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Characterization and antimicrobial activity of lemon peel mediated green synthesis of silver nanoparticles

Abstract: For a long time, nanoparticle biosynthetic discipline is still under development and is known to have a big impact on numerous manufactures. Synthesis of nanoparticles by green methods with antimicrobial properties is of great researchers concern in the explored of new pharmaceutical and biomedical products. In this study, synthesis of silver nanoparticles (AgNPs) have been carried out using aqueous extract of lemon peel (*Citrus limon*), which acts as encapsulating cage for the silver nanoparticles. Synthesized AgNPs was monitored by formation of brown color, confirmed by UV/VIS spectra, which showed appearance of a surface plasmon resonance (SPR) band. FT-IR analysis of the bioextract after the addition of silver solution showed strong bands at 1021, 1443, 1634 and 3428 cm^{-1}. Transmission electron microscopy examination of the solution containing AgNPs demonstrated spherical particles within nanoranged from 9.3 nm to 20.3 nm with the main diameter of 15.76 nm. The biologically synthesized AgNPs showed a promising antimicrobial activity, where the maximal growth inhibition was recorded for both *Pseudomonas* (*P.*) *aeruginosa* and *Escherichia* (*E.*) *coli* – 21.5 ± 1.3 and 19.0 ± 0.20 mm, respectively. While the minimum growth inhibition was recorded for Gram positive bacteria *Bacillus subtilis* (*B.*) and *Staphylococcus* (*S.*) *aureus* – 15.0 ± 0.20 and 16.5 ± 1.50 mm, respectively. At the same time weak antifungal activity was observed for both *Aspergillus* (*A.*) *flavus* and *Candida* (*C.*) *albicans* – 14.0 ± 0.15 and 12.5 ± 0.45 mm, respectively.
Key words: green synthesis, silver nanoparticles, lemon peel extract, antimicrobial activity.

Introduction

In the fast development of nanomaterials in the quest for green, eco-friendly routes for new products often culminates in the utilization of microorganisms [1] and plant biomasses for the manufacture of sustainable nanocomposites [2; 3] and nanoparticles [4-6], which are frequently used in biological approaches [7]. Nanotechnology mainly deals with the fabrication of nanoparticles having various shapes, sizes and managing their chemical and physical parameters for further use in human benefits with their growing applications in various fields [8]. Preparation of metal nano-sized, usually ranging in size from 1 to 100 nm, is amongst the most emerging areas in the field of nanotechnology. Currently, the application of nano materials is becoming increasingly important in order to solve the problems associated with material sciences, including solar energy conversion, photonics [9; 10], catalysis [11], microelectronics [12], antimicrobial functionalities [13], and water treatment [14]. Many biological routes of AgNPs synthesis have been reported using plant extracts, such as *Citrus aurantium*, *Citrus limon*, *Capsicum annuum*, *Brassica oleracea*, *Aloe vera*, *Nigella sativa*, *Pulicaria glutinosa*, *Justicia glauca*, *Mimusops elengi L.*, and *Coffea L.* [15-26].

However, in most cases, a deep characterization of the biomolecule coating is not made. It is known that the reducing agents may include flavonoids, membrane proteins, $NAD(P)^+$ reductases, dehydrogenases, citric acid, polyphenols, and secondary metabolites [27], whereas the capping agents may be extracellular proteins, enzymes, peptides, and tannic acids [28]. The AgNPs have various and important applications. Historically, silver has been known having a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items. It has been reported that AgNPs are non-toxic to human and most effective against bacteria, virus and other eukaryotic micro-organism at low concentrations and without any side effects [29; 30]. Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents [31]. A small concentration of silver is safe for human cells, but lethal for microorganisms [32]. Antimicrobial capability of AgNPs allows them to be suitably employed in numerous household applications such as textiles disinfection in water treatment, food storage containers, home appliances and in medical devices [33]. The most important application of silver and AgNPs is in medical industry such as tropical ointments to prevent infection against burn and open wounds [34]. Nowadays, biosynthesis of silver nanoparticles (AgNPs) had gained so much attention in developed countries due to development demand of environmental friendly technology for material synthesis. Thus, this study aims to show sustainable alternatives of new antimicrobial products based on nanomaterials by utilizing lemon waste to produce AgNPs that are active toward a number of pathogenic microorganisms.

