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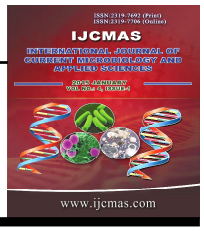


Original Research Article

Biosynthesis of Silver Nanoparticles using *Garcinia mangostana* Fruit Extract and their Antibacterial, Antioxidant Activity

Subashini Rajakannu[1]*, Sruthi Shankar[1], Sindhuja Perumal[1], Santhini Subramanian[1] and Gnana Prakash Dhakshinamoorthy[2]

[1]Department of Biomedical Engineering, SSN College of Engineering, Kalavakkam, Tamil Nadu, Chennai 603 110, India
[2]Department of Chemical Engineering, SSN College of Engineering, Kalavakkam, Tamil Nadu, Chennai 603 110, India
**Corresponding author*

A B S T R A C T

Garcinia mangostana is a medicinal plant has been used for hundreds of years in Southeast Asia as a medicine for treatment of various medical conditions. Thus, the study planned to biosynthesize silver nanoparticles using aqueous fruit extract of *Garcinia mangostana (G. mangostana)* and to evaluate their antibacterial and antioxidant activity *in vitro*. The synthesized silver nanoparticles (AgNPs) were confirmed by color transformation and Ultraviolet-visible (UV-visible) spectrophotometry. The appearance of dark brown color and UV absorption spectra range at 430nm confirmed the synthesized silver nanoparticles. The Transmission Electron Microscope (TEM) analysis showed that the sizes of the synthesized AgNPs ranged from 30 to 50nm. These biologically synthesized AgNPs were tested for antibacterial activity against three human pathogens such as *Escherchia coli, Pseudomonas auroginosa,* and *staphylococcus aureus*. The obtained nanoparticles showed significant inhibitory activity on all bacterial species. The free radical scavenging activity was assessed by DPPH (1, 1-Diphenyl-2-picrylhydrazyl) assay. The biosynthesized AgNPs showed significantly higher antioxidant activity compared to *G. mangostana* fruit extract alone. It could be concluded that the synthesis of silver nanoparticles using aqueous fruit extract of *G. mangostana* helpful for the preparation of pharmacologically useful drugs.

K e y w o r d s

Antimicrobial, Antioxidant, *Garcinia mangostana*, Silver nanoparticles, TEM

Introduction

Synthesis of noble metal nanoparticles and their description attracts an increasing interest in the field of nanotechnology because of their potential applications in various fields such as biotechnology, chemistry, physics and medicine (Jha *et al.*, 2014). Number of approaches is available for the synthesis of nanoparticles, in that synthesis by biological route (using plant extract and microorganisms) is the alternative eco-friendly method. Silver nanoparticles play a significant role in the

field of biology and medicine and show a strong toxicity over microorganisms (Klueh *et al.,* 2000). Silver nanoparticles are reported to possess antimicrobial (Samberg *et al.,* 2011), anti-inflammatory (Wong *et al.,* 2009), and antiviral (Lara *et al.,* 2010) activities. However, no reports demonstrate the aqueous fruit extract of *Garcinia mangostana* derived silver nanoparticles and its effect on human pathogens.

Garcinia mangostana (G. mangostana) linn belongs to the family of Guttiferae, has many properties such as antibacterial, anti fungal, anti oxidant, anti inflammatory, anti tumor, cosmetic uses, medicinal uses, oral and pharmacological uses. It is a tree 7-8m high, known only from cultivation in South East Asia and subsequently taken by man to other parts of the tropic (Chaverri *et al.,* 2008). Fruit rind of the *G. mangostana* contains 7–13% of tannin, and a premier source where xanthones are found in concentrated amounts (Jung *et al.,* 2006), which contribute in anti bacterial and anti oxidant properties. Green synthesis is one effective method of obtaining silver nanoparticles from the plant extract of *G. mangostana*. The reducing property of *G. mangostana* extract is due to the presence of secondary metabolites xanthone derivatives. Therefore, the study planned that preparation of silver nanoparticles using *G. mangostana* would be useful to develop new antimicrobial and antioxidant drugs.

