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# Alpha-mangostin, the Major Compound from *Garcinia mangostana* Linn. Responsible for Synthesis of Ag Nanoparticles: Its Characterization and Evaluation Studies

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## ABSTRACT

In nanotechnology, nanoparticles have terrific application in biomedicine due to its novel properties and its eco-friendly nature. The present study reports a facile biosynthesis of silver nanoparticles from the rind extract of *Garcinia mangostana*, a medicinal plant which acts as a reducing agent. The Surface Plasmon Resonance (SPR) of the silver nanoparticles was characterized using UV-V is spectrophotometer and observed at 460 nm. The nanoparticles were found as spherical and the particle size ranges from 30-40 nm from the Scanning Electron microscopy. The XRD data (X-ray diffraction) shows the crystalline nature and the presence of elemental silver in the synthesized nanoparticles were observed using EDX (Energy dispersive X-ray spectrophotometer) analysis. The interaction of nanoparticles with the extract of *G. mangostana* was carried out by FT-IR (Fourier Transform Infra red spectroscopy). Various compounds in the rind extract were identified by Thin Layer Chromatography (TLC). Using High Performance Liquid Chromatography (HPLC), the percentage of the compound was analyzed. Then the total number of compounds in methanolic extract of the rind and the molecular weight of particular compound were carried out by Gas Chromatography-Mass Spectroscopy (GC-MS). Finally, the activity of crude extract and synthesized nanoparticles was tested against the human pathogens *E. coli, Bacillus subtilis* and *Aspergillus niger*. Various concentrations of the nanoparticle in colloidal form were used to analyze the activity starting from 20 μL for bacteria and 100 μL for fungi. The silver nanoparticle of the rind extract of *Garcinia mangostana* exhibited strong bactericidal and fungicidal activity which was observed by clear zone of inhibition and it has wide application in the field of medicine.

**Key words:** Rind, silver nanoparticles, mangostin, *Garcinia mangostana*, HPLC antimicrobial activity

## INTRODUCTION

Medicinal plants constitute a very important "National Resource" because India has one of the richest plant based ethno-medical tradition in the world and Nanotechnology is the combination of chemistry, physics and biology that deals with creating objects less than 100 nm in dimension. In ancient days nanoparticles have been prepared by physical and chemical methods but in the past few years research on nanoparticles have been emerging particularly, metallic nanoparticles

[i.e.] silver nanoparticle shows commercial demand (Satyavani *et al.*, 2011) due to their valuable properties like, sensors (Mehta *et al.*, 2010), micro-electronics (Bar *et al.*, 2009; Schimd, 1992), photo catalysis (De Heer, 1993; Bawendi *et al.*, 1990), lithography (Kayanuma, 1988; Xia *et al.*, 1999) especially antimicrobial activity (Shipway *et al.*, 2000) these potential applications used to develop the novel technology and provide a new scope in the field of medicine.

*Garcinia mangostana* Linn, belongs to the family Clusiaceae/Guttiferae. It is a tropical plant commonly known as mangosteen found in Southeast Asia. The plant is rich in xanthones (Sondi and Salopek-Sondi, 2004) and is known to contain a wide range of naturally-occurring polysaccharide (Bennett *et al.*, 1989). Xanthone compounds have anti-inflammatory (Merza *et al.*, 2004), antioxidant (Chomnawang *et al.*, 2005), anti-proliferate (Jung *et al.*, 2006; Ji *et al.*, 2006) immunostimulatory (Pedraza-Chaverri *et al.*, 2009), antiplasmodial (Matsumoto *et al.*, 2003) and strong antibacterial activity.