Materials and methods

Chemicals. Chemicals and reagents used in the following experiments were of analytical grade. Silver nitrate ($AgNO_3$) salt was purchased from Techno Pharmchem, India. All the media components were from Oxoide, India.

Microbial strains. Antimicrobial activity was assayed against a panel of microorganisms certified by American Type Culture Collection (ATCC) and National Collection of Pathogenic Fungi (NCPF), including three Gram-positive bacteria *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538, and against Gram-negative *Pseudomonas aeruginosa* ATCC 9027 and *Escherichia coli* ATCC 7839, a fungus (yeast) – *Candida albicans* (NCPF-stock laboratory strain) filamentous fungi – *Aspergillus flavus*. Cultures were inoculated into specific broths and incubated at 37 °C for 24 h.

Collection of lemon peel. The lemon *Citrus limon* was purchased from two popular markets at Rafha governorate, Northern Border region in the Kingdom of Saudi Arabia. The collected lemon was washed, air-drying and subjected for peel obtaining in the laboratory under aseptic conditions.

Preparation of lemon peel biological extract. The collected lemon peel was washed and boiled in distilled water for 10 min at 90°C. 100 g of the lemon peel were crushed in 200 mL of distilled water; resulting extract was filtered through a clean cloth and treated with equal volumes of chilled ethanol. Resulting precipitate was lyophilized into a powder for further experiments.

The green synthesis of silver nanoparticles (AgNPs). AgNPs were synthesized by bioreduction of Ag^+. 5 mL of the fresh suspension of lemon peel extract (greenish in color) were added to 45 mlL 0.002 M $AgNO_3$ solution in 100 mL conical flasks at the room temperature in darkness for some period. The emulsion color turned to dark brown after adding of 1 mM $AgNO_3$ and stirring at room temperature.

Characterization of silver nanoparticles (AgNPs) as follows

UV/VIS spectral analysis. The synthesized silver nanoparticles were characterized spectrophotometrically using ultraviolet UV/VIS spectroscopy analyses as function of time at room temperature using Perkin Elmer UV/VIS spectrometer. Samples of aliquots (0.2 ml) of the suspension were diluted with 2 ml deionized water and subsequently measured UV/VIS spectra of the resulting diluents.

Fourier transform infrared spectrometer (FT-IR). FT-IR measurements were carried out in order to obtain information about chemical groups present around AgNPs for their stabilization and conclude the transformation of functional group due to reduction process. The measurements were carried out using JASCO FT-IR-3600 infra-red spectrometer by employing KBr Pellet technique.

Transmission electron microscopy (TEM). The size and morphology of the synthesized nanoparticles were recorded by using TEM model JEOL electron microscopy JEM-100 CX. TEM studies were prepared by drop coating silver nanoparticles onto carbon-coated TEM grids. The Film on the TEM grids were allowed to dry, the extra solution was removed using a blotting paper.

Assay of antimicrobial activity of the synthesized AgNPs. Antimicrobial activity was determined by the disk diffusion method [35], using cell suspensions with concentrations equilibrated to a 0.5 McFarland standard for bacterial and unicellular fungal strains and loopful for the filamentous fungi. After incubation at appropriate incubation conditions, the plates were investigated and the diameters of inhibition zones were recorded.

Statistical analysis. All experiments were performed in triplicate and the values were expressed as mean ± SD using Microsoft Excel 2016.

Results and discussion

Buzzing of nanotechnology in each and every aspect of science and technology has been booming at a tremendous rate now a day. Nanotechnology has different applications in the field of physical, chemical and medical sciences; it has now started revolutionizing the drug delivery sciences [36].

