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Green synthesis of plant extract supported silver nanoparticles using *Citrus limonum* peel, leaf and seed extract and their antibacterial activity

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Key words: Silver nanoparticle, *Citrus limonum*, Green synthesis, Plant extract, Bark, Leaves, Seed

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Abstract

The synthesis of silver oxide nanoparticles (AgNPs) through the use of plant extracts is a remarkably simple, cost-effective, efficient, and environmentally friendly approach. In recent years, there has been a surge in the exploration of eco-friendly methods for synthesizing AgNPs, with researchers addressing the potential of extracts derived from various plant components, including leaves, stems, roots, and fruits. The current work concentrated on the green synthesis of silver nanoparticles (AgNPs) through the use of aqueous *Citrus limonum* bark, leaf and seed extract, optimizing the different experimental factors required for the formation and stability of AgNPs. FTIR spectra confirmed that *Citrus limonum* bark; leaf and seed extract acted as both reducing and surface passivation agent for the synthesized AgNPs. The morphology, size, and elemental composition of AgNPs were investigated by SEM analysis, which showed crystalline spherical silver nanoparticle by XRD analysis. In addition, the antimicrobial and antioxidant properties of this bioactive silver nanoparticle were also investigated. The AgNPs showed excellent antibacterial activity against one (Gram-positive) pathogenic bacteria *Bacillus cereus* and (Gram-negative) *Pseudomonas aeruginosa*. These results indicated a simple, fast, and inexpensive synthesis of silver nanoparticles using the *Citrus limonum* bark, leaf and seed extract that has promising antibacterial activity.

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Introduction

The broad spectrum of nanotechnology is important in the major fields of biology, chemistry, physics, and material sciences. Nanotechnology deals with the study of materials at the nanometers (Singh *et al.*, 2009; Elumalai *et al.*, 2010; Marambio-Jones and Hoek, 2010). The nanomaterials can be synthesized by different methods including chemical, physical, irradiation, and biological methods. The development of new chemical or physical methods has resulted in environmental contaminations, since the chemical procedures involved in the synthesis of nanomaterials generate a large amount of hazardous by-products (Zhang *et al.*, 2008). Thus, there is a need for "green nanotechnology" that includes a clean, safe, ecofriendly, and environmentally nontoxic method of nanoparticle synthesis, and in this method there is no need to use high pressure, energy, temperature, and toxic chemicals (Jeong *et al.*, 2005; Savithramma *et al.*, 2011). The biological methods include synthesis of nanomaterials from the extracts of plant, bacterial, fungal species, and so forth. The synthesis of nanoparticles from the plant extracts is considered to be a process (Saxena *et al.*, 2010; Schultz *et al.*, 2008; Varadan *et al.*, 2010). Chemical methods of nanoparticle synthesis have adverse environmental impacts due to some toxic chemicals that may adsorb on their surface. Raja *et al.* (2011) reported that chemical methods are an environmental burden. Thus, green synthesis provides an environmentally safe and sustainable alternative one step route for preparation of nanoparticles. From previous researches, green synthesis does not use high temperature, pressure or energy (Muchanyereyi-Mukaratirwa *et al.*, 2017; Ahmed *et al.*, 2016; Nyoni *et al.*, 2019). Again, some recent studies have reported on the potential use of microorganisms in eco-friendly synthesis of nanoparticles as the next generation of nanomaterials in multidisciplinary applications (Suba *et al.*, 2022; Crooks *et al.*, 2001; Vijayaraghavan and Nalini, 2010).

In green synthesis, plant extracts have been found to be fast reducing agents of metal ions in comparison with microorganisms, hence the selection and use of plant extracts in this work. The main phytochemicals present in plant extracts are flavonoids, terpenoids, ketones, amides, aldehydes and carboxylic acids. These phytochemicals take part in the reducing metal ions to form their respective nanoparticles, and also act as capping agents (Prathna *et al.*, 2011). Silver nanoparticles are synthesised by the reduction of silver ions to neutral silver atoms. This is achieved by the reduction of silver ions by a reducing agent *Citrus limonum* bark, leaf and seed is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants, and these materials could be used as reducing agents in the synthesis of AgNPs. In this research, AgNPs were synthesised through a green synthesis method using the extracts from *Citrus limonum* bark, leaf and seed. Antimicrobial activity of the synthesised AgNPs against the isolated clinically significant bacterial strains such as namely, *Bacillus cereus* and *Pseudomonas aeruginosa* was studied in this work.

Materials and methods

Citrus limonum bark, leaf and seed were collected from Local Fruit Agricultural Land in Ambasamdrum and used for generating silver nanoparticles as shown in Fig. 1.

Fig. 1. Tree of *Citrus limonum* of bark, leaf and seed

Dextrose agar (PDA) of Himedia make was used to maintain as well as to propagate the Bacteria culture. Peptone and Dextrose were used to make medium for observing fungal growth in broth. Silver nitrate ($AgNO_3$) salt, Methanol other chemicals was purchased from Ranbaxy make.

