



A Comparative Study of Biosynthesized Silver-Nanoparticles from *Citrus maxima* Peel, Pulp and Seed: A Special Retrospect for Antimicrobial Activity

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Silver nanoparticles (AgNPs) have been used in various medicinal products because of its anti-microbial properties. This research study has reported a simplistic, cost effective and eco-friendly method for the synthesis of Silver nanoparticles.

Objective: The objective of present study was to compare the synthesis of silver nanoparticles (AgNPs) from various parts of *Citrus maxima* fruit like pulp, peel and seed.

Methodology: The synthesized nanoparticles were characterized by the use of UV-visible spectroscopy, fourier transform infrared spectroscopy and transmission electron microscopy. Time-dependent synthesis of AgNPs was studied spectrophotometrically. UV-visible spectrophotometer was used to confirm the synthesis of AgNPs which showed maximum absorption at 410 nm, 420 nm and 430 nm respectively.

Expected Results: Fresh peel extract exhibited the highest concentration of silver nanoparticles in comparison to pulp and seed. Fourier-transform infrared spectroscopy (FTIR) spectra analysis confirmed the presence of possible functional groups in AgNPs which can be responsible for reduction of nanoparticles. Morphological characters of AgNPs were analyzed using transmission electron microscopy (TEM) depicting the particles size as 12.58-47.80 nm. The antibacterial

property of synthesized AgNPs was analyzed viz *Escherichia coli* (MTCC 1687) and *Staphylococcus aureus* (MTCC 902), specify them to be effective against both gram positive and gram negative bacteria.

Conclusion: These results suggested that the fresh peel extract of *Citrus maxima* is a high-quality bioreductant for the synthesis of silver nanoparticles and have prospective for various biomedical applications.

Keywords: Silver nanoparticles; silver nitrate; citrus maxima; biosynthesis; antimicrobial activity.

1. INTRODUCTION

Nanotechnology dealt with the particles size ranging from 1-100 nm that is approximately in one dimension [1]. Nanobiotechnology is an integrative field involved both nanotechnology and biological sciences that utilizes of biological systems to fabricate nanoparticles (NP) [2]. NPs synthesis through chemical and physiochemical methods are proved hazardous to the environment [3]. Nanobiotechnology includes the synthesis of nanoparticles (NPs) through an environmental friendly process as green synthesis that is done with the help of biomolecules present in plant extract [4]. NPs are also synthesized by using microorganisms but through plant is much easier and the most preferring technique in comparison to microorganisms as it comparatively safer and ecofriendly in nature [5,6]. To avoid the harmful effect of these chemicals we use biological approach. Also, biologically synthesized NPs are getting active interest of researchers because numerous biological chemical compounds present in plants that assist in green synthesis [7].

Nanoparticles reflect various identical properties developing on their morphological characters and also on the stabilizer [8]. Protein and enzymes are the biomolecules that assist in increasing the stability of nanoparticles [9]. Due to the unique physiochemical qualities of silver at the nano level, the synthesis of silver nanoparticles (AgNPs) are getting much more attention during recent years [10].

It has been reported that AgNPs have antibacterial, antifungal and antiviral qualities on a large scale. They possess the ability to perform and modify the cell membrane of bacteria and may lead in cell death [11].

Synthesis of AgNPs through biological method using citrus fruit is one of the approaches where there is depletion of silver ions. For production of AgNPs, citrus fruit extract itself works as

reducing agent, therefore this technique of synthesis is environment friendly and safe. Synthesis of NPs using naturally available substances offers a number of advantages in clinical and pharmaceutical applications [12]. The study of AgNPs has been done on a broad spectrum particularly as a part of modern anticancer medium in order to treat cancer cell more effectively [12]. AgNPs are synthesized by using crude of *C. maxima* fruit [13,14] but still the synthesis of AgNPs is not done by taking fresh peel, pulp and seed of *C. maxima* fruit separately. In present work we formulated AgNPs by using peel, pulp and seed of *C. maxima* fruit and compared yield and quantity of AgNPs among respective extracts as well as the antibacterial activity of synthesized NPs was also studied.

2. METHODOLOGY

2.1 Chemical and Reagents

All the chemicals used were of analytical grade, including silver nitrate (AgNO_3), purchased from Sigma-Aldrich Co., (St Louis, MO, USA) and Fisher scientific, Waltham, MA 02451, United States.

