

Green Synthesis of Silver Nanoparticles from *Punica granatum* L. and Its Antimicrobial Activity

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ABSTRACT

Green synthesis of nanoparticles was done from *Punica granatum* L. by using Silver nitrate ($AgNO_3$) from the exocarp, mesocarp and the juice of Aarakta, a local variety of Maharashtra, India. In this work, we have explored an inventive contribution for synthesis of silver nanoparticles using the peel (exocarp and mesocarp) and the seed juice of pomegranates. The procedures for development of stable silver nanoparticles were characterized. The synthesis of nanoparticles was confirmed through UV-Visible spectroscopy. The interaction between nanoparticles with functional groups was confirmed by using FT-IR and PCA was performed. The antimicrobial activity of these pomegranate synthesized silver nanoparticles was evaluated against different microorganisms viz. *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. Out of the three microorganisms, *Pseudomonas aeruginosa* was found to be sensitive for the extractions of pomegranate and formed the antibiotic zones nearer to standard antibiotic, Ampicillin at a quantity of 40 μ l per sample. From this study it is clear that, pomegranate nanoparticles can be used as potential antibacterial agents in future therapeutics.

Keywords: Aarakta, *Pseudomonas aeruginosa*, *Proteus vulgaris*, Pomegranate peel, Pomegranate juice

INTRODUCTION

The rapidly emerging branch of applied science, Nanotechnology has broad applications ranging from medicine to numerous industries. The tiny particles with their textural advantage are capable of extending extensive applications towards human welfare. Different types of nanoparticles are synthesized from various biological materials ranging from microorganisms [1-4] to plants [5-8]. The green synthesis of silver nanoparticles from pomegranate is reported by various groups of researchers [9-14]. The major plant parts used as substrates for the synthesis of nanoparticles are peel and seed juice. Certain studies used leaves as the starting material for the nanoparticle synthesis [10].

Punica granatum L., commonly known as pomegranate is a unique fruit known from ancient times and cultivate across Mediterranean region. Its cultivating area also extends to Himalayas and California and Arizona of USA. The seed taste, color and texture make the pomegranate as a special fruit and have a valuable global market. The alternative medicine in India from ancient times used pomegranate juice and peel for curing many diseases. Ayurveda extensively studied the medicinal properties of pomegranate and studied its action against many diseases.

The study of silver nanoparticles in pomegranate has been done previously [9,10,12,13] where as certain studies were proved with gold nanoparticles [9,11,14]. Most of these nanoparticles were characterized by UV-Spectrophotometry, FT-IR, SEM, EDAX, XRD and TEM.

The application of nanoparticles as antimicrobial agents has been studied extensively in microorganisms [3,4] and plants [5,6,8]. The antimicrobial activity of nanoparticles synthesized from pomegranate is of great importance in alternative medicine. *Pseudomonas* [9,13], *Bacillus* [10,12,13], *Staphylococcus* [10,13], *Proteus* [10,13] and *Klebsiella* [12] species were considered previously to study the antimicrobial activities of silver and gold nanoparticles synthesized from pomegranate peel, seed juice and leaves. The study of these microorganisms enhances the rate of treatment while curing the diseases associated with them.

The current study has focused on the green synthesis of silver nanoparticles from a locally available pomegranate variety “Aarakta” by using silver nitrate. The characterization of these nanoparticles were done through UV-Spectrophotometry, FT-IR and tested for their antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.

MATERIALS AND METHODS

Plant material

The plant materials were selected as pomegranate fruits and were collected from the local market of Loni, Maharashtra. Aarakta variety of pomegranate is chosen for further study. Aarakta is a dark-red colored fruit possessing dark-red seeds with sweet taste. Pomegranate fruits were collected and evaluated in the laboratory of Biotechnology, P.V.P. College, Loni, Maharashtra, India.