Rind of *Garcinia mangostana* used for the synthesis of silver nanoparticles. The rind extract contains various compounds (Jung *et al.*, 2006) like tannins and saponins (Ngamsaeng *et al.*, 2006). Among them, particularly some compounds may be act as reducing agent of silver ions. After synthesis, the nanoparticles were confirmed using UV-Vis spectra (Cruz *et al.*, 2010) and the shape was outhunted by using scanning electron microscopy (Ramgopal *et al.*, 2011). The major compound in the rind extract which is responsible for nanoparticle synthesis is analyzed using Thin layer chromatography. It is an important technique for finding potential new compounds for therapeutic use. Then, the responsible agent for silver nanoparticle synthesis was studied by HPLC (Bhaskara *et al.*, 2011) and Mass Spectroscopy (MS). The possible functional groups of silver nanoparticle were identified by FT-IR. The nature and elements present in nanoparticle were detected using XRD (Mallikarjuna *et al.*, 2012) and EDX (Cruz *et al.*, 2010), respectively. Finally, screening of antimicrobial activities was carried out for the crude extract and silver nanoparticle from the rind (Kannan and Subbalaxmi, 2011). Due to the unique properties of the rind, *Garcinia mangostana* has been used in Indian folklore as a remedy for skin diseases, dysentery and for healing wounds (Ji *et al.*, 2006). In the present investigation, this study was undertaken with the aim to isolate, purify the bioactive compound responsible for synthesis of silver nanoparticle and to characterize the nanoparticles from the rind extract of *Garcinia mangostana*.

## MATERIALS AND METHODS

The total experimental work was carried out at Sri Paramakalyani Center for Environmental Sciences, MS University from November 2010 to November 2011.

**Materials:** All the chemicals used for extraction and screening of phytochemicals were of analytical grade from Merck Limited, Mumbai, India. Silica gel used for Thin-layer Chromatography (TLC) was bought from Loba Chemie Pvt. Ltd. Mumbai, India. Mueller Hinton Agar, Nutrient agar and Nutrient broth used for antimicrobial assay was purchased from Himedia Laboratories, Mumbai, India. Standard Mangostin (97% purity) was purchased from C-Tech Environmental lab Chennai, Tamil Nadu. The rind of *Garcinia mangostana* was purchased from a local market at Courtallam and authentically confirmed by Dr. Wessley, C-Tech, Environmental lab Chennai.

### Methods

**Preparation of the extract:** Rind of *Garcinia mangostana* was washed thoroughly to remove the impurities and air dried for 10-20 days. Then it was grinded well in ordinary flour mill and the grinding was repeated for 2-3 times to obtain fine powder and sieved using a 20-mesh size

to get particles of uniform size range. From this, various concentrations like 2.5, 5 and 7.5 g were taken and boiled in 100 mL distilled water at 40-50°C. The resultant extract was filtered with the help of Whatman No. 1 filter paper and stored at 4°C for further studies.

**Phytomediated silver nanoparticles:** Synthesis of silver nanoparticles using the rind of *Garcinia mangostana* requires 1 mM silver nitrate and the rind extract was taken from 7.5 g of rind powder. For the reduction of silver ions, 10 mL of rind extract was mixed with 90 mL of aqueous silver nitrate solution. After 15 min, a brown color is obtained which indicates the formation of silver nanoparticle. Then the reduced solution was centrifuged at 10,000 rpm for 30 min. The supernatant was discarded and pellet obtained was centrifuged with water repeatedly to get pure nanoparticles.

**Characterization studies**

**UV-visible spectral analysis:** The initial characterization of silver nanoparticles was analyzed using UV-Visible spectroscopy. The reduction of silver ions was monitored periodically by measuring the spectra of the test samples and it was observed on a Perkin Elmer UV-Vis spectrophotometer with the scanning speed of 240 nm $min^{-1}$ and 1 nm resolution. The range of wavelength is from 300-700 nm. Double distilled water was used as the blank.

**Scanning electron microscope:** SEM is a powerful tool to characterize and find out different shapes of nanoparticles by forming a focused beam of electrons that was scanned over the sample and some desired signal was collected to form a particular image. Silver nanoparticles were prepared by carbon-coated copper grids.

**Energy dispersive X-ray (EDX):** It is an analytical technique to identify the elemental composition of the specimen. It utilizes x-rays that are emitted from the specimen when bombarded by the electron beam. It is the most useful instrument for investigations of boundary equation. This particular element analysis was carried out in Philips XL-30 to confirm the presence of elemental silver.