C. maxima fruits of *Rutaceae* family were collected from local market of Bareilly and the identified fruit specimen was deposited in the Department of Botany, IFTM University, Moradabad, Uttar Pradesh India.

2.2 Preparation of *C. maxima* Fruit Extract

C. maxima (pomelo) fruit extract was prepared according to the procedure as stated by Caio Henrique Nasi de Barros *et al.* [15]. In short, Fresh *C. maxima* fruit was washed with distilled water and green outer skin of peel was removed and cut it into small pieces. 5 g of peel was added in 100 ml distilled water and stirred at 100 rpm for 30 min (80°C). After that, the extract was filtered with the help of whatman filter paper of pore size 11 μm and the resulting filtrate was

stored at 4°C for further use. The same procedure was applied to prepare pulp and seed extracts of *C. maxima* as shown in figure 1.

2.3 Preparation of 1 mM AgNO₃

To prepare 1 mM AgNO₃ solution, 0.017 g of AgNO₃ was dissolved in 100 mL of distilled water and kept in dark place.

2.4 Synthesis of AgNPs

C. maxima peel extract (5 mL) was added to 1 mM of AgNO₃ solution (18 mL; 1:3.6) then incubated at 55°C for 30 min. The formation of AgNPs was confirmed by the colour change of the solution, the same procedure was applied to synthesize AgNPs from pulp and seed, instantly UV-vis spectra was recorded and it is found that all three samples i.e. pulp, peel and seed show different values.

2.5 Optimization Parameter

A series of experiments were performed to investigate the effect of different parameters viz. concentration of AgNO₃, amount of plant extract, pH and temperature on the formation of AgNPs and the optimum condition was confirmed using the UV visible spectroscopy.

2.6 Characterization of AgNPs

Characterization of AgNPs was done using UV-visible spectroscopy (Schimadzu) in the wavelength range of 300- 600 nm. FTIR spectra for green synthesized AgNPs were recorded on a Agilent Cary 630 FTIR Spectrometer (Range: 4000-450 cm⁻¹) and the recorded spectra was used to identify the bio groups attached on the surface of synthesized AgNPs [16,17]. Morphological characters of AgNPs were analyzed by using TEM (Jeol JEM 1400, Jeol Ltd. Tokyo, Japan).

2.7 Antimicrobial Activity of Biosynthesized AgNPs

Agar well diffusion method was performed to analyze the antimicrobial activity of the biologically synthesized AgNPs against Gram positive and Gram negative bacteria species. In this method we used nutrient agar media (2.52g/90ml) for the growth of bacteria. The strain used in the study were *Escherichia coli* (MTCC 1687) and *Staphylococcus aureus*

(MTCC 902). Petri dishes plated with nutrient agar media were used to spread bacterial strains. The well (6 mm diameter) soaked peel extract, AgNO₃ and AgNPs were individually put down on petri dishes and incubated at 37 °C for 18 hours, subsequently each inhibition zone was measured with the help of ruler.

3. RESULTS

3.1 Visual Observation and UV-Vis Spectroscopy

The early prophecy of AgNPs formation was primarily monitored by colour change. These colour changes were observed visually after the addition of AgNO₃ with respective extract, please see Fig. 2 A. The green route synthesized AgNPs using *C. maxima* fruit aqueous extract was confirmed by the UV-vis spectrum analysis at scale ranging from 300-600 nm. The maximum absorption peak of AgNPs using *C. maxima* pulp, peel and seed were observed at 410, 420 and 430 nm respectively (Fig. 2 B). Among pulp, peel and seed extracts, peel extract mediated AgNPs show high intensity of UV-vis spectra indicating maximum yield of AgNPs, so we chose peel for further study.

3.2 Effect of AgNO₃ Concentration

Maximum absorbance for different concentrations (0.5 mM, 1.0 mM, 2.0 mM, 3.0 mM) of AgNO₃ is shown in Fig. 3. Results expressed that 0.5 mM is an appropriate concentration of AgNO₃ because it showed the maximum synthesis with the high peak at 420 nm. Thus AgNO₃ of 0.5 mM concentration was considered to be optimum for further analysis.