Preparation of seed peel and juice extract

The pomegranate fruits were washed thrice before starting the experimental part. The parts of the fruit viz. exocarp, mesocarp and seeds were separated manually. These parts weighing 10 g were selected for the synthesis of nanoparticles [15,16]. The coat part, i.e., exocarp and mesocarp were cut into small pieces and the juice was prepared from the seeds by using a mixer. All these samples were boiled in 75 ml of deionized water for 3 min. These extractions were then cooled and filtered. An extract of 50 ml from each sample is collected and stored in the refrigerator. These filtrates were used to study the nanoparticle synthesis and characterization.

Synthesis of silver nanoparticles

The nanoparticles from the pomegranate were synthesized by adding 5 ml of exocarp, mesocarp and seed extracts separately to 100 mL of 1 mM Silver nitrate. This mixture was allowed to react at room conditions for 10 to 15 min. The synthesis of nanoparticles was confirmed by observing the color change by comparing the before and after boiling stages of the solution [17].

Characterization of silver nanoparticles

The samples were centrifuged at 5000 rpm about one or two times to remove the unwanted content present in the extraction solutions mixed with silver nitrate. The samples were dried in hot air oven for about 15 min and the silver nanoparticles were obtained in powder form at a temperature of 100°C. The presence of nanoparticles was then confirmed technically by using Systronics made UV-visible spectroscopy with a quartz cuvette at a wavelength ranging 800-400 nm. The major functional groups of those nanoparticles synthesized were identified by FT-IR method. Principal component analysis (PCA) was performed by using the software Unscrambler X (version. 10.4.1; CAMO ASA, Norway) to understand the multiple variations [18].

Antibacterial activity

The microorganisms tested in this study are *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* were taken from the mother slant [19] and prepared the working test culture. By using these working test cultures, all the antibacterial tests were conducted. The modified agar well-diffusion method was performed to evaluate the antibacterial activities of the seed extracts. A freshly grown culture was serially diluted. An amount of 0.1 ml of prepared cells were aseptically spread onto the surface of nutrient agar plates and then left to dry for 30 min. Wells (4 mm in diameter) were made in media using a sterilized steel borer. Then each well was filled with 10 µl, 20 µl, 30 µl, 40 µl of silver nanoparticle samples. These plates were left at room temperature for 30 minutes to allow the process of diffusion of materials in media. Plates were incubated at 37°C for 18–24 h. After 24 h, zone of inhibition was observed. As a positive reference, the standard antibiotic Ampicillin was used.

RESULTS

Synthesis of nanoparticles

The pomegranate exocarp and mesocarp samples were separately treated with 1 mM AgNO₃ solution. Initially, both

of these solutions appeared safranin (Figures 1a and 1b) in color, but following the addition of AgNO_3 turned the color into dark brown. Following 15 to 25 min of reaction, the synthesis of nanoparticles occurred due to the reduction of Ag^+ ions. The Pomegranate juice extract appeared pink and following the addition of AgNO_3 solution the color turned to pale yellow (Figure 1c). However, the process of color change is delayed in the case of seed juice, which took around 25 min to undergo reduction.

Characterization of silver nanoparticles

Exocarp silver nanoparticles

In the exocarp, a mass of different substances was blended together which was an aqueous silver nitrate solution and *Punica granatum* exocarp extract. The sample was observed by UV-visible spectrophotometer for its maximum absorbance, which was found at 556 nm, confirming the reduction of silver ions (Figure 2a).

FT-IR measurements were carried out to identify the nature of the product and also to identify functional groups present in pomegranate exocarp extract for the reduction of Ag^+ ions to silver nanoparticles (Figure 2b). Peaks appeared at 3200-3600, 1714, 1641, 1392, 1217 and 1278. The band at 3200-3600 cm^{-1} indicates phenolic OH. The band at 1714 cm^{-1} indicate -C=O stretching vibrations carbonyls. The band at 1641 cm^{-1} corresponds to -C=C- stretching vibrations of alkanes. The band at 1392 cm^{-1} represents -C-N- stretch. The band at 1217 cm^{-1} and 1278 cm^{-1} indicate -C-O stretch that represents strong absorption.

