

Original Research Article

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Synthesis of Silver Nanoparticles from Different Citrus Fruit Peel Extracts and a Comparative Analysis on its Antibacterial Activity

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ABSTRACT

Keywords

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Greener way of synthesizing nanoparticles has emerged as an alternative method, as it is eco- friendly and cost effective. The present work investigated the synthesis of silver nanoparticles with the aid of citrus fruit peel extracts (CFPE) under two different conditions such as sun dried and oven dried form. Characterisation of silver nanoparticles was carried out using UV-Visible spectroscopy. The colourless reaction mixture turned brown and displayed UV-Visible spectra characteristic of silver nanoparticles. The present study revealed that silver nanoparticles prepared from sun dried *Citrus sinensis* and mixed fruit peel extracts showed significant antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhimurium* which are common food related pathogens.

Introduction

Nanoparticle synthesis in recent years has received considerable attention due to their unique properties and potential applications (Swathy *et al.*, 2014).

The biological molecules undergo highly controlled assembly in order to make themselves suitable for nanoparticle synthesis. These nanoparticles are reliable and eco-friendly when compared with nanoparticles synthesized by other methods. The use of environmentally benign materials for the synthesis of nanoparticles offers numerous benefits in pharmaceutical and biomedical applications (Jain *et al.*, 2009), as toxic chemical substances are not employed in their

synthesis. The size and shape of the nanoparticles forms the basis for its usage as antimicrobial agents. Antibacterial activity of silver nanoparticles on antibiotic resistant, non-resistant and multidrug resistant strains disrupts bacterial enzyme activity, capable to penetrate bacterial cell membrane, creates structural imperfections thereby preventing bacterial proliferation (Caroling *et al.*, 2013). Extensive work has been carried out on the biological synthesis of nanoparticles by using plant extracts (Subbaiya *et al.*, 2014, Prathiba *et al.*, 2015, Kirubha *et al.*, 2015). Very few studies are available on the biosynthesis of silver nanoparticles from peel extracts (Aysha *et al.*, 2014, Kokila *et al.*, 2015) and seed

extracts (Pandit, 2015). The present study illustrates the biofabrication of silver nanoparticles from fruit waste materials especially citrus fruit peel extracts. Characterisation of silver nanoparticles was done by using UV-Visible spectroscopy, which gives a preliminary confirmation of silver nanoparticles. An attempt has been made to compare the antibacterial activity of silver nanoparticles prepared from different citrus fruit peel extracts and mixed fruit peel extracts.

Materials and Methods

Preparation of fruit peel extracts

Collection of citrus fruit peels and powder preparation

Fruits such as *Citrus sinensis*, *Citrus limon* and *Citrus limetta* were obtained from the local market. Then the fruit peels were removed and cleaned thoroughly using distilled water to remove the dust particles adhering to the surface of the fruit peel. Then the fruit peels were kept for drying in two different conditions, one in microwave oven and the other under sunlight. In microwave oven it was dried at 160°C for 5 minutes and under sunlight it was dried for a period of 3-5 days. The dried peels were powdered and extract preparation for each type of fruit peels were done separately for both the techniques. Mixed fruit peel extract was also prepared in both the cases. Biosynthesis of silver nanoparticles (Caroling *et al.*, 2013, Aysha *et al.*, 2014, Kiruthika *et al.*, 2016)

Five grams of powdered peel of each type of citrus fruit was added to 50ml of distilled water and boiled (water bath) for 15 minutes at 50°C. Then the extract was filtered through Whatmann No.1 filter paper and stored at 4°C for further use (as per standard procedures with slight modifications). Synthesis of silver

nanoparticles involved addition of 2ml of 1mM AgNO₃ solution to 8ml of fruit peel extract and it was kept under dark condition for 24 hours at room temperature. One tube was kept as a control without addition of 1mM AgNO₃ solution. The same method was adopted for all other extracts.

Characterisation of silver nanoparticles

Silver nanoparticles are soluble in distilled water and the colour changes were observed visually. A dark brownish colouration indicates the formation of silver nanoparticles. The reduction of pure Ag⁺ ions was monitored by measuring the UV spectrum of the reaction medium after overnight incubation after diluting a small aliquot of the sample in distilled water. The silver nanoparticles synthesized was analysed with the help of UV-Visible spectrophotometer (300 – 600 nm range).