Preparation of extract

Twenty grams fresh *Citrus limonum* bark, leaf and seed were washed with tap water and then washed with distilled water, air dried and then they were finely cut and soaked in 100 ml boiling distilled water for 5–10 min and filtered through Whatman filter paper no. 42. This extract was used for generating silver nanoparticles. This Extract is always used fresh.

Preparation of silver nanoparticles using extract

Sterile deionized triple - distilled water was used to synthesis a stock solution from $AgNO_3$, which were utilized in the next dilutions, later. AgNPs were made according to the method described by Gurunathan *et al.* (2013); Sujatha *et al.* (2013). Zina Albahadly *et al.* (2019). First, it was taken 20 ml of aqueous extract of bark, leaves and seeds *of Citrus Limonum* that were filled with sterile distilled water to a total 50 ml. After that, the solution is added to 5 ml of $AgNO_3$ solution and then exposed for 5 days under room condition. The yellow color of mixture solution was turned to dark yellow, when the solution put in incubation for 24 hours, which are indicating that silver nanoparticle were formatted indicates the formation of silver nanoparticle and the absorbance of silver nanoparticle in the solution were monitored by using UV-vis spectroscopy.

Selection of bacterial strains

For this study, a total of two pathogenic bacteria were chosen. Two of them were strains of bacteria, such as *Pseudomonas aeruginosa* (Gram-negative) and *Bacillus cereus* (Gram-positive), two pathogenic germs. The bacterial strains utilized in this study were initially acquired from Sri Paramakalyani College's Department of Microbiology in Alwarkurichi. Throughout the research period, the juvenile bacterial cultures were prepared and used. After being made, the nutritional broth was divided across many tubes. These tubes were subsequently sterilized. After being taken from the institute, the pure bacterial strains were injected. The cultures were employed for the tests after the tubes were incubated for 24 and 48 hours at 37°C.

Antibacterial activity

The synthesis of silver nanoparticle, *Citrus Limonum* peel, leaf, and seed extract, and control were tested against the chosen bacterial strains to determine their antibacterial properties (Hae Kim *et al.*, 2007; Sujatha *et al.*, 2013). Each sterile petriplate was filled with 20ml of sterilized agar medium, which was then left to harden. Using a sterile cotton swab, the test bacterial cultures were equally distributed over the suitable media. Then, utilizing a sterile cork borer, a 0.5cm well was created in the medium. 200µl of each of the pathogenic bacteria *Bacillus cereus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative) (synthesis of silver nanoparticle, *Citrus limonum Peel*, Leaf and Seed extract, and control) were then transferred into distinct wells. For 24 and 48 hours, these plates were incubated at 37°C. Following the incubation period, the diameter of the inhibitory zone surrounding each well was measured and the findings were examined.

Results and discussion

UV-Vis spectrophotometer

The biosynthesis of silver nanoparticles by using the supernatant of Bark Extract, Leaf extract and Seed Extract of *Citrus limonum* can be easily monitored by UV-visible spectrophotometer shows in Fig. 2-4. The UV-vis absorption spectrum is strongly depends on the shape of the synthesized nanoparticles. The synthesis of silver nanoparticle was measured in UV-vis spectrum at different time intervals from 30 min to 24 hrs. The absorbance of silver nanoparticle was gradually increased from 30 min to 24 hrs. At 24 hrs, the surface plasmon resonance band was observed respectively at 470 nm (Fig. 2-4). The decreased absorbance at 48 hrs incubation reveals the reaction was completed at 24 hrs. The 30 min to 24 hrs Bark Extract, Leaf extract and Seed Extract of *Citrus*

limonum derived silver nanoparticle are also shows intense peak at 470 nm (Fig. 2-4). The low absorbance of silver nanoparticle at 48 hrs reveals the silver nanoparticle synthesis process was completed at 24 hrs. The similar peak was observed for nanoparticle synthesized by using *K. pneumoniae*, *E. coli*, Sanghi *et al.* (2009), and *R. paultris* Bai *et al.* (2009). Moreover the plasmon bands are broadened with an absorption end in the longer wavelengths, which may be due to the size distribution of the particles Mulvaney *et al.* (1996). The silver nanoparticle synthesized by 30 min to 24 hrs Bark Extract, Leaf extract and Seed Extract of Citrus limonum were monitored by UV-vis spectrum at different time intervals from 30 min-24 hrs.