Materials and Methods

Materials

Silver nitrate was purchased from Sigma Chemicals Company. All the other chemicals were purchased from Labchem Products, Chennai. The fresh fruit of *G. mangostana* (*G. mangostana* L.) were purchased from local market in Chennai,Tamil Nadu. The bacterial cultures such as *Escherichia coli* (*E.coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Staphylococcus aureus* (*S. aureus*) were obtained from Lifetech Research Centre, Chennai, India.

Preparation of plant extract

The rind of the fresh fruit of *G. mangostana* is separated and washed thoroughly with distilled water. Then it is dried at 50^{o}C in a hot air oven. The dried rind was ground in a domestic mixer and stored at 4^{o}C for further processing. About 2.5g is weighed and transferred into 500ml beaker containing 100ml of distilled water, mixed well and it is boiled in water bath for 1 hour. The extract obtained was filtered using Whatmann No.1 filter paper and the filtrate collected in a 250ml Erlenmeyer flask is cooled to room temperature and stored for further use (Ravichandran *et al* ., 2011).

Bio synthesis of silver nanoparticles

Aqueous solution (1 mM) of silver nitrate ($AgNO_3$) was prepared and used for biosynthesis of silver nanoparticles. 10 ml of *G. mangostana* fruit extract was added into 95 ml of aqueous solution of 1 mM silver nitrate for reduction into Ag^+ ions. It is then boiled for 15 minutes at 80°C. Reduction of silver nitrate to silver ions was confirmed by the color change from light to dark brown. The formation of silver nanoparticles was also confirmed by UV-visible spectrophotometric analysis. The fully reduced solution was centrifuged at 5000 rpm for 20 minutes. The particles settled down are thoroughly washed with distilled for 2 or 3 times to remove the extract from it and dried in hot air oven. The prepared silver nanoparticles are then stored for further purposes (Rajasekar *et al.,* 2013*)*.

Characterization of silver nanoparticles

UV–Visible spectra analysis

UV–Visible spectral analysis was done by using Jasco V-630 and the sample was scanned between 300 and 700 nm at a scanning speed of 300 nm/min. 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 into deionized water. One milliliter of the sample was pipetted into a test tube and diluted with 4 ml of deionized water and subsequently analyzed at room temperature. The nanoparticle solution showed maximum absorbance at 430 nm (Leela and Vivekanandan, 2008).

Transmission electron microscopy (TEM)

Samples for TEM analysis were prepared by placing a drop of the silver colloidal solution on a TEM copper grid. The films on the TEM grids were dried and the excess solution was removed using blotting paper. The instrument was operated at an accelerating voltage of 80KV. TEM measurements were performed and the size, shape of the bioreduced silver nanoparticles was obtained from TEM image (Elavazhagan and Arunachalam, 2011).

Anti-bacterial studies

Anti-bacterial activity of aqueous *G. mangostana* fruit extract and silver nanoparticles was determined by agar well diffusion method for *Escherichia coli, Pseudomonas aeuruginosa and Staphylococcus aureus.* The medium was sterilized at 120°C in autoclave. Than the medium was transferred into sterilized Petri plates and kept at 37°C for solidification. The bacterial strains were spread on the Petri plates using loop. On each plate, a single well of 3 m diameter was made using a gel punch. The *G. mangostana* extract and its silver nanoparticles (5ul) were added into the wells. The plates were incubated at 37°C for 24 hrs. The experiments were carried out in triplicates and the zone of inhibition was measured (Chakraborthy, 2008).