Assessment of antibacterial activity of silver nanoparticles

Test pathogens

Antibacterial activity of synthesized silver nanoparticles was tested against *Salmonella typhimurium* (MTCC 10312), *Escherichia coli* (MTCC 3224) and *Pseudomonas aeruginosa* (MTCC 6204). The bacterial strains employed in this work were procured from Microbial Type Culture Collections (MTCC) centre, Institute of Microbial Technology (IMTECH), Chandigarh.

Silver nanoparticles as antibacterial agent

The bacterial strains obtained from MTCC were used to determine the antibacterial activity of the silver nanoparticles. The experiment was carried out in Mueller Hinton agar by disc diffusion method. The inoculum was prepared and compared with Mac

Farland’s standard (0.5). Then the test culture was spread on to the agar surface. Discs with different concentrations (10µL, 20µL, 30µL) of silver nanoparticles (from peel extracts), silver nitrate and extract without silver nitrate were placed on the agar surface.

The same technique was followed for all the 8 extracts such as *Citrus sinensis* - sun dried and oven dried, *Citrus limon* -sun dried and oven dried, *Citrus limetta* – sun dried and oven dried and mixed peel extracts - sun dried and oven dried (Figs. 1 and 2). The plates were incubated at 37°C for 24 hours.

Results and Discussion

Synthesis of silver nanoparticles from citrus fruit peel extracts was visually confirmed by colour change after 24 hours of incubation at room temperature. The peak value between 400 -500 nm ranges indicates the presence of silver nanoparticles by UV- Visible spectrophotometer analysis. Among all the

citrus fruit peel extracts, the silver nanoparticles of *Citrus sinensis* (sun dried) (Fig. 1) extract showed good antibacterial activity against all the bacterial strains used. Mixed fruit peel extract (sun dried) also showed good antibacterial activity.

The antibacterial activity was confirmed on the basis of zone size exhibited by standard antibacterial agent (Ceftriaxone- 30 µg was taken as common antibacterial agent).

UV – Visible spectroscopy is a simple and fast way to confirm the formation of silver nanoparticles. Synthesis of nano sized particles with antibacterial properties is of great interest in the development of new pharmaceutical products. Among the different types of extracts prepared, sun dried *Citrus sinensis* and mixed fruit peel extracts showed good antibacterial activity. Studies show that the positive charge on the Ag+ ions is crucial for its antimicrobial activity (Figs. 3–6).

Table.1 Zone of inhibition shown by silver nanoparticles synthesized from citrus Fruit peel extracts against *Pseudomonas aeruginosa*

Microorganism	Silver nanoparticles from		Concentration and Zone size (Diameter in mm)		
			10µl	20µl	30µl
<i>Pseudomonas aeruginosa</i> (MTCC 6204)	<i>Citrus sinensis</i>	SD	10	18	21
		OD	8	14	20
	<i>Citrus limon</i>	SD	9	14	21
		OD	8	12	19
	<i>Citrus limetta</i>	SD	9	15	21
		OD	7	13	20
	Mixed peels	SD	12	16	23
		OD	10	15	21

Table.2 Zone of inhibition shown by silver nanoparticles synthesized from citrus Fruit peel extracts against *Salmonella typhimurium*

Microorganism	Silver nanoparticles from		Concentration and Zone size (Diameter in mm)		
			10µl	20µl	30µl
<i>Salmonella typhimurium</i> (MTCC 10312)	<i>Citrus sinensis</i>	SD	10	17	22
		OD	9	15	20
	<i>Citrus limon</i>	SD	9	13	20
		OD	8	11	19
	<i>Citrus limetta</i>	SD	7	13	20
		OD	7	11	19
	Mixed peels	SD	12	17	22
		OD	10	13	21

Table.3 Zone of inhibition shown by silver nanoparticles synthesized from citrus Fruit peel extracts against *Escherichia coli*