Mesocarp silver nanoparticles

In the case of mesocarp extract combined with silver nitrate, the maximum absorbance was found at 506 nm indicating silver reduction (Figure 3a). FT-IR measurements for mesocarp extract showed peaks and appeared at 3200-3600, 2083, 1645 and 1215 (Figure 3b). The band at 3200-3600 cm^{-1} indicates phenolic O-H. The band at 2083 cm^{-1} indicates $\text{-C}\equiv\text{C-}$ stretch of alkyne. The band at 1645 cm^{-1} represent -C=C- stretch of conjugated amide. The band at 1215 cm^{-1} indicates -C-O- stretch.

Seed juice silver nanoparticles

In the case of seed juice extraction, the silver nitrate addition synthesized nanoparticles and the sample showed maximum absorbance at 453 nm confirming the reduction of silver ions (Figure 4a). FT-IR measurements for pomegranate juice showed the peaks at 3200-3600, 2088, 1643 and 1217 (Figure 4b). The band at 3200-3600 cm^{-1} indicates phenolic OH. The band at 2088 cm^{-1} indicates $\text{-C}\equiv\text{C-}$ stretching vibration of alkynes. The band at 1643 cm^{-1} corresponds to -C=O- stretch of conjugated amide. The peak at 1217 cm^{-1} indicates -C-O- stretching which represent strong absorption.

PCA

A combined FT-IR and PCA analysis was performed and the data matrix was decomposed to check the source of data variability which was concentrated in to the first PCs [18]. The scatter plots of PC1 (variability – 69%) \times PC2

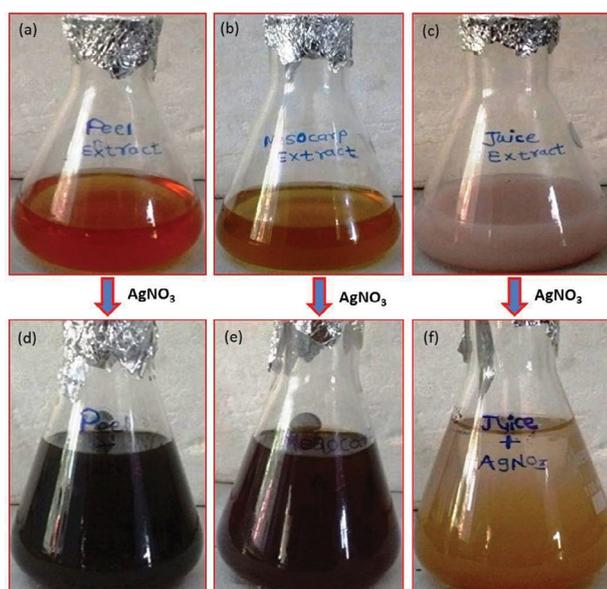


Figure 1: The synthesis of silver nanoparticles from pomegranate, Aarakta - (a) exocarp extract; (b) mesocarp extract; (c) seed extract; (d) exocarp and AgNO_3 solution after 15 min; (e) mesocarp and AgNO_3 solution after 15 min; (f) seed juice and AgNO_3 solution after 25 min

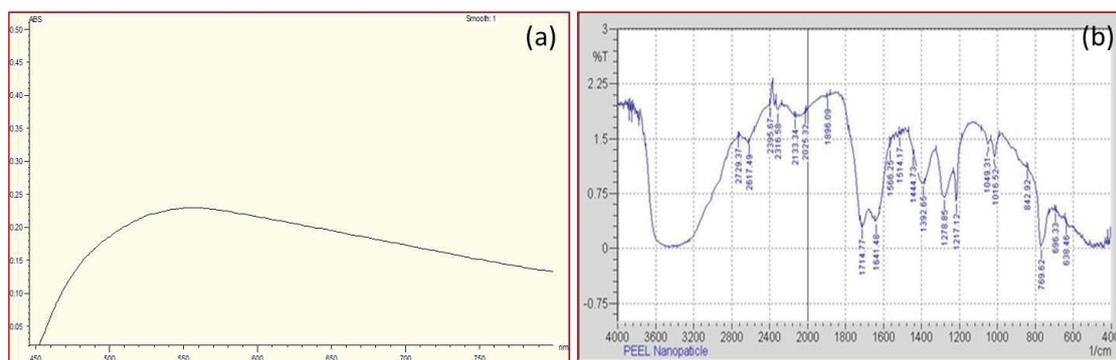


Figure 2: Characterization of exocarp silver nanoparticles by (a) UV- visible spectrophotometry and (b) FT-IR