3.3 Effect of Amount of Fruit Extract

Different concentrations of peel extract (2 mL, 5 mL, 8 mL, 10 mL) were optimized and observed that the higher concentration of AgNPs was formed in 8 ml, it was affirmed by spectrophotometer (see Fig. 4). Thus the 8 ml fruit extract was considered to be optimum and chosen for further analysis.

3.4 Effect of pH

The suspension was calibrated for various pH, as it is one of the significant criterion in the formation of AgNPs. Lowest silver nanoparticles

synthesized at pH 5 and pH 11. pH 7 was considered to be optimum as highest nanoparticle formation was observed at this pH with highest absorbance at 420 nm (see Fig. 5).

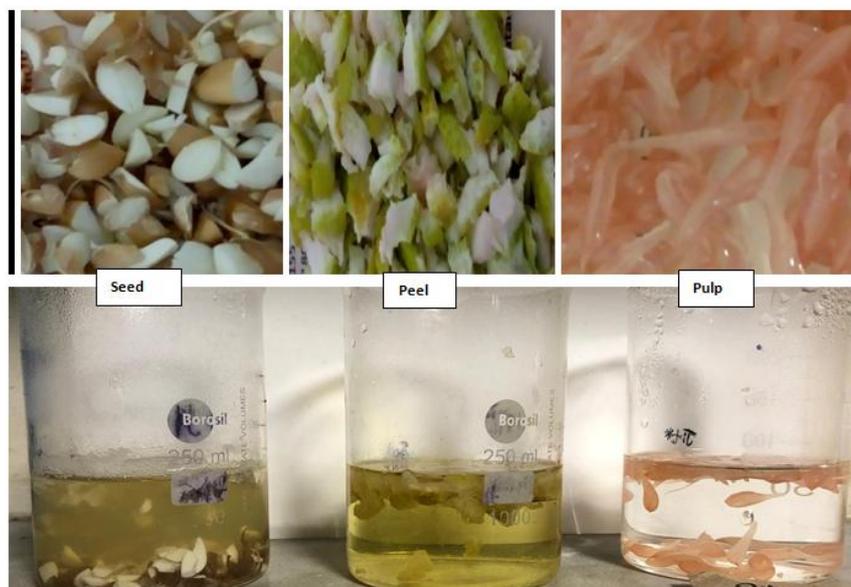


Fig. 1. Preparation of extract from seed, peel and pulp of *C. maxima*

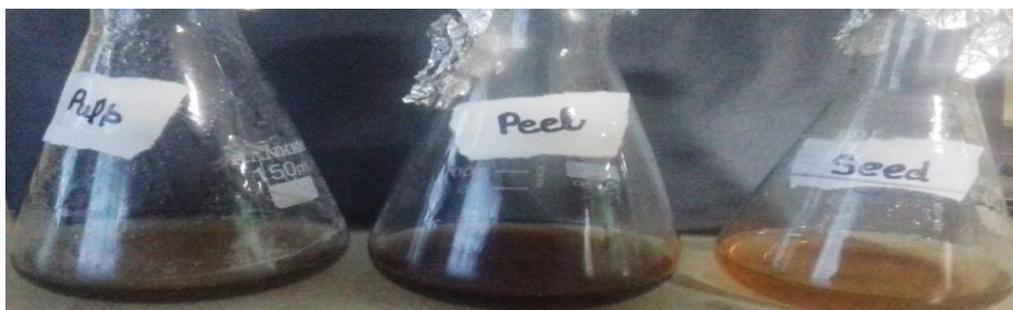


Fig. 2A. Change in color showing reduction of silver particles

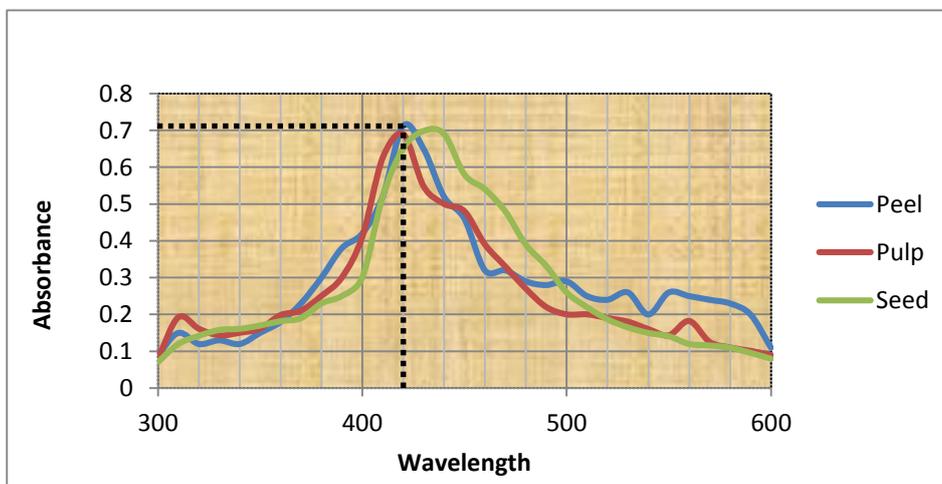


Fig. 2B. UV-Vis absorption spectra of fresh peel, pulp and seed mediated AgNPs

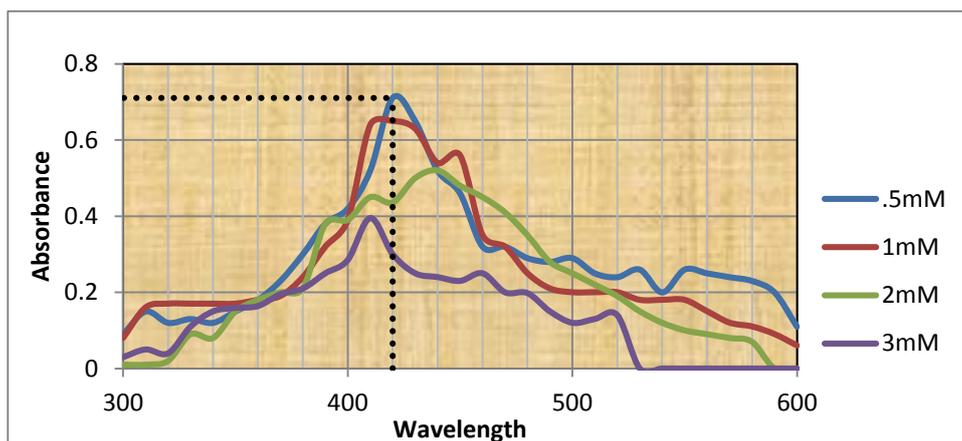


Fig. 3. Determination of effect of various concentration of silver nitrate

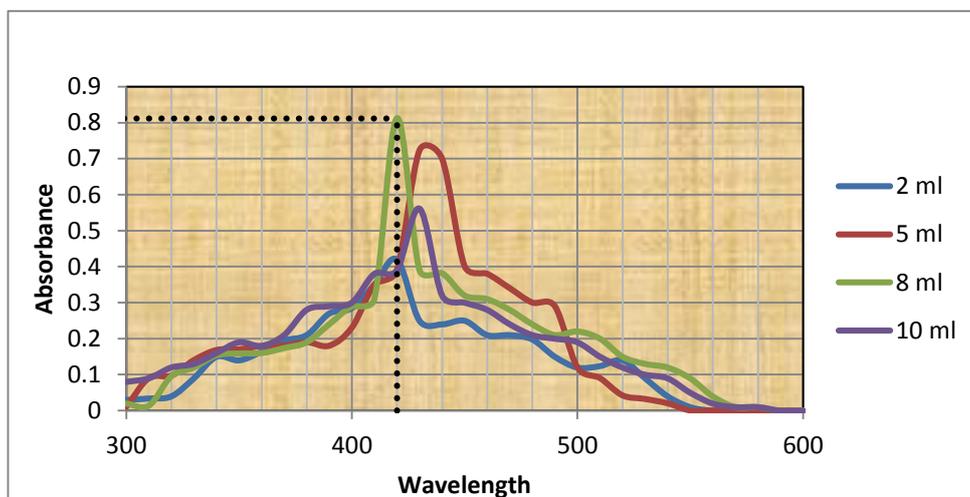


Fig. 4. Determination of effect of various concentrations of extract