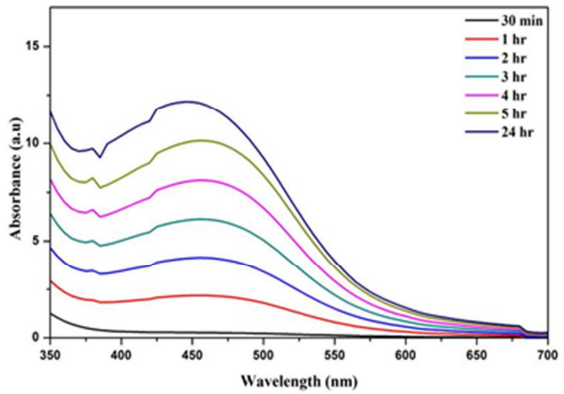

Fig. 2. UV-Vis spectra recorded the synthesis of silver nanoparticles. The silver nitrate 1mM was added to the bark extract of *Citrus limonum* and incubated at different growth periods like 30min – 24 h

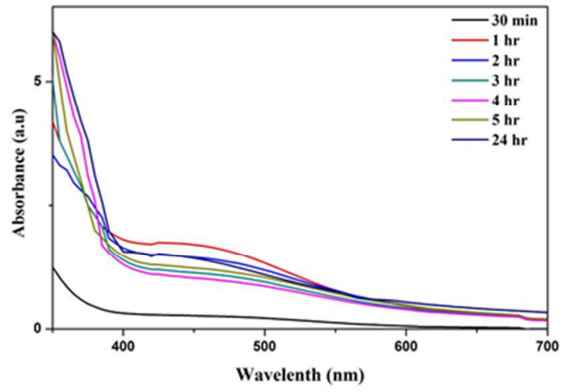

Fig. 3. UV-Vis spectra recorded the synthesis of silver nanoparticles. The silver nitrate 1mM was added to the leaf extract of *Citrus limonum* and incubated at different growth periods like 30min – 24 h

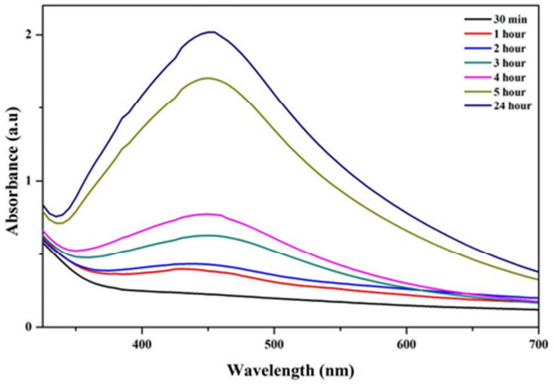

Fig. 4. UV-Vis spectra recorded the synthesis of silver nanoparticles. The silver nitrate 1mM was added to the seed extract of *Citrus limonum* and incubated at different growth periods like 30min – 24 h

It is considered that the nanosized silver nanoparticles should have a wider band gap than the bulk material due to the quantum confinement of the electron-hole pair that forms owing to absorption of the suitably energetic photon. The larger energy difference causes a shift in the visible absorbance spectrum of silver Kumar *et al.* (2013). The optical excitation of electrons across the band gap is strongly allowed, producing an abrupt increase in absorptive at the wavelength corresponding to the gap energy. This feature in the optical spectrum is known as the optical absorption edge Boldish *et al.* (1998). The exact position of the resonance band depends on a number of factors such as dielectric constant of the medium, size and shape of the particles, type of the capping agent as well as to refractive index of the surrounding in the medium Pandian *et al.* (2011). UV-visibly indicates the structure of silver nanoparticles is very stable and could be stored for a long time period with no corrosion in ambient conditions.

X-ray diffraction (XRD)

The XRD pattern of synthesis of silver nanoparticles synthesized by using *Citrus limonum* plant bark, leaf and seed water extract are shown in Fig. 5-7. There are four intense peaks was observed in the whole

spectrum of 2Θ values ranging from 20-80. The XRD spectrum shows three intense peaks at of 38.4°, 40.1, 66.2° and 76.8° corresponds to (1 1 1), (2 2 0) (222) and (3 1 1) set of planes indicates the silver nanoparticles are pure crystalline in nature (Fig. 5).

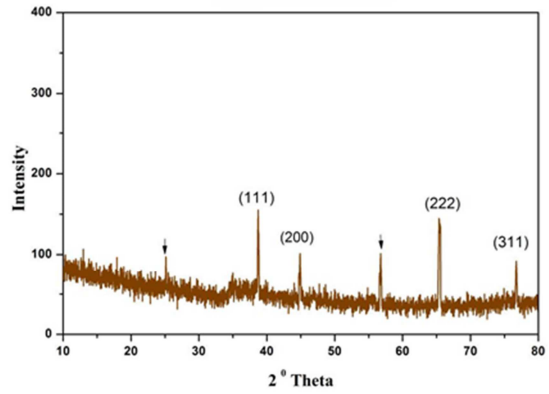

Fig. 5. XRD of the air-dried silver nanoparticles synthesized using bark extract *Citrus limonum*