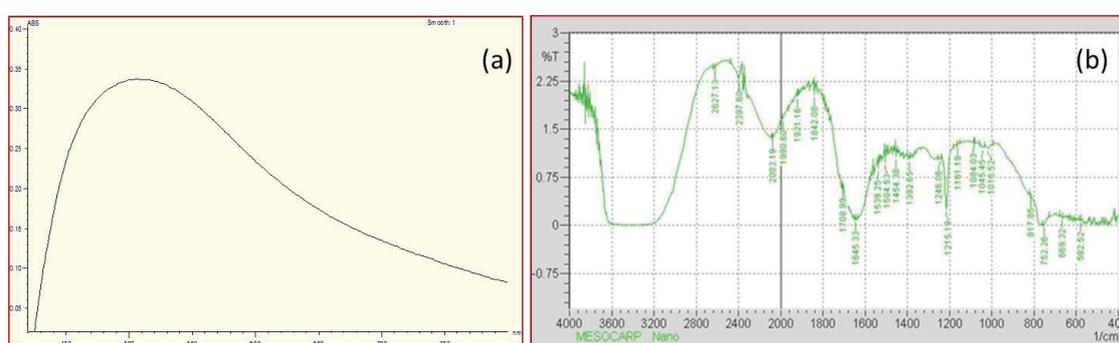


Figure 3: Characterization of mesocarp silver nanoparticles by (a) UV-visible spectrophotometry and (b) FT-IR

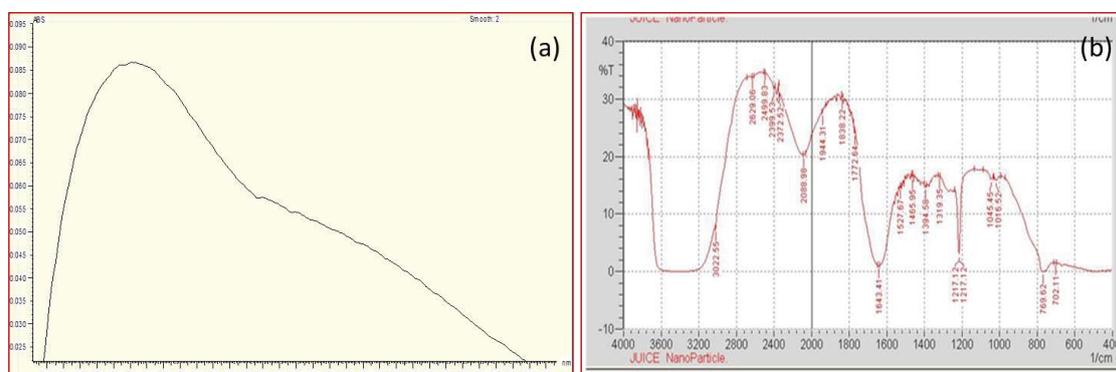


Figure 4: Characterization of seed juice silver nanoparticles by (a) UV-visible spectrophotometry and (b) FT-IR

(variability – 25%), PC2 (Variability – 25%) × PC3 (Variability – 6%) and PC1 (Variability – 69%) × PC3 (6%) were depicted and shown in Figures 5a-5c. The PC1 × PC2 scatter plot (Figure 5a) shows that the exocarp and mesocarp samples grouped separately in the negative side of PC1, whereas the seed samples were in the positive side. In the scatter plot PC2 × PC3 (Figure 5b), the seeds remained in the negative side, while the peel parts appeared in the positive side. In the Figure 5c, the scatter plot between PC1 and PC3 shows that the exocarp and mesocarp are grouped towards negative direction, whereas the seeds were directed towards positive side. In summary, PC1 (exocarp) has the highest coefficient value for the rest of the components. The first two principal components, PC1 and PC2 contain 94% of the total variation.