Antioxidant property

The antioxidant activity was determined by *in vitro* DPPH (1, 1-Diphenyl-2-picrylhydrazyl) free radical scavenging assay (Ara and Nur, 2009). The free radical scavenging capacity of the aqueous extract of *G. mangostana* and Biosynthesized AgNPs was determined using DPPH assay. DPPH solution (0.004% w/v) was prepared in 95% methanol. The extract of *G. mangostana and* Biosynthesized AgNPs was mixed with 95% methanol to prepare the stock solution (10mg/100mL). From stock solution 2mL, 4mL, 6mL, 8mL & 10mL were taken in five test tubes & by serial dilution with same solvent were made the final volume of each test tube up to 10mL whose concentration was then 20μg/mL, 40μg/mL, 60μg/mL, 80 μg/mL & 100μg/mL respectively. Freshly prepared DPPH solution (0.004%w/v) was added in each of these test tubes containing *Extract and biosynthesized AgNPS (*20 μg/, 40μg/mL, 60μg/mL, 80μg/mL, 100μg/mL) and after 10 min, the absorbance was taken at 517 nm using a UV–visible spectrophotometer). Control sample was prepared without adding any extract and nanoparticles. 95%methanol was used as blank (Subashini and Rakshitha, 2012).

The Scavenging activity of DPPH (%) was calculated by using the following equation

% DPPH radical scavenging = [(Absorbance of control -Absorbance of test Sample) / (Absorbance of control)] x100

Statistical analysis

Values are the mean ± SD; (n=3). The levels of significance were:*P<0.05. Data within the groups are analyzed by paired sample T test in SPSS20.

Results and Discussion

The present research work used *G. mangostana* fruit for the biosynthesis of silver nanoparticles and studied their effect on microbial growth. The fruits of *G. mangostana* was dried and ground to fine powder before subjecting to crude phytochemical extraction. As the *G. mangostana* extract was mixed with aqueous solution of 1mM silver nitrate it started to change colour to reddish brown due to reduction of silver ions; which indicates the formation of silver nanoparticles which is represented in (Figure 1A & B). The color turned to reddish brown and this change has been observed in several investigations (Khandelwal *et al.,* 2010; Saxena *et al.,* 2010)

The results of phytochemical analysis of the extract and AgNps are shown in (Table 1), which indicates the presence of secondary metabolites such as Tannins, Saponins, Flavonoids, Proteins, Anthraquinones, Carbohydrates and Ascorbic acid, Phenol. The presence of phenolic compounds constitutes a major group of compounds that act as primary antioxidants which are mainly responsible for the reducing property of the extract (Obolskly *et al.,* 2009).

The reduction of silver ions to silver nanoparticles by *G. mangostana* extract was measured using UV-visible spectrophotometry. It was observed that the initial color of silver nitrate treated *G. mangostana* extract turned from light to dark brown after the reaction. The color transformation of *G. mangostana* extract treated silver nitrate might be due to vibrations in surface Plasmon of silver (Abdel Aziz *et al.,* 2014). The synthesized silver nanoparticles maximum range was measured using UV-visible spectrophotometry. The peak located at 430nm (Figure 2) indicates the reduction of Ag+ ions which further confirmed the formation of silver nanoparticles. It is correlated to the findings of (Caroling *et al.,* 2013), who have reported that the noble metal silver displays characteristic absorbance at around 410-430nm. It has been suggested that Polyol components, flavonoids and terpenoids are mainly responsible for the reduction of silver ions (Huang *et al.,* 2007). Thus the study suggests that aqueous active components like flavonoids, xanthones, and tannins of *G. mangostana* extract might reduce silver ions.

Transmission electron microscopy analysis proved the formation of silver nanoparticles, shown in (Figure 3). TEM analysis reveals that the AgNPs are predominantly spherical in morphology which correlated with the results of (Ravichandran *et al.,* 2011). The green synthesis of noble nanoparticles using plant or fruit extracts lead to the formation of crystalline nanoparticles with variety of size and shapes ranges from 1 to 100nm. In the present study the TEM analysis revealed that the size of AgNPs ranged between 30 and 50nm.