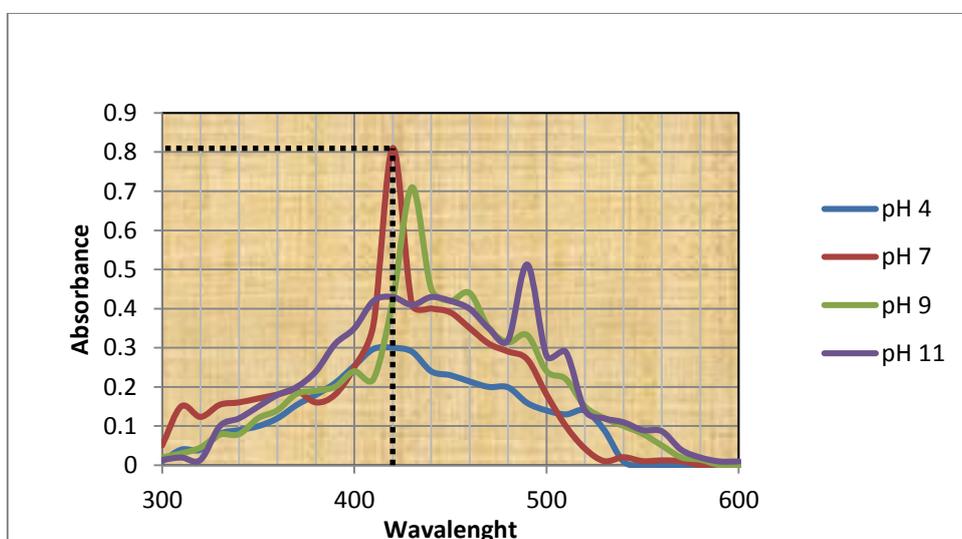


Fig. 5. Determination of effect of pH

3.5 The Effect of Temperature

The effect of Temperature (35°C, 55°C, 80°C and 100°C) on the formation of AgNPs was analyzed. This was authenticated by studying the UV-Vis spectroscopy. As shown in Fig. 6, the maximum emergence of AgNPs was noticed at 100°C temperature with highest absorbance peak at 410 nm (see Fig. 6).

3.6 FTIR Study of Synthesized AgNPs

To examine the characters of biologically functional groups, FTIR spectroscopy has been used and these groups are found in *C. maxima* fruit peel and also related in the formation of NPs. The FTIR spectrum of synthesized AgNPs showed exquisite peak detected at 3417.38 cm⁻¹, 1639.11cm⁻¹ and light peak detected at 2082.28 cm⁻¹, 1383.89cm⁻¹, 1064.55cm⁻¹ and 709.42cm⁻¹. The exquisite peak at 3417.38 denote O-H stretching for alcohols and phenols, 1639.11 may be attributed to the C=C stretch bonding function of alkene groups. Although light peak at 2082.28cm⁻¹, 1383.89cm⁻¹ and 1064.55cm⁻¹ possibly attributed to the C≡C stretching, C-H and C-O stretching which denoted the existence of alkyne, aldehyde and primary alcohol functional group (Fig. 7).

3.7 TEM Study of Synthesized AgNPs

TEM was used to visualize the morphology of synthesized AgNPs through *C. maxima* peel extract. Fig. 8 shows that most of the synthesized particles were spherical in shape. The maximum and minimum size of synthesized individual AgNPs were found 47.80 and 12.58 nm, respectively.

3.8 Antimicrobial Activity of Peel, AgNO₃ and AgNPs

AgNPs are applied in the medical sector on a large scale and have prohibitory effect on several microorganisms. The antibacterial property of *C. maxima* peel extract, AgNO₃ and synthesized AgNPs was calculated. The antimicrobial activity of these samples and the enlargement of the distinct zone were analyzed using the same amount of AgNO₃, fruit extract and AgNPs as shown in Table 1 & Fig. 9.

The result indicated that, the fruit extract alone represents a remarkable positive effect to the experimented microorganisms (low clear zone) against *S. aureus* (Gram positive bacteria) and

E. coli (Gram negative bacteria). AgNO₃ also exhibits a remarkable positive effect to the experimented bacteria (low clear zone). Although AgNPs exhibit the highest antimicrobial property against the tested microorganism (high zone).