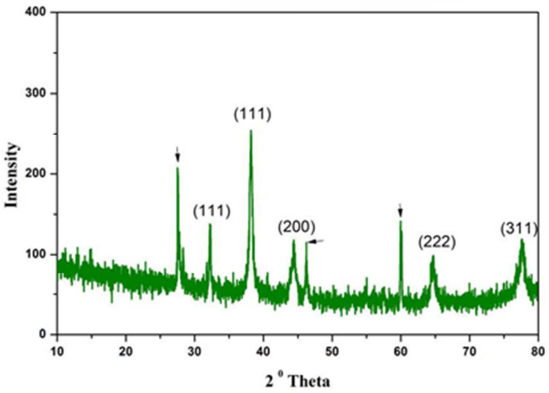

Fig. 6. XRD of the air-dried silver nanoparticles synthesized using leaf extract *Citrus limonum*

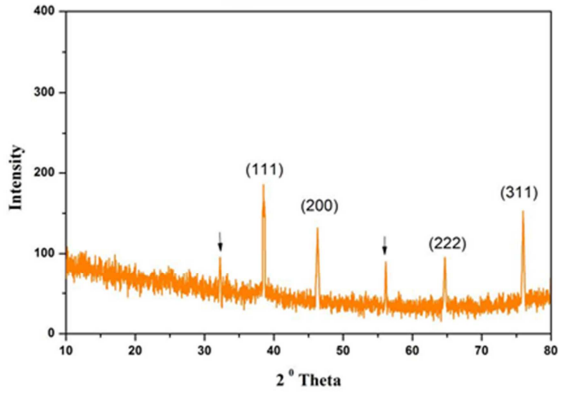

Fig.7. XRD of the air-dried silver nanoparticles synthesized using seed extract *Citrus limonum*

Similarly, the bark derived silver NPs also are showed (Fig. 6) intense peaks at 32.7 °, 38.4 °, 65.1°, 44.6 ° and 78.6 ° corresponding to the (1 1 1), (1 1 1), (2 0 0) and (3 1 1) respectively also reveals the silver are crystalline in nature and seed derived silver nanoparticles are showed (Fig. 7) instance peaks are 38.7, 46.4, 65.3 and 75.9 corresponding to the (1 1 1), (2 0 0), (2 2 2) and (3 1 1) respectively. The broadening of Bragg peaks indicates the formations of nanoparticles are crystalline in nature Bai *et al.* (2009). These peaks are matched with CdS a peak which was published by Joint Committee for Powder Diffraction standards (JCPDS File no.10-454) for CdS nanoparticles. The mean size of silver nanoparticle was calculated using Debye-Scherrer equation by determining the width of (1 1 1) Bragg's reflection. In general, line broadening in X-ray powder diffraction measurements occurs in very small crystallites, which is the result of combined effects of the crystallite size, non-uniform strain, and instrumental broadening Wang *et al.* (2009).

Fourier transform infrared spectroscopy (FTIR)

FTIR measurements were carried out to identify the possible biomolecules responsible for the reduction of silver nitrate of the bioreduced silver nanoparticles synthesized by *Citrus limonum*. A number of vibration bands can be seen in the region 4000 – 500 cm^{-1}. Absorption spectrum observed in the region of 2000 – 400 cm^{-1} is 3270 cm^{-1}, 2975 cm^{-1}, 1591 cm^{-1}, 1300 cm^{-1}, 1031 cm^{-1}, and 819 cm^{-1} for *Citrus limonum* bark (Fig. 8), 3276 cm^{-1}, 2933 cm^{-1}, 1602 cm^{-1}, 1327 cm^{-1}, 1066 cm^{-1} and 824 cm^{-1} for *Citrus limonum* leaf (Fig. 9) and 3280 cm^{-1}, 2926 cm^{-1}, 1634 cm^{-1}, 1535 cm^{-1}, 1336 cm^{-1} and 1043 cm^{-1} for *Citrus limonum* seed (Fig. 10).

The absorption peaks located at around 3270 cm^{-1}, 3276 cm^{-1} and 3280 cm^{-1} can be assigned to the -C≡C-H:O-H Stretching vibrations due to the amines terminal linkages in polypeptides respectively. Sanghi *et al.* (2009) have reported the proteins can bind to silver nanoparticles either through free amine groups or cysteine residues vital role in the protein.