**X-ray diffraction (XRD):** The nature of silver nanoparticles was analyzed using X-Ray diffraction. These were performed using the instrument BRUKER D8 advance model. The X-ray patterns were obtained in 2 theta configuration and the range was selected from 20° and 80°. The obtained result was compared and confirmed with the standard file of XRD.

**Fourier transform infra red spectroscopy (FT-IR):** The IR spectrum was carried out to recognize the chemical change of the functional group which was involved in bioreduction. The powdered form of silver nanoparticles from the rind powder was subjected to analysis. Potassium bromide (KBr) coated Germanium is used for beam splitting and the measurements were carried out by FT-IR Shimadzu. The range of reflection mode is from 4000 to 400 $cm^{-1}$ with a resolution of 4 $cm^{-1}$.

**Identification of phytochemicals:** Phytochemicals are secondary metabolites synthesized by plants. The presence of various secondary metabolites such as steroids, flavanoids, alkaloids, terpenoids, quinones and glycosides in the crude extract of *Garcinia mangostana* was analyzed by standard method (Sadasivam and Manickam, 1996).

**Screening of bioactive compound:** Thin layer chromatography was carried out using a thin layer of silica gel (60F254). The thickness of the chromatographic plate coated with silica gel was 0.2 mm. All chromatograms were developed in a glass beaker at room temperature. Among different solvent systems tested by trial and error method, toluene: methanol (8:2 v/v) was found as suitable mobile phase for separation of compounds.

**Determination of purity of bioactive compound:** The purpose of HPLC was to confirm the purity of a compound and to provide the quantitative results. It was equipped with an UV-visible spectrometer set at 210 nm. The chromatographic separation was performed at room temperature, C18 analytical column (25 cm X 4.6 mm, 5 μm) injection volume 20 μL at flow rate of 1 mL $min^{-1}$ and the mobile phase was Acetonitrile: Water (80:20).

**Gas chromatography-mass spectrometry (GC-MS):** GC-MS is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. The instrument used was Shimadzu GC-17A equipped with QP-5000 mass spectrometry and the column was packed with 5% diphenyl dimethylsiloxane. The temperature of column was 70°C and the injected volume is 0.1 mL. Mass spectrometer is an accurate method to determine the molecular mass of the bioactive compound. It ionize, accelerate, deflect and detect the ionized molecules separately and gives the mass of the compound. The spectra were recorded in the range of 40-500 nm.

**Inoculum preparation:** The bacterial strains used were *E. coli*, *Bacillus subtilis* and fungal strain used was *Aspergillus niger*. The strains were obtained from the Department of Microbiology, Kamaraj College, Thoothukudi, Tamil Nadu. Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller Hinton Broth (MHB) for bacteria and Potato Dextrose Broth (PDB) for fungi. The cultures incubated for 24 and 48 h at room temperature for bacteria and fungi.

**Antimicrobial activity:** Agar well diffusion method (Perez *et al*., 1990a) for antibacterial susceptibility was carried out according to the standard method to assess the presence of antibacterial activity of the crude extract and nanoparticle. The concentration of the extract and nanoparticle used in the experiment was 20, 40, 60 and 80 μL. Wells of about 6 mm diameter were made aseptically using gel puncture instrument. The plates were swabbed with *E. coli* and *B. subtilis*. Then the plates were incubated at 37°C for 24 h and control plates were maintained separately. Antibacterial activity was evaluated by measuring the diameter of the zone of inhibition around the well.

Antifungal activity of crude extract and nanoparticle was tested against *Aspergillus niger*. Disc diffusion method for antimicrobial susceptibility testing was carried out according to Kirby Bauer method (Drew *et al*., 1972). The concentration of the extract used in the experiment was 100 μL. 15 mL of Potato Dextrose Agar medium was mixed with the crude extract and poured into the petri plate and the fungal culture was transferred to the centre part of the medium and it was incubated at room temperature for 48 h. Control plates were maintained separately. The antifungal activity of the silver nanoparticle was carried out using various concentrations (100, 200 and

300 μL) of nanoparticle. The fungus was spread on medium and the discs impregnated with nanoparticle were placed over the inoculated plate. After 48 h incubation, the ability of the fungus to spread in the medium was observed.