Antimicrobial activity

The exocarp, mesocarp and seed juice samples showed significant antimicrobial activities against different bacterial samples. The organisms, i.e., *E. coli*, *P. aeruginosa* and *P. vulgaris* were found sensitive towards these samples (Figure 6). The standard antibiotic used in this study, i.e., ampicillin showed a maximum zone of inhibition (24 mm) against *E. coli* and a minimum zone of inhibition for *P. aeruginosa* (17 mm).

The exocarp extract of nanoparticles when treated the microorganisms with the concentrations of 10 to 40 μl each, the corresponding zones of inhibitions were ranged from 8 to 16 mm (Figures 6a-6c and Table 1). The mesocarp samples

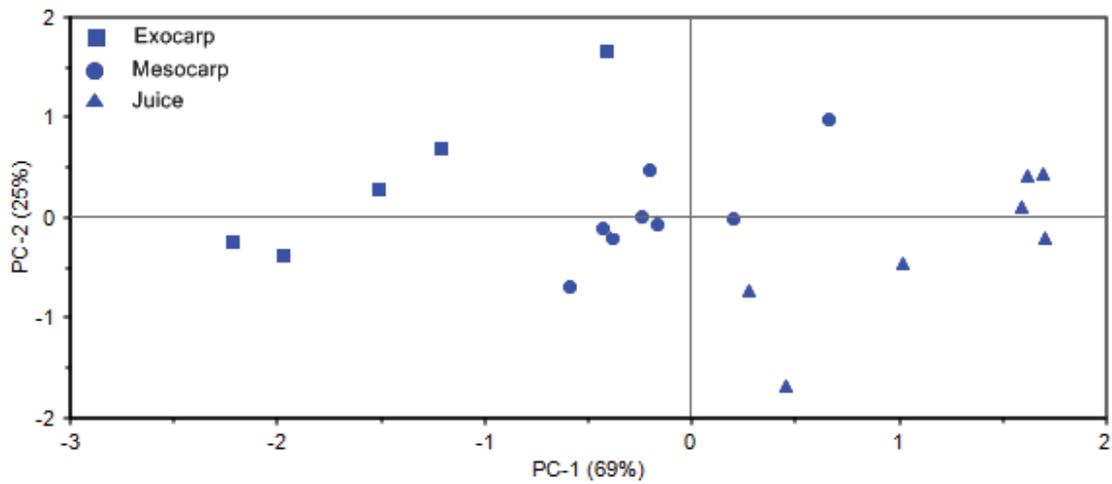


Figure 5a: PCA score plots (PC1 × PC2) for pomegranate peel and juice

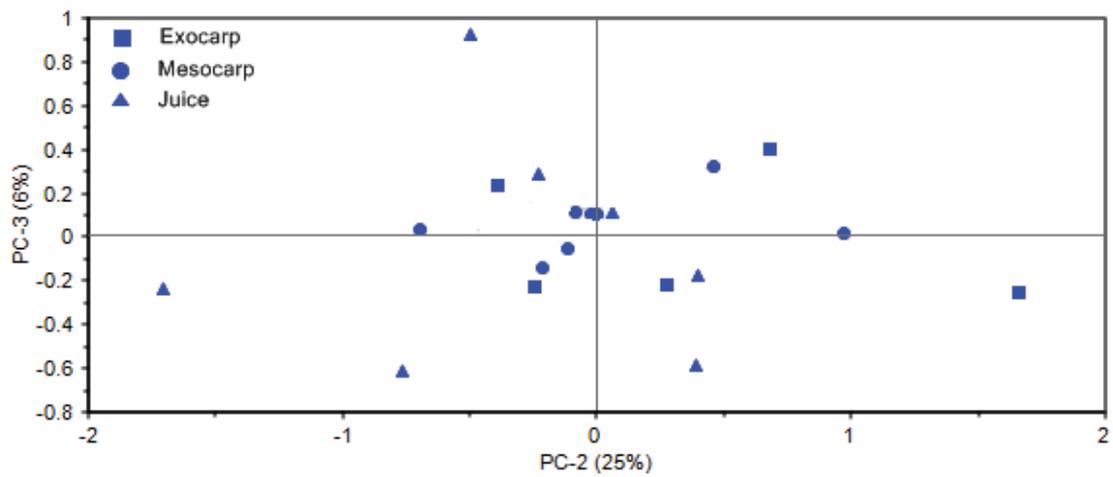


Figure 5b: PCA score plots (PC2 × PC3) for pomegranate peel and juice

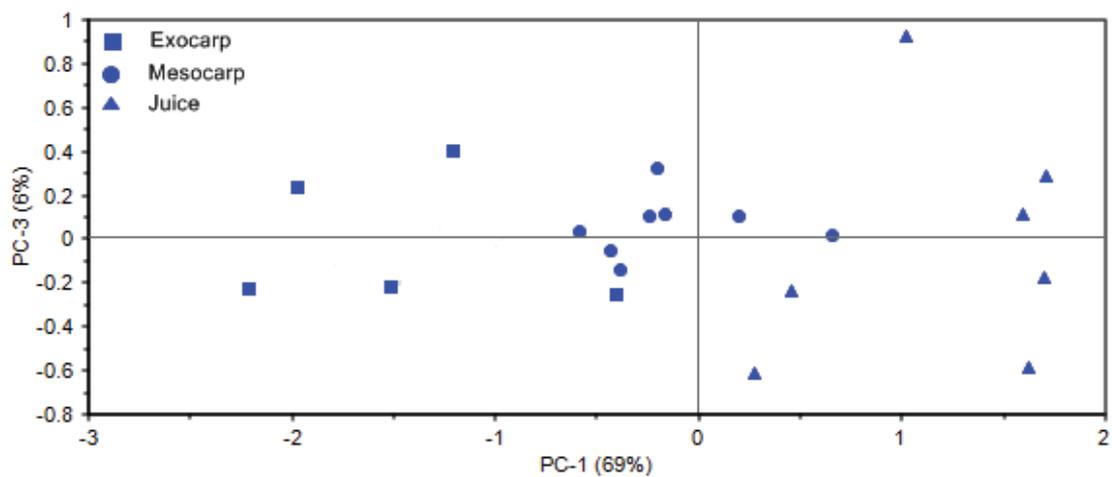


Figure 5c: PCA score plots (PC1 × PC3) for pomegranate peel and juice

formed the zones of inhibitions that were ranged from 9 to 16 mm (Figures 6d-6f and Table 1). The seed juice samples showed the zones of inhibitions with a range from 8 mm to 16 mm (Figures 6g-6i and Table 1). However, no single sample showed the antimicrobial activity more than the standard antibiotic used in this study.