Biological synthesis of nanoparticles by plant extracts is at present under exploitation as some researchers worked on it [37; 38] and testing for antimicrobial activities [39-41]. For the last two decades, extensive work has been done to develop new drugs from natural products because of the resistance of microorganisms to the existing drugs. Nature has been an important source of a products currently used in medicinal practice [42].

Biosynthesis of silver nanoparticles by lemon peels bioextract. In the present study, synthesis of AgNPs by the lemon peel bioextract was carried out. Silver nitrate used has distinctive properties such as good conductivity, catalytic and chemical stability. The formation of AgNPs was found to be successful as suggested by initial changes in color. It is well known that AgNPs exhibit brown color (Figure 1a, b) in aqueous solution due to excitation of surface plasmon vibrations in AgNPs. It is well known that silver nanoparticles exhibit yellowish-brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles [43-45]. Reduction of the silver ion to silver nanoparticles during exposure to the peel extracts was followed by color change and as well as by UV/VIS spectroscopy.

Characterization of the synthesized silver nanoparticles AgNPs. Reduction of the silver ion to silver nanoparticles during exposure to the peel extract was followed by color change and as well as by UV/VIS spectroscopy. It is generally recognized that UV/VIS spectroscopy could be used to examine size and shape-controlled nanoparticles in aqueous suspensions. UV/VIS spectra that were recorded at different intervals for monitoring the reaction, the appearance of a surface plasmon resonance (SPR) band increased in intensity with time. It also reveals the production of silver nanoparticles within 1 h. The UV/VIS absorption spectra recorded from the silver nanoparticles solution after 1.5 h of reaction (Figure 2). Metallic silver nanocrystals generally show a typical optical absorption peak at approximately 3 keV due to the surface plasmon resonance [46-48]. This result confirmed that the produced nano-structures are pure silver. Therefore, metallic nanoparticles have spectrum in the UV/VIS region [49].

a b

Figure 1 – Differential colouring dependent of AgNPs synthesis.
Note: a – extract of lemon peel (colorless), b – synthesized AgNPs (brown color)

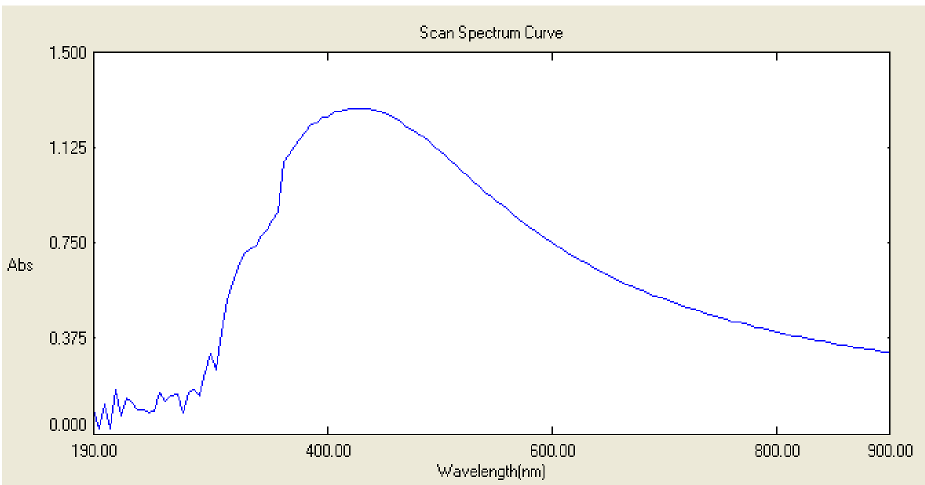

Figure 2 – UV/VIS spectra of reduced Ag ions to AgNPs with lemon peel bioextract

Analysis of FT-IR absorption spectra of the bioextract after the addition of silver solution revealed the strong bands at 1021, 1443, 1634 and 3428 cm^{-1}. The band at 1021 cm^{-1} corresponded to C-N stretching vibrations of amine. The band at 1443 cm^{-1} corresponded to C-H and OH bending and 3428 cm^{-1} was attributed to characteristic of –NH stretching of amide (II) band. The weaker band at 1634 cm^{-1} corresponded to amide I, arisen due to carbonyl stretch in proteins (Figure 3).