## RESULTS AND DISCUSSION

UV-Visible Spectroscopy is the primary method to indicate the bioreduction of silver from the aqueous silver nitrate solution to silver nanoparticles. 1 mM silver nitrate was used for the reduction of silver by rind extract of *Garcinia mangostana* at different concentrations (2.5, 3.5 and 7.5 g). Among the three concentrations, 7.5 g was suitable for nanoparticle synthesis because, the biosynthesis started within few minutes and maximum yield of silver nanoparticle was obtained. After 1 h, the intensity of colour increased from pale yellow to brown (Perez *et al*., 1990b). This current report coincides with the results done by the leaf extract of the same plant (Veerasamy *et al*., 2011) and also in the extract of Citrus lemon (Parashar *et al*., 2009; Prathna *et al*., 2011). The secondary metabolites like flavanoids, phenols, steroids, quinones etc and some of the proteins present in *G. mangostana* may also be responsible for the biosynthesis. The distinct peak of silver nanoparticle was observed at 460 nm due to surface plasmon vibrations in nanoparticles (Song and Kim, 2009; Forough and Farhadi, 2010). The broad peak in Fig. 1a. Represents that the silver nanoparticles are polydispersed and the reaction was maximum at 24 h incubation.

FT-IR measurements were carried out to recognize the possible functional groups of silver nanoparticles obtained from the rind of *Garcinia mangostana* was shown in Fig. 1b. The extract of the rind was responsible for the reduction of silver ions and also the capping agents responsible for the stability of the bioreduced silver nanoparticles.

The absorbance peak for silver nanoparticle was observed at 3315, 3199, 1672, 1456, 1400, 1336, 1191, 1122 and 601.The band at 3315 $cm^{-1}$ corresponds to O-H or N-H stretch contains alcohol, phenols, primary, secondary amines or amides, respectively (Prathna *et al*., 2011). The peak at 3199 $cm^{-1}$ belongs to O-H stretch of carboxylic acid. The prominent band obtained at 1672 $cm^{-1}$ assigned to carbonyl peak C = O stretching indicating carboxylate content in plant based samples (Prathna *et al*., 2011). The peaks near 1456 and 1400 were assumed as C-C stretching of aromatics (Dubey *et al*., 2010) or C-H bend alkanes, respectively. The band at 1336 $cm^{-1}$ assigned to N-O symmetric stretch nitro compounds. The peak 1191 $cm^{-1}$ and 1122 $cm^{-1}$ represents C-O group of polyols such as hydroxyl flavones and hydroxyl xanthones (Cruz *et al*., 2010; Huang *et al*., 2007; Roy *et al*., 2010). Mainly the polyols play a prominent role in the reduction process (Veerasamy *et al*., 2011) and the band 601 $cm^{-1}$ belongs to C-Br stretch.

The SEM image confirmed that the synthesized particles were in nano level and the range of size obtained from 20 to 50 nm spherical in shape as shown in Fig. 1c. The particles get aggregated with one another and the image was taken after 24 h, this similar result was discussed by (Chandran *et al*., 2006).

Figure 1d shows the XRD pattern of silver nanoparticles using rind of *Garcinia mangostana*. The peaks were recorded from 20°-80° at 2θ scale and predict the diffraction peaks at 38°, 46°, 64° and 77° corresponds to the crystal facets of (111) (200) (220) and (311). It has some unassigned peaks found in the graph which was due to the presence of biomolecules in the nanoparticle. As the crystallite size gets smaller, the peak gets broader due to instrumental profile, microstain etc. The report clearly exhibits that the silver nanoparticles were in crystalline nature. The obtained XRD spectra was compared and confirmed with the standard spectra of silver which was published by JCPDS file 04-0783. Thus the XRD pattern proved the presence of silver nanoparticles due to the crystal facets.