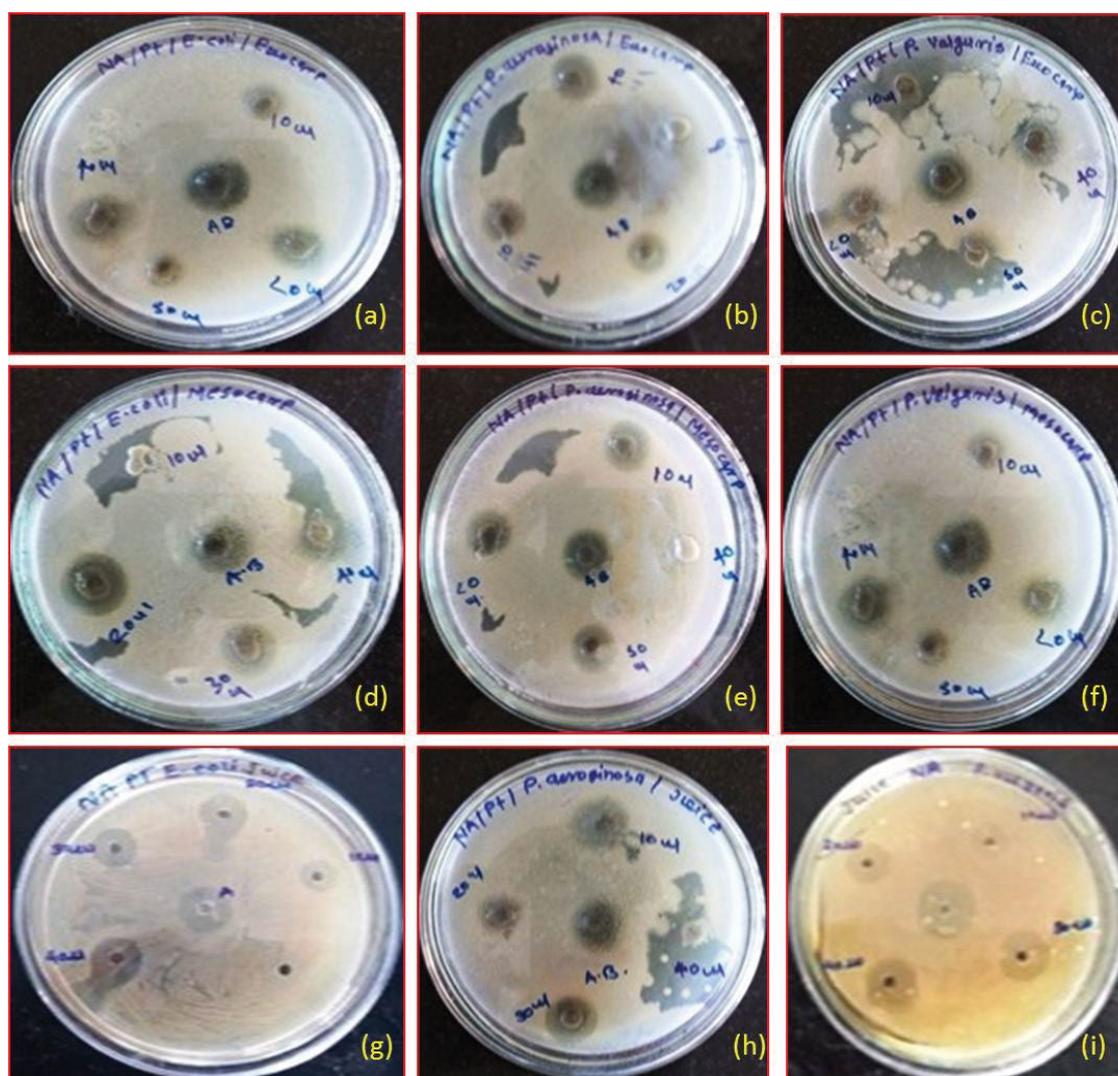


Figure 6: Antimicrobial activity of Aarakta exocarp against (a) *E. coli*, (b) *P. aeruginosa*, (c) *P. vulgaris*; mesocarp against (d) *E. coli*, (e) *P. aeruginosa*, (f) *P. vulgaris*; seed juice against (g) *E. coli*, (h) *P. aeruginosa*, (i) *P. vulgaris*

Table 1: Antimicrobial activity of exocarp, mesocarp and seed juice of Aarakta variety (Zone of inhibition measured in mm)

Bacteria treated	Exocarp sample (µl)				Mesocarp (µl)				Seed Juice (µl)				Ampicillin (µl)			
	10	20	30	40	10	20	30	40	10	20	30	40	10	20	30	40
<i>E. coli</i>	9	11	14	18	10	12	14	16	9	10	14	16	10	13	16	24
<i>P. aeruginosa</i>	9	12	13	15	9	13	13	15	8	12	14	15	9	14	15	17
<i>P. vulgaris</i>	8	13	12	13	8	13	12	13	8	10	12	13	9	13	15	20