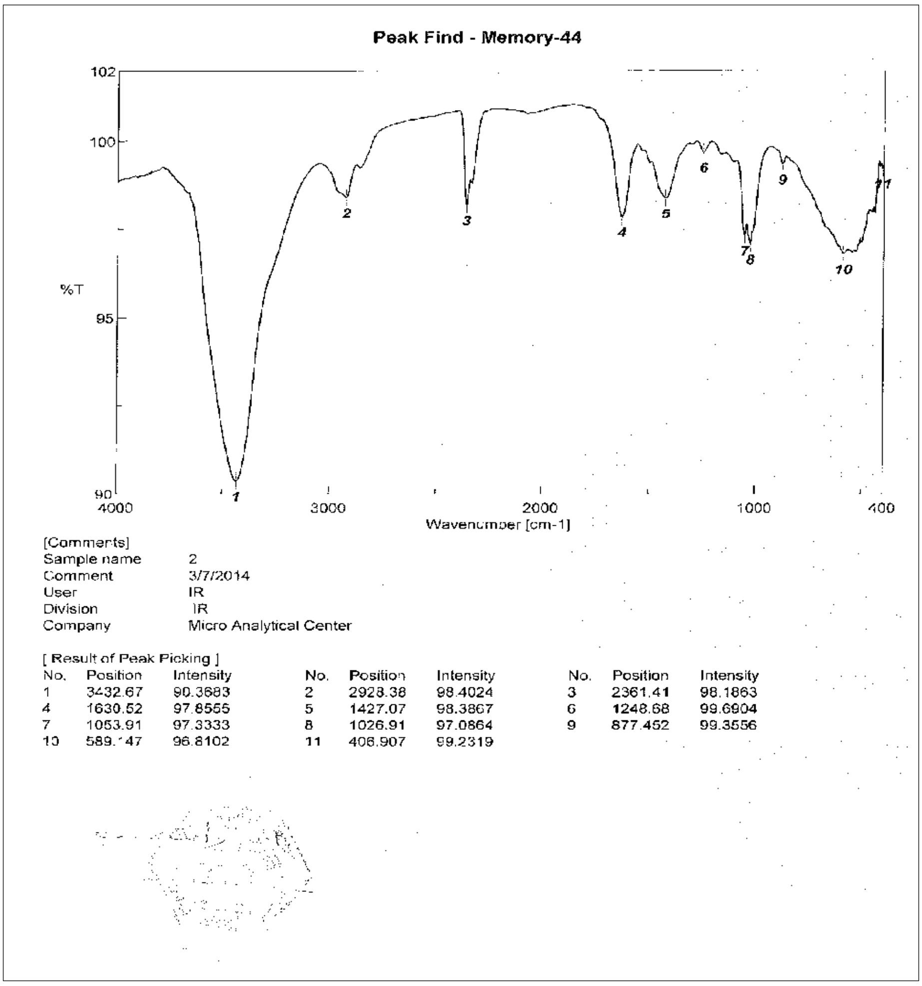

Figure 3 – FT-IR spectra of silver nanoparticles from lemon peel bioextract

FT-IR analysis of the synthesized AgNPs after the addition of silver solution revealed the strong bands at 1021, 1443, 1634 and 3428 cm^{-1}. The positions of these bands were close to that reported formative proteins. The FT-IR results indicate that the secondary structure of proteins was not affected as a consequence of reaction with Ag^{+} ions or binding with AgNPs [49].

Transmission electron microscopy (TEM) examination of the solution containing AgNPs demonstrated spherical particles within nanoranged from 7.4 nm to 18.5 nm with the main diameter of 11.60 nm (Figure 4).