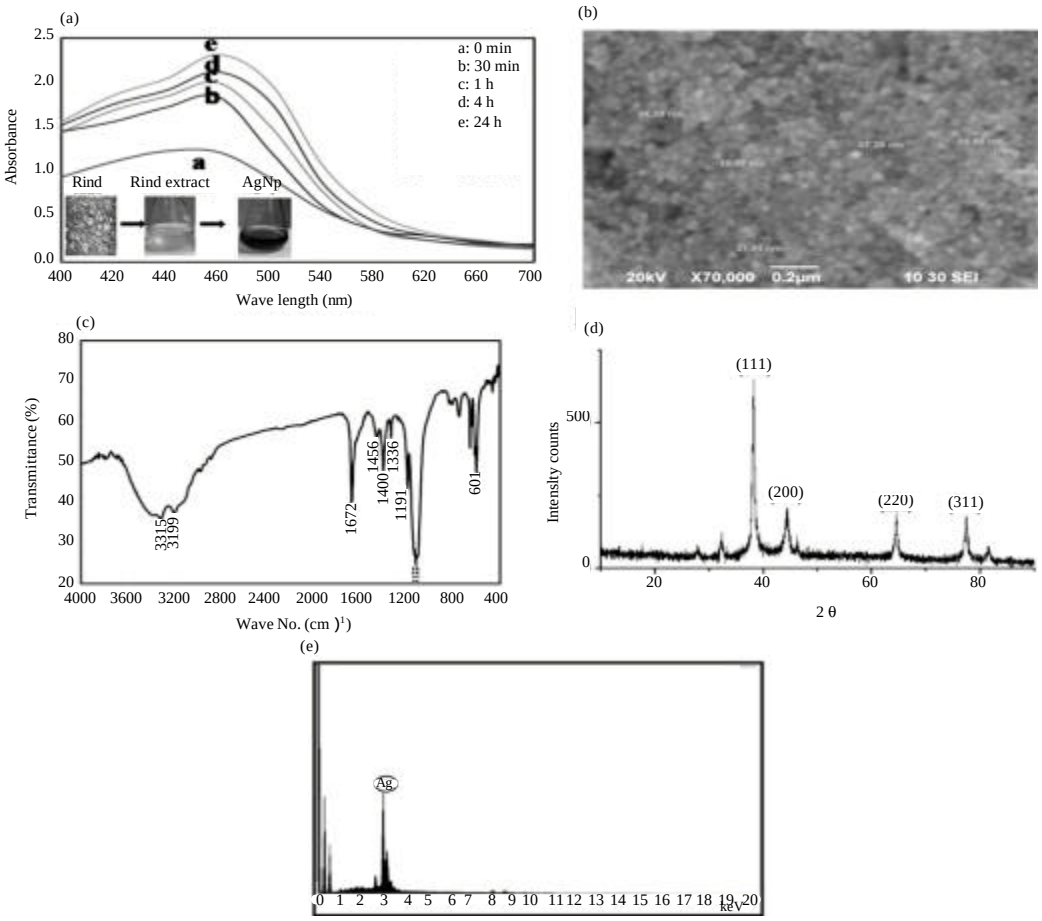


Fig. 1(a-e): Characterization studies for silver nanoparticles from the rind of *Garcinia mangostana*, (a) UV visible spectrum analysis of biologically synthesized silver nanoparticle, (b) FT-IR spectrum of purified silver nanoparticles from the rind of *Garcinia mangostana*, (c) SEM image of silver nanoparticles at X 70, 000 magnifications, (d) XRD pattern of the air dried silver nanoparticles and (e) EDX pattern for silver nanoparticles

The peak observed around 3 KeV predicts the binding energy of AgL which proves the conformation of pure silver due to surface plasmon resonance (Kalimuthu *et al*., 2008; Prathna *et al*., 2011). Some weaker atoms like Cl and O also appeared due to minor impurities shown in Fig. 1e.

Phytochemical analysis of rind extract from *Garcinia mangostana* is given in Table 1.