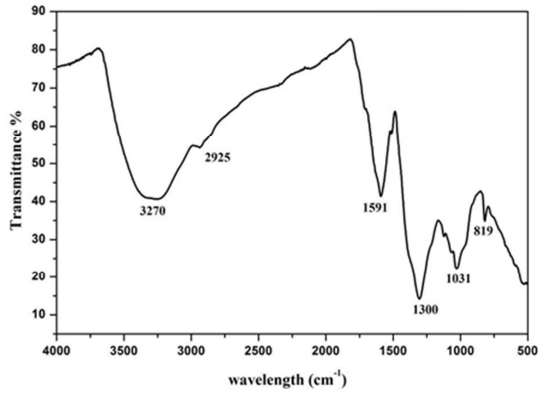

Fig. 8. FTIR Spectrum of silver nanoparticles using bark extract of *Citrus limonum*

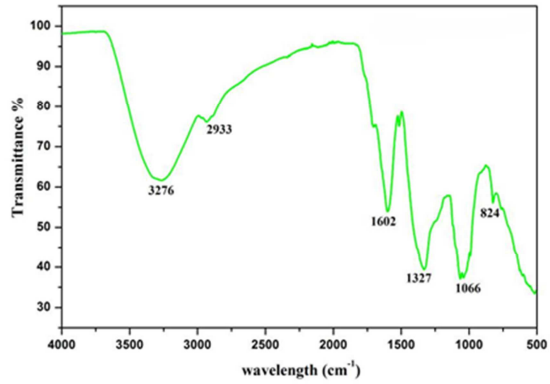

Fig. 9. FTIR Spectrum of silver nanoparticles using leaf extract of *Citrus limonum*

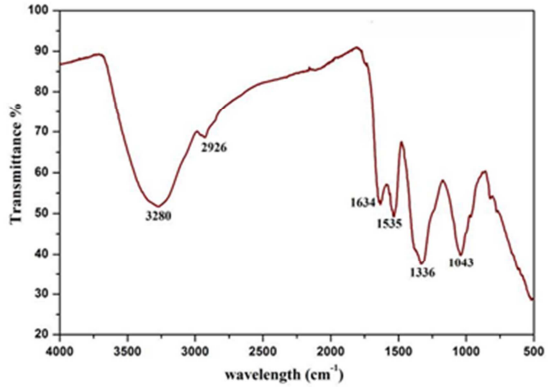

Fig. 10. FTIR Spectrum of silver nanoparticles using seed extract of *Citrus limonum*

The current report may be cystein residues play a vital role in the silver nanoparticle. The weaker band was observed at 2929 cm^{-1} correspond to the C-H stretching vibrations alkanes groups. In *Citrus limonum* derived silver nanoparticles, the bands can be existing at 2926 cm^{-1}, 1634 cm^{-1}, 1535 cm^{-1}, 1336 cm^{-1} and 1043 arises C-H stretching, C-C Stretch (in ring), C-H (-CH_2X) and C-N Stretch vibrations due to aromatic groups, alkens, alkens halides and aliphatic amins, respectively as shown in Table 1. Previously, Bai *et al.* (2009) reported that the aliphatic and aromatic groups were involved in the synthesis of silver nanoparticle by using *R. palustris*. The band seen at 796 cm^{-1} and 557 cm^{-1} and are identified as alkyl halides and arises due to C–Cl stretching. The evidence suggests that the biological molecules can possibly carry out the function for the formation and stabilization of the silver nanoparticles in the aqueous medium.

Table 1. FTIR analysis and functional groups of extract of bark and seed of *Citrus limonum*

Citrus limonum extracts	Wavenumber (cm^{-1})	Functional Groups
Bark extracts	3270 cm^{-1}	-C≡C-H:O-H Stretch
	2725 cm^{-1}	C-H Stretch
	1591 cm^{-1}	C-C Stretch (in ring)
	1300 cm^{-1}	C-H Wag (-CH_2X)
	1031 cm^{-1}	C-N Stretch
	819 cm^{-1}	=C-N Bend
Leaf extract	3276 cm^{-1}	-C≡C-H:O-H Stretch
	2933 cm^{-1}	C-H Stretch
	1602 cm^{-1}	C-H Stretch (in ring)
	1327 cm^{-1}	C-H Wag (-CH_2X)
	1066 cm^{-1}	C-N Stretch
	824 cm^{-1}	=C-N Bend
Seed extracts	3280 cm^{-1}	-C≡C-H:O-H Stretch
	2926 cm^{-1}	C-H Stretch
	1634 cm^{-1}	C-C Stretch (in ring)
	1535 cm^{-1}	C-C Stretch (in ring)
	1336 cm^{-1}	C-H Wag (-CH_2X)
	1043 cm^{-1}	C-N Stretch

Scanning electron microscope (SEM)

Scanning electron microscope shows the surface morphology and size of the silver nanoparticles at different magnification such as 10,000X and 20,000X and operated at an accelerating voltage of 30 Kv. *Citrus limonum* treated with the silver nanoparticle are well-dispersed (Kokila *et al.*, 2015). The particles were approximately in the range of 1 µm (Scale bar). SEM image had shown (Fig. 11 and 12) individual nanoparticles as well as number of aggregates.