4. DISCUSSION

During the past few years, the research work in the field of Nanobiotechnology, the biosynthesis of AgNPs is growing gradually because of widespread application in various areas including catalysis, antimicrobial activity and biosensing imaging etc. Biosynthesis is an optional technique to synthesize NPs by using plant extract as an eco-friendly method to safeguard from toxic compound. In this study AgNPs were synthesized from different parts of *C. maxima* fruit (pulp, peel & seed). A change in colour from yellowish to dark brown was observed when AgNO₃ was added with the fruit extract (Fig. 2A). This change in color indicates the completion of the process of NPs formation and the incidence of change in colour of the solution to brown colour is known as surface plasmon resonance [18,19]. The ratio diversified from one plant to another, for instance, in the leaf of *P. guajava* plant, a combination of 1 mL plus 9 mL exhibit maximum peak at 440 nm [20].

UV-vis spectroscopy was performed for primary characterization of AgNPs that gives us assurance of NPs synthesis. Absorption peak from UV-vis spectra was used to check the size and stability of NPs. UV-vis spectra was recorded immediately just after the reduction of AgNO₃ with the fruit extract and the maximum absorption of pulp, peel and seed were noticed at 410 nm, 420 nm and 430 nm respectively. In this study we found different absorption values in all three samples i.e., extract from pulp, peel and seed among which peel mediated AgNPs show the high intensity spectra. According to Niluxsshun MCD *et al.* the increased intensity indicates that the number of nanoparticle increases due to reduction reaction between silver ions and phytochemicals exist in the aqueous solution [21]. Parmar D *et al.* also reported that Citrus peel has the highest number of Phytochemicals as compared to pulp and seed [22]. Different position of functional groups are illustrated with the help of FTIR spectroscopy that possibly exist in *C. maxima* peel extract which is in good correlation with those found in the literature[16], these functional groups play important role to make biosynthesized NPs more secure physically.

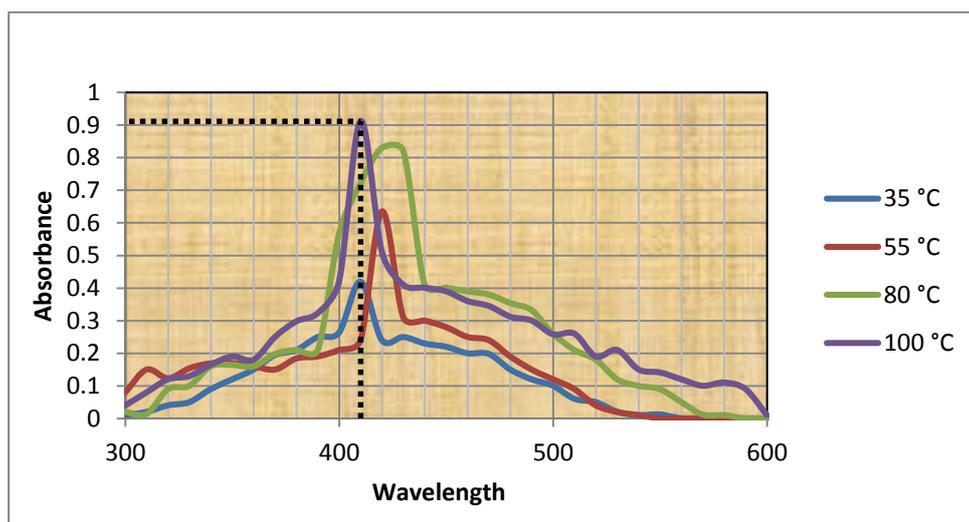


Fig. 6. Determination of effect of temperature

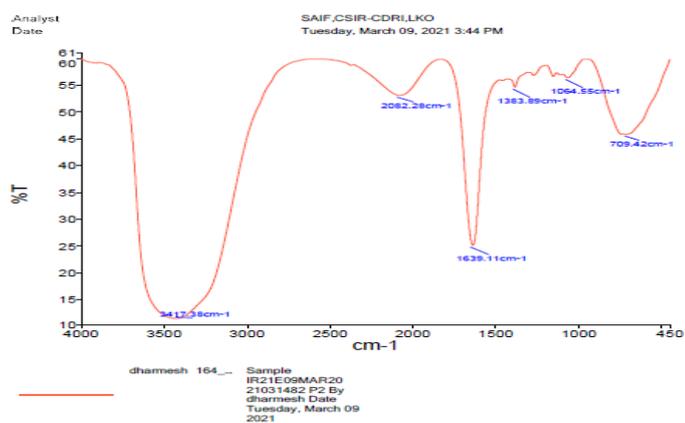


Fig. 7. FT-IR spectrum of Citrus maxima fruit peel extract mediated green synthesized AgNPs