The antimicrobial activity of *G. mangostana* fruit extract and biosynthesized AgNPs was determined by agar well diffusion method to distinguish the antimicrobial activity. Antimicrobial activity was carried out on three different pathogens, such as of *Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus* (Rai *et al.,* 2009).

Table.1 Phytochemical screenings of plant extract and bio synthesized nanoparticles

S. No	Chemical Constituents	Phytochemical Screening	
		Plant extract	AgNps
1.	Tannins	+	+
2.	Saponins	+	+
3.	Flavonoids	+	+
4.	Proteins	+	+
5.	Steroids	+	+
6.	Anthraquinones	+	+
7.	Phenol	+	+
7.	Carbohydrates	+	-
8.	Ascorbic acid	+	+

+ present; - absent

Figure.1A *G. mangostana* extract **B.** Biosynthesized AgNPs

Figure.2 UV–visible spectra of synthesized AgNPs

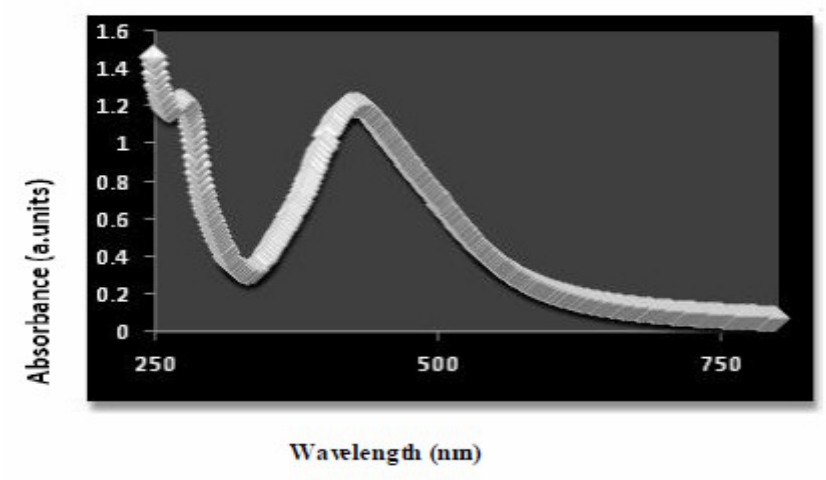


Figure.3 TEM analysis of Silver nanoparticles synthesized by *G. mangostana* extract at 39000x magnification showing particle size less than 50nm