Microorganism	Silver nanoparticles from		Concentration and Zone size (Diameter in mm)		
			10µl	20µl	30µl
<i>Escherichia coli</i> (MTCC 3224)	<i>Citrus sinensis</i>	SD	10	15	21
		OD	8	14	20
	<i>Citrus limon</i>	SD	9	15	21
		OD	8	13	19
	<i>Citrus limetta</i>	SD	9	14	20
		OD	7	14	20
	Mixed peels	SD	11	15	22
		OD	10	15	20

SD- Sun dried, OD- Oven dried

Fig.1 Sun dried citrus fruit peel powder



Fig.2 Oven dried citrus peel powder

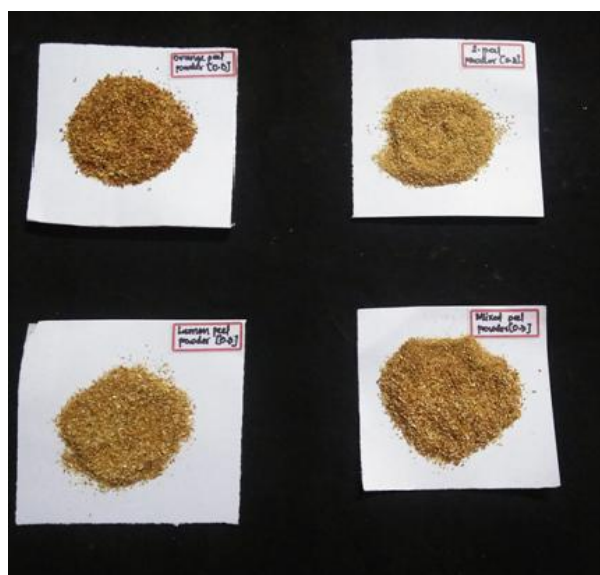


Fig.3 UV-Vis Characterisation of AgNPs of *Citrus limetta*

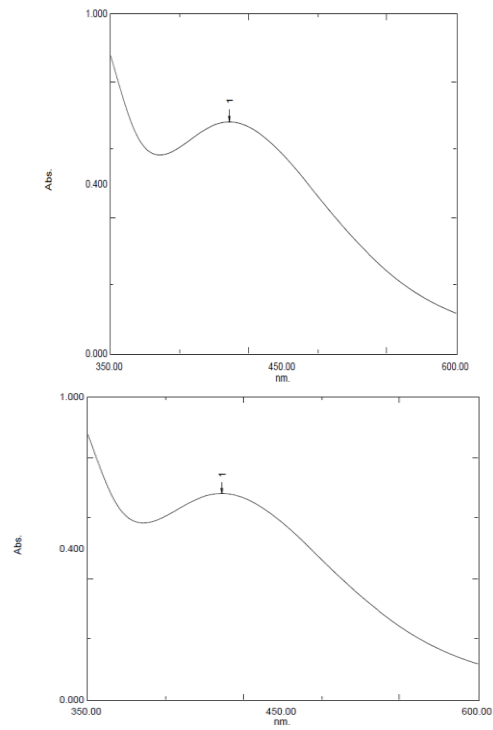


Fig.4 UV-Vis Characterisation of AgNPs of *Citrus limon*

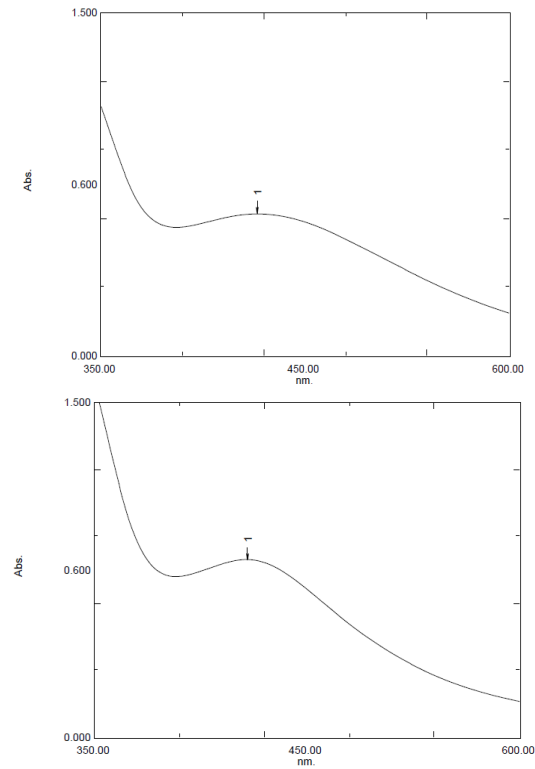


Fig.5 UV-Vis Characterisation of AgNPs of *Citrus sinensis*

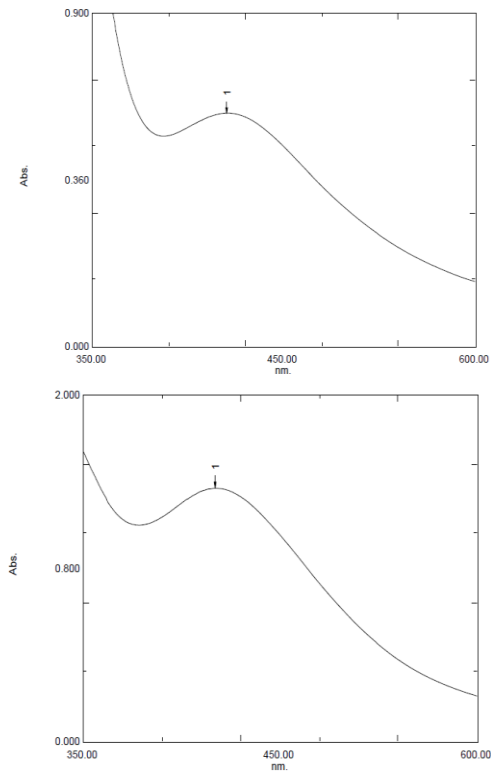


Fig.6 UV-Vis Characterisation of AgNPs of Mixed Extract

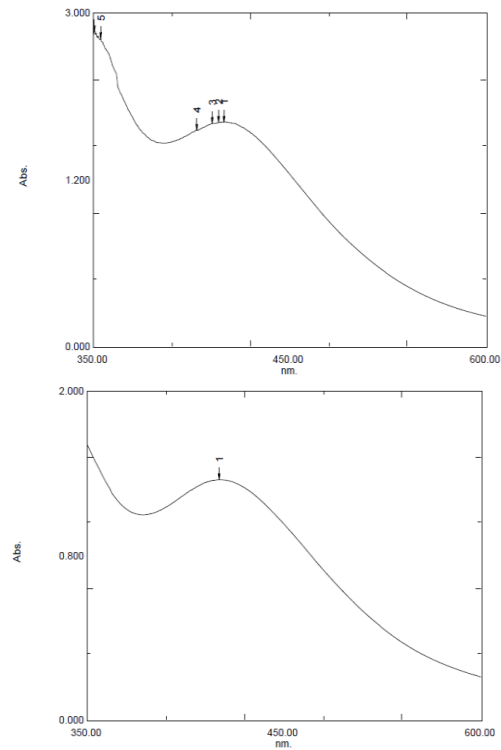
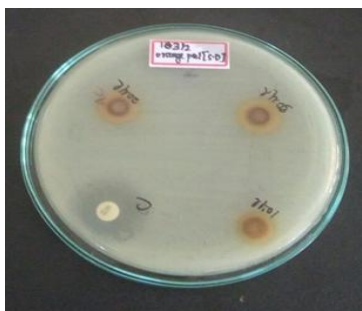


Fig.7 AgNPs of mixed peel extract (SD) showing antibacterial effect on *Pseudomonas aeruginosa*



Fig.8 AgNPs of mixed peel extract (SD) showing antibacterial effect on *Salmonella typhimurium*



They not only interact with the surface of the microbial membrane but can also penetrate into the cell (Banker *et al.*, 2010).

Thus the present study is a preliminary work on the biosynthesis of silver nanoparticles from citrus fruit peel extracts and further it was found that, silver nanoparticles obtained from sun dried *Citrus sinensis* and mixed fruit peel extracts showed good antibacterial activity (Figs. 7 and 8; Tables 1–3).

In future, these methods can be employed in drug delivery system as it is cost effective when compared with the usage of conventional antibiotics and also in food packaging to prevent spoilage of food materials but only after performing an intrinsic study on the preparatory methods.

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