The presence of secondary metabolite suggests the pharmacological activities of *Garcinia mangostana*. Numerous studies prove the positive relationship of consumption of fruits and vegetables containing phenolics in the prevention of aging related diseases caused by oxidative stress (Kaur and Kapoor, 2001; Vinson *et al*., 2001). Phenolics have been recognized as natural antioxidants and have been extensively studied as antimicrobial agents, for their antiallergenic and anti-inflammatory properties along with their antimutagenic action (Rice-Evans *et al*., 1996).The

Table 1: Phytochemical analysis

| Secondary metabolites | Presence/Absence |
|---|---|
| Alkaloids | Absent |
| Coumarin | Absent |
| Flavanoids | Present |
| Glycosides | Present |
| Phenols | Present |
| Quinones | Present |
| Steroids | Present |

antibacterial and anti-inflammatory activities of rind extracts of *G. mangostana* were due to the flavonoid content especially novel flavanols and metabolites play role in the synthesis of nanoparticles mainly due to the flavanoids.

Thin layer chromatography was carried out to screen the bioactive compound and finally the percentage of particular compound was analyzed using HPLC with three different solvents like hexane, methanol and acetone. It is a highly sensitive method of detection and quantification of any chemicals in a particular sample using ultraviolet and visible absorbance, HPLC chromatogram of the standard compound mangostin is shown in Fig. 2a, b shows the HPLC chromatogram of the methanol extract of the rind. The bioactive compound mangostin was obtained with a single large peak at a retention time of 7.09 min, which was compared with the standard graph for confirmation shown in Fig. 2b. Mangostin present in all three extracts but methanol shows high yield. This particular bioactive compound mangostin was also reported in the fruit of *G. mangostana* (Walker, 2007). Some minor peaks were also observed which represents the various compounds in the rind extract. Compared to other compounds mangostin occurred 60% in the rind, therefore this compound may be responsible for the reduction of $Ag^+$ in nanoparticle synthesis.

Gas chromatography identified the total compounds present in the rind extract shown in Fig. 2c and also the molecular weight was analyzed by using the mass spectra Fig. 2d. Identification of the marker constituents was generally done by comparing their retention times with those of the reference standards injected under identical conditions in GC-MS. The sample was submitted to gas chromatography coupled with a mass spectrophotometer, Major five compounds were identified by GC-MS, retention time and molecular fragmentation. The chromatographs and the spectral analysis showed the presence of five compounds in the rind extract which was given below:

- Pentane 1,3 epoxy-methyl-(CAS) 2-isopropyl oxetane
- Undecane (CAS) n-Undecane hendecane
- Tetradecane, 1-iodo-tetradecyl iodide myristyl iodide 1-iodotetradecane
- Decanoic acid (CAS) capric acid decoic acid caprynic acid
- Iso-octane (ethenyloxy)-(CAS) Isooctyl vinyl ether

The HPLC data suggested that the compound mangostin is responsible for biosynthesis of nanoparticle. But three different types of compounds namely α-mangostin, β-mangostin and γ-mangostin are present in the rind extract with molecular weight 410, 424 and 396, respectively. The mass spectral data showed that the molecular weight of the compound is 410. Therefore, it is confirmed that α-mangostin is particularly responsible for the synthesis of silver nanoparticle.

