fig. 4 : Efect of different time intrevals on synthesised silver nanoparticles.

Besides that, the ratio of rind extract to silver nitrate solution was altered to investigate the optimum composition to maximize the yield of silver nanoparticles. The absorbance shows that there is no much effect in the formation of silver nanoparticles with change in the quantity of extract. Maximum absorbance is shown at 433 nm in E 3 - 5:3 (Figure 5).

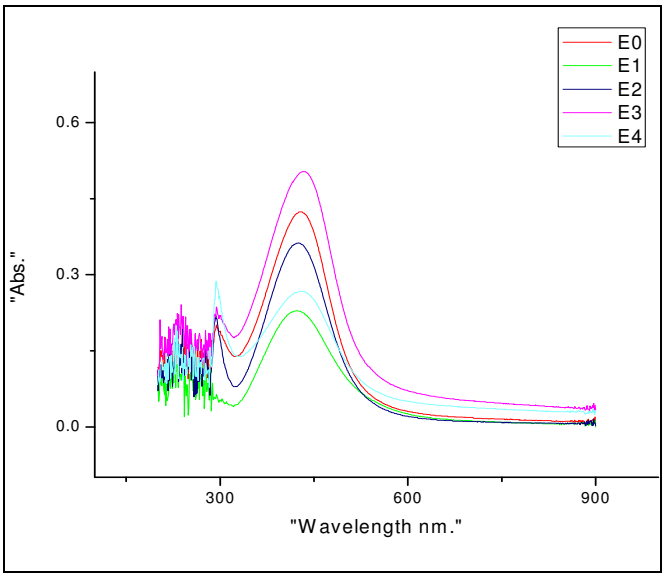

Fig. 5 : Effect of variation in concentration of extract to $AgNO_3$.

In total, the optimized condition was, time = 15 min, pH = 7, concentration of silver nitrate = 0.01 M, and the concentration ratio of silver nitrate and *G. mangosteen* rind extract =5:2.

The antibacterial activity of synthesized AgNPs is compared with Silver nitrate, standard amoxicillin, plant extract, water, against bacteria Staphylococcus sp. and Pseudomonas. Against both bacteria, AgNPs showed better zone of inhibition than the plant extract and silver nitrate tried alone (Table 1). The AgNPs from *G. mangostana* rind extracts displayed a significant zone of inhibition against both gram positive (Staphylococcus) and gram negative bacteria (Pseudomonas). It was observed that the inhibition zone against gram negative bacteria is 15.5 mm higher than that of the Amoxicillin, standard tried (Figure 6).

In order for silver to have any antimicrobial properties, it must be in its ionized form. In its ionized form, silver is inert but on coming in contact with moisture it releases silver ions (Ahmed *et al.,* 2016). The interaction of the free silver ions of silver nitrate with vital enzymes of bacteria provides antibacterial activity. Metallic silver, silver nanoparticles and sparingly soluble silver salts releases the silver ions when they come in contact with water. These ions were biochemically active agents. These silver ions will react with sparingly soluble salts, which precipitate or remain in colloidal dispersion and will also undergo complexation with proteins and other bio-molecules if they were released in the media (Karthika, 2017). The antimicrobial activity of silver nanoparticles strongly depends on the concentration of the silver nanoparticles present in the reaction mixture (Balavijayalakshmi and Ramalakshmi, 2017). The Gram-positive bacteria showed lower zone of inhibition while Gram-negative bacteria showed better results. In Gram-positive bacteria the rigid thicker peptidoglycan layer may be a reason for less zone of inhibition and it prevent the entering of nanoscaled particles in to the cell wall (Saravanakumar *et al*., 2017).

Table 1 : Zone of inhibition of Silver nanoparticles of *G. Mangostana* rind extract against bacterial pathogens *Pseudomonas* and *Staphylococcus.*

Treatments	Zone of Inhibition (mm)	
	Pseudomonas	***Staphylococcus.***
Silver nanoparticle (SNP)	15.5	13
Silver nitrate(SN)	13.5	4.5
Amoxicillin(A)	12	25
Plant extract (PE)	6.5	1.5
Water (W)	0	0

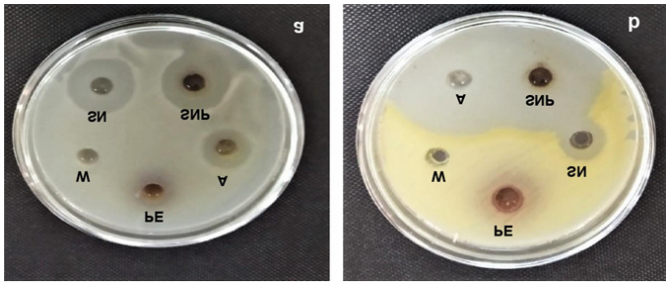

Fig. 6 : Zone of inhibition of Silver nanoparticles of *G. mangostana* rind extract against bacterial Pathogen (a) Pseudomonas (b) Staphylococcus

CONCLUSIONS

The current study exposed a simple approach to the green synthesis of silver nanoparticles using *G. mangostana* rind extract (aqueous). The biosynthesised silver nanoparticles using *G. mangostana* rind extract possess excellent antibacterial activity. The antibacterial activity is demonstrated by a considerable zone of inhibition against pseudomonas and staphylococcus. The nanoparticles were characterized using UV-Vis spectroscopy and the peak was obtained at the wave length of 410, 413, 414, 429, 433 nm in various treatments. The present study showed a simple, rapid and economical method to synthesis silver nanoparticles. Hence the use of plant extract for biosynthesis of silver nanoparticles can form an immense impact in coming decades.

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