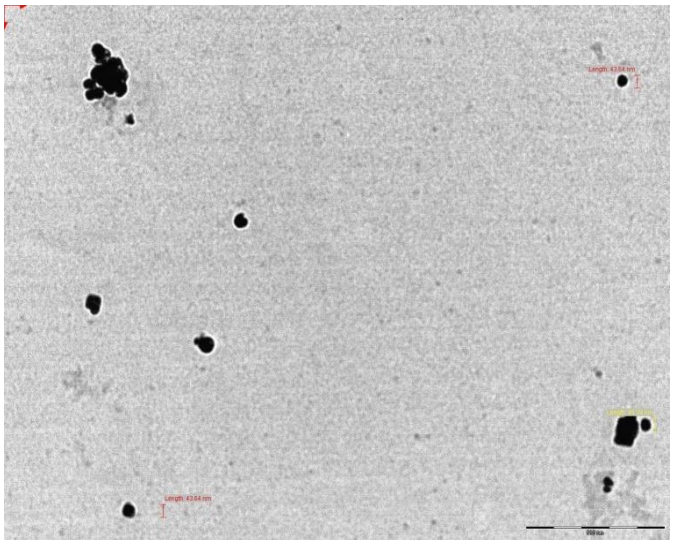
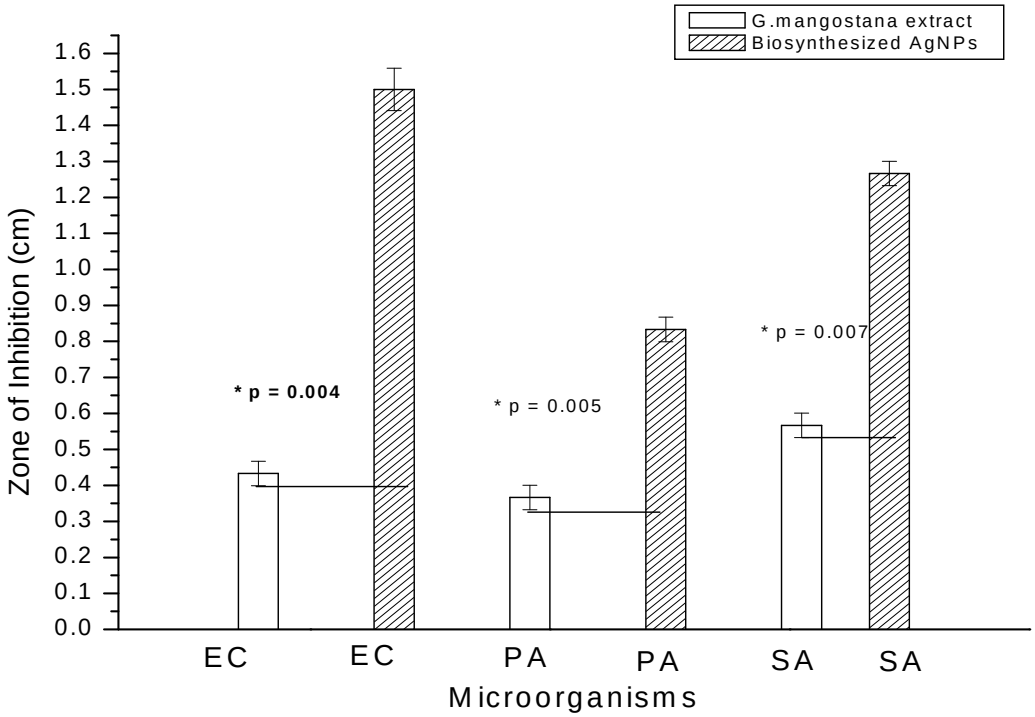
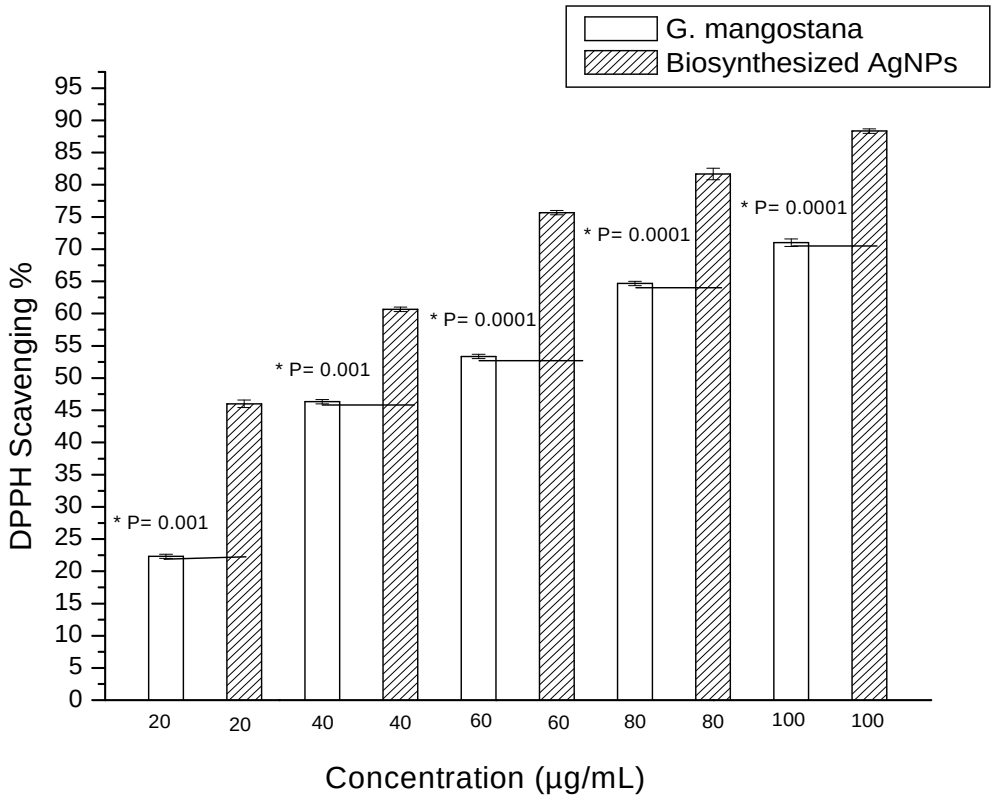