It illustrates the particles are spherical in shape and aggregates into the particles are no well-defined morphology. Silver nanoparticles of sizes 30-45 nm (Fig. 11) for *Citrus limonum* bark extract and 32 nm to 53 nm (Fig. 12) for *Citrus limonum* leaf extract respectively. The present report is good agreement with silver nanoparticles was reported by using the green synthesis method.

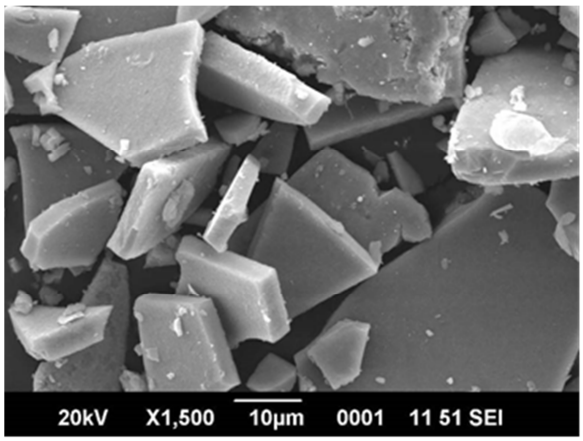

Fig. 11. SEM analyses of silver nanoparticles. SEM images shows some spherical-shaped and poly dispered silver nanoparticles synthesized using bark extract of *Citrus limonum*.

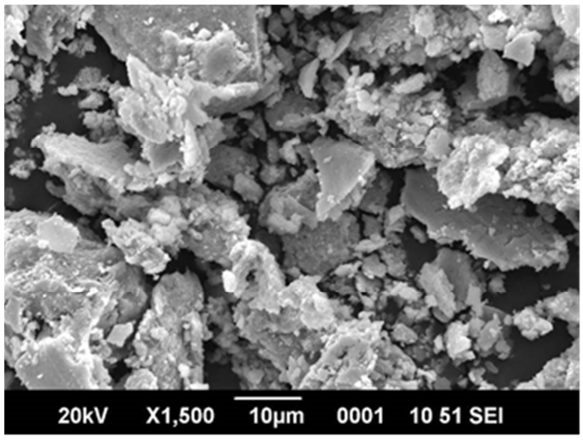

Fig. 12. SEM analyses of silver nanoparticles. SEM images shows some spherical-shaped and poly dispered silver nanoparticles synthesized using leaf extract of *Citrus limonum*

Silver nanoparticles antimicrobial activity and its clinical applications

Using the well-diffusion approach, the antibacterial activity of the silver nanoparticle synthesized using this method was investigated against two distinct kinds of bacteria, *Bacillus cereus* and *Pseudomonas aeruginosa*. As seen in Fig. 13, the concentration of silver nanoparticle was adjusted to 25 µl, 50 µl, 75 µl, and 100 µl. The assessment of green approach silver nanoparticleµ antibacterial activity against Citrus limonums bark, leaf, and seed extracts was conducted in vitro using the well diffusion method, as Fig. 13 illustrates.

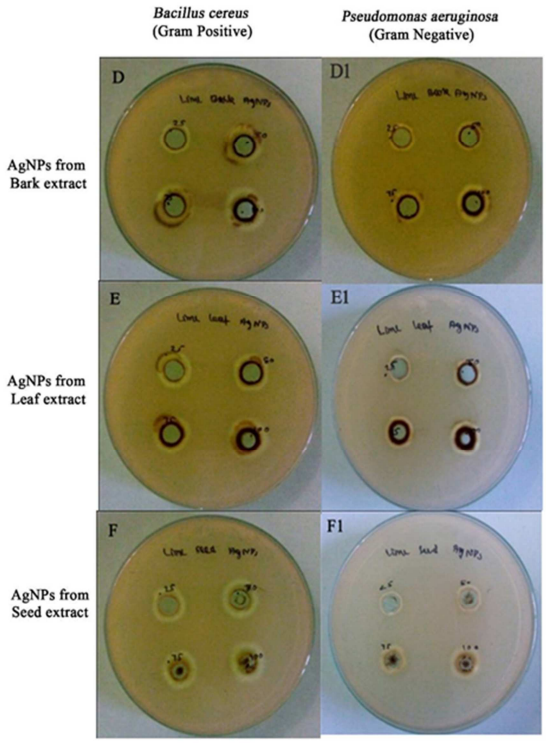

Fig. 13. Antibacterial activity of AgNPs synthesized from bark extract, leaf extract and seed extract of *Citrus limonum*

Standard antibiotics including Bacteria, *Bacillus cereus*, and *Pseudomonas aeruginosa* were also tested for their antibacterial efficacy.