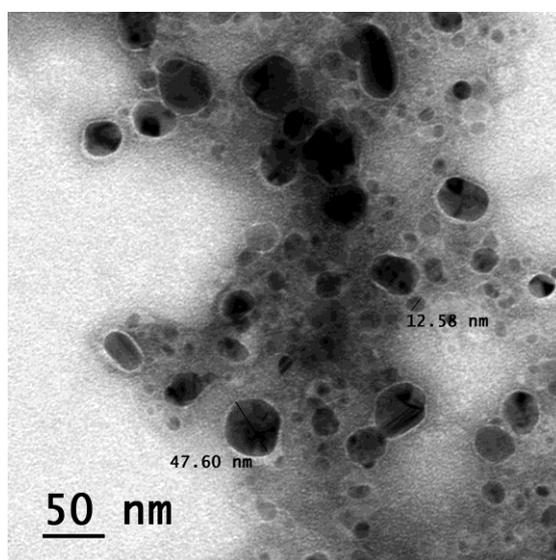


Fig. 8. TEM micrograph of the silver nanoparticles, the scale bar corresponds to 50 nm.



Fig. 9. Antimicrobial activity of peel, AgNO₃ and AgNPs

Table 1. Antimicrobial activity of peel, AgNO₃ and AgNPs

S.No	Pathogens	Zone of Inhibition (mm)		
		Peel	AgNO ₃	Nanoparticle
1.	<i>E Coli</i>	8	9	21
2.	<i>S aureus</i>	7	7.5	18

As we obtained the maximum concentration of AgNPs from peel extract, so we chose peel to synthesize AgNPs with optimum parameters, in this procedure firstly we did synthesis of AgNPs with the different concentration of AgNO₃ and we found that 0.5 mM concentration of AgNO₃ was most suitable for synthesis. The result is consistent with the study of Mrinal Kashyap *et al.* demonstrating that 0.5 mM AgNO₃ was best concentration to synthesize silver nanoparticles [23].

After that we took the constant concentration of AgNO₃ (0.5 mM) and treated with different volume of peel extract. We observed that 8 mL peel extract was suitable for NPs synthesis. When we synthesize NPs at different pH we noticed that pH have direct effect on synthesis of NPs. The data shows that colour of the solution was not changed at pH 4 and slightly changed at pH 11 indicated that the synthesis of AgNPs did not take place at extreme acidic and alkaline pH. Maximum concentration of AgNPs was depicted at pH 7 indicating that neutral pH is the optimum condition for NPs synthesis. Consistent with the study of Sarsar *et al.* showing that AgNPs syntheses of *P. guajava* leaf extract was optimum at pH 7 [20].

Further we analyzed the effect of temperature on synthesis of AgNPs and we found that the intensity of SPR peak increased along with an

increase in temperature, which indicated the increased formation of AgNPs. This is rationally known that the reactants are consumed very quickly with an increase in the temperature, resulting to the synthesis of smaller NPs [23,24]. Similarly, the highest SPR peak was observed at 100°C when the banana peel extract was used [25]. Moodley *et al.* also have reported that NPs synthesis can be enhanced to yield smaller size NPs by increasing the temperature [26]. In the present study, the particle size was determined as 12.58-47.80 nm using TEM. The effectiveness of synthesized NPs against different diseases depends on their morphology. Furthermore, the antibacterial property of synthesized AgNPs indicated them to be effective in case of Gram-positive and Gram negative bacteria tested viz *Escherichia coli* (MTCC 1687) and *Staphylococcus aureus* (MTCC 902). Based upon the previous studies and present data it can be hypothesized that ecologically synthesized NPs using plant extracts looks to be promising than other approaches [27].

5. CONCLUSION

Biologically synthesized NPs from plant extract has been designated as "green synthesis" and considered to be gained attention due to its non-pathogenic, providing single step technique, economical protocol, and eco-friendly in nature. During this work, we have developed an easy to

use method to synthesize AgNPs direct from fresh peel extract of *C. Maxima* fruit and established its antimicrobial properties.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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