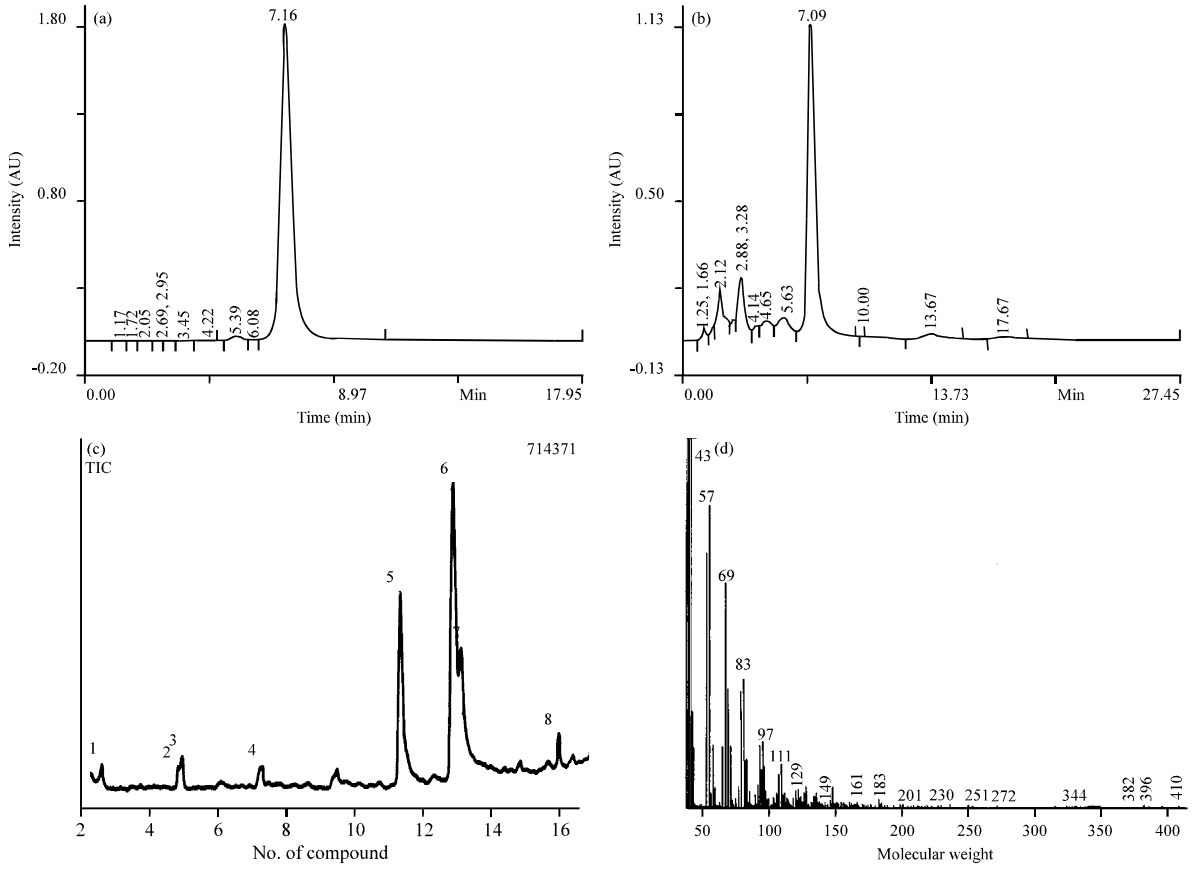


Fig. 2(a-d): Characterization studies for the crude extract (rind) of *Garcinia mangostana*, (a) Standard mangostin and its retention time was 7.16, (b) The maximum peak is the retention time for the compound mangostin, (c) The peaks are the total compounds present in the crude rind extract and (d) The molecular weight 410 confirms the compound alpha-mangostin

Antibacterial activity of the rind extract was tested against gram negative and gram positive strains such as *E. coli* and *B. subtilis* shown in Fig. 3d, e and activity of silver nanoparticle was shown in Fig. 3a, b. By agar well diffusion method, significant activity of the silver nanoparticle was observed against the tested bacterial strain. The unique character of gram negative bacteria is, it has double membrane surrounding each bacterial cell. This outer membrane excludes certain drugs and antibiotics from the cell. So these may be the reason for the susceptibility of the gram negative bacteria. The active compounds present in the rind extract were also responsible for antibacterial activity (Ushimaru *et al.*, 2007). Among the various compounds, α-mangostin plays major role in the antimicrobial activity (Iinuma *et al.*, 1996; Sundaram *et al.*, 1983; Suksamrarn *et al.*, 2003; Chomnawang *et al.*, 2005). This strong activity might be helpful in preventing various diseases.

Antifungal activity of silver nanoparticle was tested against *Aspergillus niger* shown in Fig. 3f and for rind extract by disc diffusion method shown in Fig. 3c. The activity of silver nanoparticle and rind extract was observed by the zone of inhibition. The activity is may be due to the disruption of cell wall. Most antifungal agents target the formation or function of ergosterol, the major sterol