In *Citrus limonum* bark, the zone of inhibition formation is mediated by synthesizing silver nanoparticle, and Table 2 displays the different concentrations of *Bacillus cereus* and *Pseudomonas aeruginosa*, which are 9±0.577, 10±0.577, 11±0.577, and 12±0.577, and 3.333±0.333, 4.666±0.333, 5.333±0.333, and 6.166±0.440. Fig. 14 depicts the zone of inhibition, and Fig. 15 shows a comparison of two distinct species.

Table 2. Antibacterial activity of AgNPs synthesized from bark extract, leaf extract and seed extract of *Citrus limonum*

AgNPs from lime plant extract	Test organisms	Zone of inhibition (mm)			
		25 µl	50 µl	75 µl	100 µl
AgNPs from bark extract	*Bacillus cereus*	9 ± 0.577	10 ± 0.577	11 ± 0.577	12 ± 0.577
	Pseudomonas aeruginosa	3.333 ± 0.333	4.666 ± 0.333	5.333 ± 0.333	6.166 ± 0.440
AgNPs from leaf extract	*Bacillus cereus*	13 ± 0.577	14 ± 0.577	14.666 ± 0.666	15.666 ± 0.333
	Pseudomonas aeruginosa	4 ± 0.577	4.333 ± 0.333	5.333 ± 0.333	6 ± 0.577
AgNPs from seed extract	*Bacillus cereus*	13 ± 0.577	13.666 ± 0.333	14.333 ± 0.333	15.666 ± 0.333
	Pseudomonas aeruginosa	3.666 ± 0.333	5 ± 0.577	5.666 ± 0.333	6.666 ± 0.333

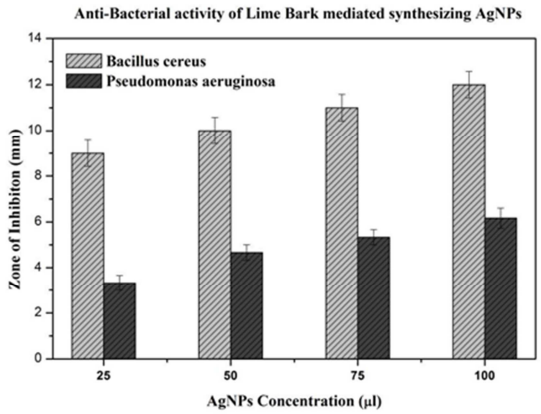

Fig. 14. Antibacterial activity of AgNPs synthesized from bark extract of *Citrus limonum*

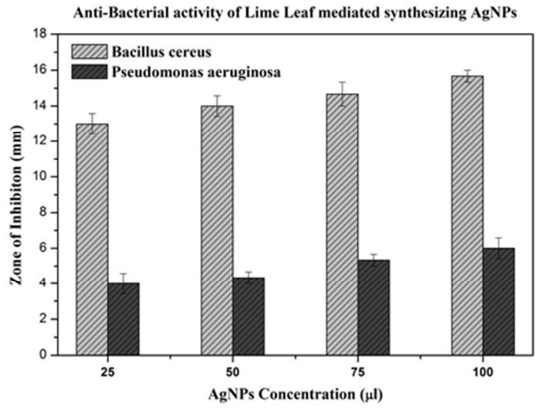

Fig. 15. Antibacterial activity of AgNPs synthesized from leaf extract of *Citrus limonum*

The growth of two distinct bacterial species was inhibited by silver nanoparticle produced from *citrus limonum* leaves, as seen in Fig. 16. The inhibition values of 13±0.577, 14±0.577, 14.666±0.666, 15.666±0.333 and 4±0.577, 4.333±0.333, 5.333±0.333, and 6±0.577 were represented by Table 2. Fig. 14 displays the contrast suppression of two distinct bacterial species (*Bacillus cereus* and *Pseudomonas aeruginosa*) with varying concentrations of *Citrus limonum* seed-mediated synthesis of silver nanoparticle. The zone of inhibition for *Pseudomonas aeruginosa* and *Bacillus cereus* at various doses of silver nanoparticle from *Citrus limonum* seed water extract (Fig. 16) is depicted in Fig. 15, which corresponds to the table. 3.666±0.333, 5±0.577, 5.666±0.333, 6.666±0.333, and 2, 13±0.577, 13.666±0.333, 14.333±0.333, 15.666±0.333. Presumably, *Citrus limonum* Bark, Leaf, and Seed extract was employed since it exhibited antibacterial properties, which need to be reflected in a larger inhibitory zone. However, because of their extraction method and reduced concentration during the experiment, they alone exhibit very little activity (Praphu and Paulose, 2012).