DISCUSSION

Knowledge management in business organizations and firms is the most sought after aspect today. One of the important aspect is the synthesis of nanoparticles from pomegranate was recently studied [9-14] in more detail form by characterizing them through SEM and TEM. Abdelmonem [9] synthesized silver and gold nanoparticles by using pomegranate peel polyphenols. They characterized the nanoparticles by UV-Spectrophotometry and TEM. Nisha et al. [10] showed the activity of silver nanoparticles synthesized from pomegranate peel and seeds. These silver nanoparticles were studied thoroughly by UV, SEM, EDAX, XRD and FT-IR. The same authors performed antimicrobial activity against *Pseudomonas*, *Bacillus cereus*, *Staphylococcus albus* and *Proteus*. Dash and Bag [11] synthesized gold nanoparticles from pomegranate juice and studied its catalytic activity. They characterized the gold nanoparticles by HRTEM, XRD and FT-IR. Gnanajobitha et al. [12] prepared and characterized silver nanoparticles from seed juice and tested its activity against *Bacillus subtilis*, *Klebsiella planticola*. The characterization of the

silver nanoparticles was done through UV, SEM, EDAX, XRD and FT-IR. Nisha et al. [13] used pomegranate leaves for the synthesis of silver nanoparticles and studied antimicrobial activity against *Pseudomonas*, *Bacillus cereus*, *Staphylococcus albus* and *Proteus*. Eliz et al. [14] showed the process of green synthesis from pomegranate seed extracts. They synthesized gold nanoparticles.

Currently studying nanoparticles were compared with the other works to compare their existence and characterization. The newly synthesized silver nanoparticles were confirmed their presence at 556 nm following the addition of silver nitrate to the exocarp, mesocarp and seed samples individually. The other authors also confirmed the appearance of silver nanoparticles around 540 nm. The appearance of peaks in FT-IR was comparable with other researchers and ranged from 1217 to 3600 cm^{-1} . The presence of phenolic OH, vibration carbonyls, alkanes and –C-O- stretch represented strong absorptions.

The antimicrobial activities of different microorganisms were comparable with Nisha et al. [10]. They showed the antibiotic zones for peel against *Pseudomonas* and *Proteus* as 16 mm and resistant, respectively. By using the seed extracts, they obtained the antibiotic zones against *Pseudomonas* and *Proteus* as 13 mm and resistant, respectively. While using the leaf extracts [13] they showed the antibiotic zones for *Pseudomonas* and *Proteus* as 15 mm and resistant, respectively. In the previous studies, the *Proteus* strains were found resistant against the action of peel, seed or leaves. But, in our study, we found that, *Proteus vulgaris* is not resistant to pomegranate exocarp, mesocarp and seed juice. At a concentration of 40 μl , all these samples showed an antibiotic zone of 13 mm, giving useful information in respect to the treatment of *Proteus vulgaris*. The current results confirmed the previous results of Nisha et al. [10,13] in respect to *Pseudomonas*. The antibiotic action of exocarp, mesocarp and seed juice against *E. coli* showed the zone of inhibitions about 16-18 mm at a concentration of 40 μl . However, it is not compatible with the standard antibiotic, Ampicillin which had a maximum zone of inhibition, i.e., 24 mm.

CONCLUSION

The advent of nanotechnology is supporting not only the Nano medicine directly but also showing its impact in the alternative medicine indirectly. The association of plant Phytochemistry and nanotechnology will assure a secured manner of curing diversified diseases in nearby future. The current study proved that, the pomegranate exocarp, mesocarp and seed juice are capable of producing nanoparticles in association with silver nitrate. These nanoparticles showed a tremendous antimicrobial activity against *E. coli*, *P. aeruginosa* and *P. vulgaris*. However, their antimicrobial activities were comparatively less with the standard antibiotic Ampicillin. This study gives a path for those aspirants who are willing to work with pomegranate nanoparticles and their antimicrobial activities.

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