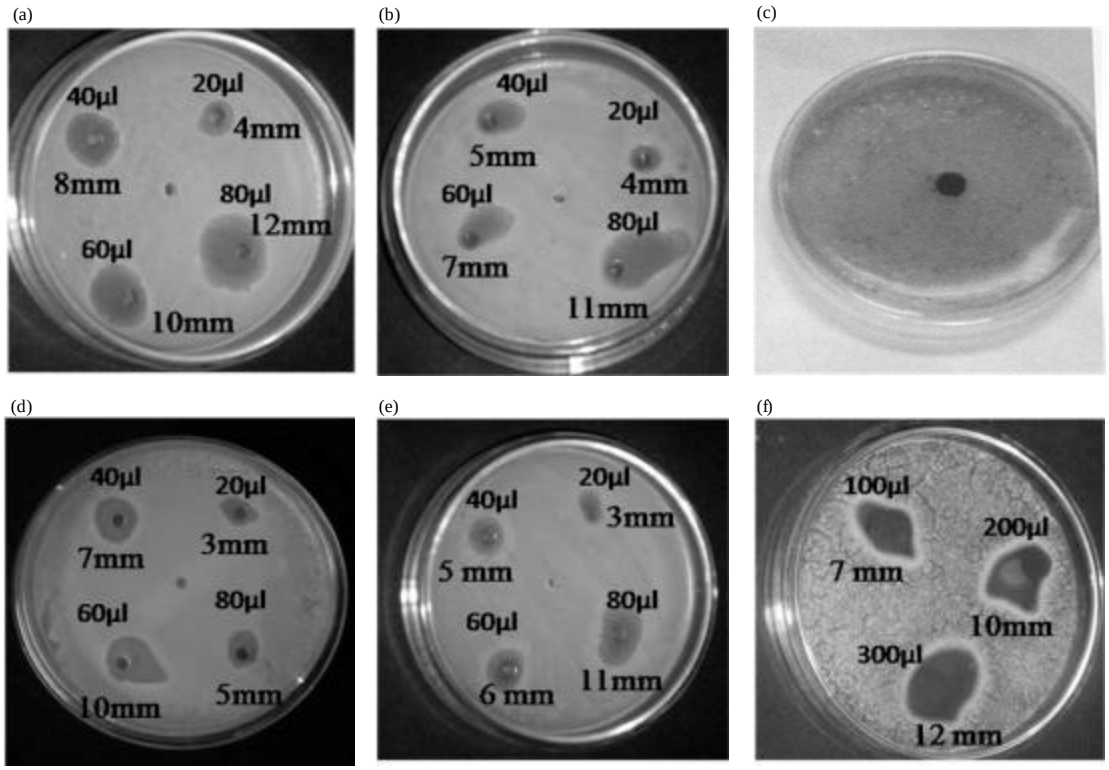


Fig. 3(a-f): Antibacterial and Antifungal Activity for the crude extract and silver nanoparticle, (a, b) Antibacterial activity of rind extract against *E. coli* and *Bacillus subtilis*, (c) Antifungal activity of rind extract against *Aspergillus niger* using potato dextrose medium shows the inhibition of growth after 48 hours of incubation, (d, e) Antibacterial activity of silver nanoparticle against *E. coli* and *Bacillus subtilis* and (f) Strong activity of silver nanoparticle against *Aspergillus niger* using Potato dextrose medium

present in the cell membrane (Ghannoum and Rice, 1999). These agents also affect the synthesis of triglycerides and phospholipids. Finally, the stability of the membrane is lost leading to damage of the cell.

## CONCLUSION

The present work concluded that *Garcinia mangostana* rind extract can be successfully used for the synthesis of silver nanoparticle as a green route. The size of particles was in the range of 30-40 nm and the morphology of silver nanoparticles was observed from the SEM imaging. FT-IR analysis indicates the possible biomolecules responsible for the reduction process is the poly phenolic group and other flavanoids and carboxylate group act as a shielding agent. Structural analysis by XRD and EDX pattern strongly suggest the formation of elemental silver, various compounds were found in the rind extract and among them the bioactive compound α-mangostin plays a vital role in the reduction of silver ions and stabilization of nanoparticles. These nanoparticles proved the excellent antimicrobial activity by the strong zone of inhibition, if increased concentration of sample

loaded it shows the decreased growth of strains. Due to its intense activity in microbiological studies can be used in the treatment of common diseases and this study shows the simple, eco-friendly process for the synthesis of silver nanoparticles.

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