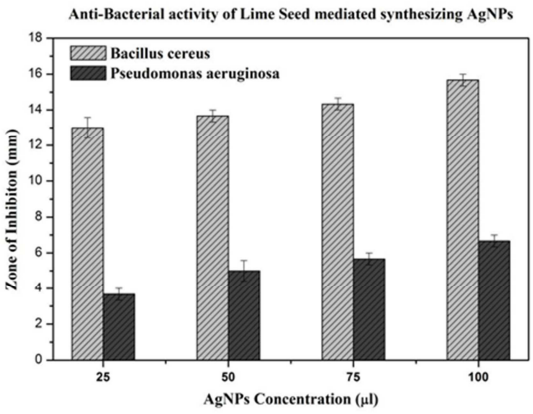

Fig. 16. Antibacterial activity of AgNPs synthesized from seed extract of *Citrus limonum*

Compared to *Pseudomonas aeruginosa*, silver nanoparticle has been shown to be more efficient against *Bacillus cereus*. Additionally, it was discovered that the bactericidal action of silver nanoparticle increased with dosage (Table 2). More

inhibition was seen in the synthesized silver nanoparticle made with *Citrus limonum* extract (Bark, Leaf, and Seed extracts) than in the other samples (Table 2). The different sizes and forms of nanoparticle could be the cause of this. According to research, silver nanoparticle bactericidal ability is totally dependent on their dose and particle size, and they are more effective against Gram-negative bacteria than Gram-positive ones (Sharma *et al.*, 2009; Savithreamma *et al.*, 2011).

It is clear that these nanoparticle exhibit strong antibacterial activity against the Gram-positive and Gram-negative bacteria that were tested. It was shown that these nanoparticle, when utilized in very low concentrations, are capable of inhibiting the growth of microbial strains due to their bactericidal and inhibitory behavior. AgNPs may have an antibacterial effect on these particles because of their capacity to increase cell membrane permeability, produce free radicals and interact with thiol groups, influence cellular signaling, and stop the formation of biofilms (Rai *et al.*, 2009; 2012). Literature review, four different mechanisms have been put up to explain how antimicrobial activity of AgNPs works. These include AgNPs' interactions with cell membranes, changes to membrane permeability, and disruptions to respiratory chain enzymes. gradual release of nanoparticle into the cells, which may limit transcription by binding silver particles and negatively impact cellular enzyme activity; release of subcellular components due to interaction between nanoparticle and the plasma membrane, which results in cell death, and the production of free radicals when silver ions impact the cell membrane (Prabhu and Poulose, 2012, Rizzello and Pompa, 2014).

Conclusion

The extract from the bark, leaves, and seeds of *Citrus limonum* is highly concentrated in bioactive compounds, such as minerals, flavonoids, ascorbic acid, citric acid, and phenolic acids. In order to do this, we employed a straightforward, safe, and environmentally friendly method for *Citrus limonum* bark, leaves, and seeds to biosynthesize silver nanoparticle. In this study, a simple approach was used to synthesise AgNPs using the Bark, Leaf and Seed extract of *Citrus limonum* of fruits. The reduction of silver ions by lemon leaves extract resulted in the formation of stable nanoparticles with multi shaped morphologies resulted in different size range of silver nanoparticles. Silver nanoparticles synthesized by the green chemistry approach reported in this study using Bark Extract, Leaf extract and Seed Extract of *Citrus limonum* could have potent applications in biomedical and pharmaceutical applications. Furthermore, it has been demonstrated that use of a natural, renewable and low-cost biological reducing agent, such as Bark Extract, Leaf extract and Seed Extract of *Citrus limonum* can produce metal nanostructures in aqueous solution at ambient temperature, avoiding the presence of hazardous and toxic solvents. The antifungal activity of Silver. Nanoparticle derived from Extract, Leaf extract and Seed Extract of *Citrus limonum* showed enhancement in activity due to synergistic effect of silver nanoparticles and essential oil components of lemon leaves the effectively was enhanced as observed from the data. The synthesised nanoparticles were characterised using SEM, FTIR and X-ray diffraction XRD analysis, discovered that the green synthesised silver nanoparticles were well dispersed with no agglomeration. The antibacterial activity of silver nanoparticles was well demonstrated by the zone of inhibition. This method of synthesising the AgNPs is cost-effective and eco-friendly. These findings imply that the synthesized nanoparticles using green nanotechnology could be an ideal strategy to combat cancer and infectious diseases. The synthesized AgNPs proved to possess improved anticancer, antimicrobial activity in comparison with the extract.

Bark Extract, Leaf extract and Seed Extract of *Citrus limonum* has many pharmacological activities and hence was utilized for green synthesis of AgNPs. From the present study we conclude that green synthesis of silver nanoparticle from *Citrus limonum* fruit peel extracts possess very good antibacterial activity against selected

microorganisms. Moreover, they also showed synergistic effect on the antimicrobial activity against gram-positive and gram-negative microorganisms. Green synthesis of silver nanoparticles can potentially eliminate the problem of using chemical agents that may have adverse effects, thus making the nanoparticles more compatible with the eco-friendly approach. Hence, the obtained results are promising and prove to be an important step in this direction, making it a cost-effective and ecofriendly alternative to the conventional approaches.

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