Figure.4 Effect of *G. mangostana* and silver nanoparticles on the growth of bacterial species



Values are the means ± SD; (n=3). The levels of significance were*P<0.05. Data within the groups are analyzed by paired sample T test. (EC - *Escherichia coli*, PA - *Pseudomonas aeruginosa*, SA -*Staphylococcus aureus*)

Figure.5 Antioxidant activity of Aqueous and AgNPs extracts of *G. mangostana*



Values are the means ± SD; (n=3). The levels of significance were:*P<0.001 and:*P<0.0001. Data within the groups are analyzed by paired sample T test.

Biosynthesized silver nanoparticles showed clear zone of inhibition as the values are indicated in (Figure 4). *G. mangostana* derived silver nanoparticles displayed significant inhibition *(E. coli* ($p<0.004$), *P. aeruginosa* ($p<0.005$) and *S. aureus* ($p<0.007$) on the growth of bacterial species when compared with *G. mangostana* extract treated cultures. It has been reported that AgNPs attach to the surface of the cell membrane, disturbs its function and penetrates directly with the bacterial outer membrane and releases Ag ions (Patil *et al.,* 2012). This study suggests that the membrane interacting ability and its function modulating nature of silver nanoparticles could be the reason for bactericidal activity of *G. mangostana* reduced silver nanoparticles

The antioxidant activity of *G. mangostana* fruit extracts and biosynthesized AgNPs was evaluated by using DPPH scavenging assay. As shown in (Figure 5), a significant difference was observed among the respective values obtained. The DPPH values were increased in a dose dependent manner. The recorded value for the lowest concentration of the aqueous extract (20 µg/mL) was 22.33 ± 0.33 and this value was significantly increased to 71 ± 0.58, in higher concentration (100 µg/mL).

However, these values recorded 46 ± 0.58 and 88.33 ±.033 for the two concentrations of the biosynthesized AgNPs respectively indicating that the plant AgNPs possessed a significantly [20μg/mL ($p<0.001$), 40μg/mL ($p<0.001$), 60μg/mL ($p<0.0001$, 80 μg/mL ($p<0.0001$) and 100μg/mL ($p<0.0001$)] higher scavenging activity when compared to the *G. mangostana* fruit extract alone. It has been reported that the antioxidant activity of *G. mangostana* fruit extract is due to the presence of phenolics and it is responsible for redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Arasali and Kadimi, 2009). Moreover, the results also indicated that the increase in antioxidant activity of biosynthesized AgNPs, compared to plant extract suggested that the plant extract itself is responsible for activity and silver nanoparticles enhances more antioxidant activity.

In conclusion, the current study exposed a simple approach for the biosynthesis of Ag nanoparticles by using aqueous extract of the fruits of *G. mangostana* as the reducing agent. The biosynthesized nanoparticles have been characterized by TEM, and UV- VIS spectroscopy. The AgNPs are crystalline in nature and the size of silver nanoparticles is in the range 30nm-50 nm. The biosynthesized AgNPs have antibacterial activity and antioxidant effect and proved to be probable candidates for medical applications where antioxidant and antimicrobial activities are highly essential. Hence the synthesized nanoparticles have possible applications in the medical field especially in drug delivery process.

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