TEM examination of the solution containing AgNPs demonstrated spherical particles within nanoranged from 7.4 nm to 18.5 nm with the main diameter of 11.60 nm. Similar AgNPs sizes synthesized from different sources were obtained various studies [49-51].

Antimicrobial activity of synthesized silver nanoparticles (AgNPs). The biologically synthesized AgNPs showed strong antimicrobial activity against number of the tested microbial strains (Table 1).

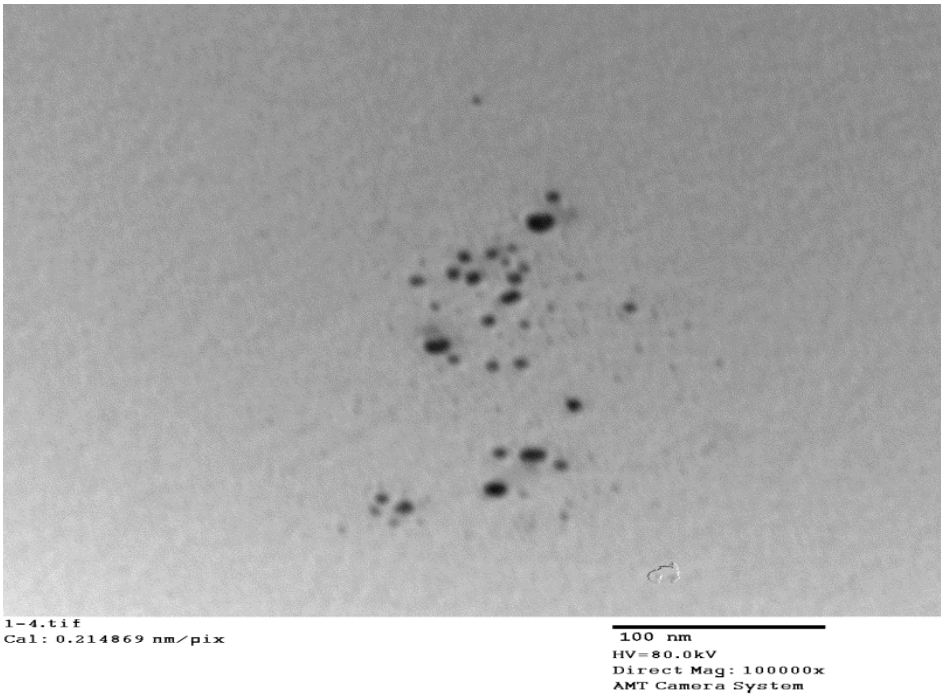

Figure 4 – TEM of the synthesized silver nanoparticles from lemon peel bioextract

Table 1 – Antimicrobial activity of silver nanoparticles from lemon peel

Agent	Mean diameter of inhibition zone (mm)					
	Gram positive bacteria		Gram negative bacteria		Unicellular fungi	Filamentous fungi
	B. subtilis ATCC 6633	*S. aureus* ATCC 6538	*P. aeruginosa* ATCC 9027	*E. coli* ATCC 7839	*C. albicans*	*A. flavus*
AgNPs	15.0 ± 0.20	16.5 ± 1.50	21.5 ± 1.3	19.0 ± 0.20	12.5 ± 0.45	14.0 ± 0.15

The maximal growth inhibition was observed in case of Gram-negative bacteria both for *P. aeruginosa* and *E. coli* with 21.5 ± 1.3 and 19.0 ± 0.20 mm, respectively. While the minimum growth inhibition was recorded for Gram-positive bacteria *B. subtilis* and *S. aureus* with 15.0 ± 0.20 and 16.5 ± 1.50 mm, respectively. At the same time weak antifungal activity was observed for both *A. flavus* and *C. albicans* with 14.0 ± 0.15 and 12.5 ± 0.45 mm, respectively.

Biologically synthesized AgNPs showed good antimicrobial activity against Gram-positive, Gram-negative bacteria as well as against unicellular multicellular fungi. Nanomaterials are the leaders in the field of nanomedicine, bionanotechnology and have a great importance in nano toxicology research. Silver exhibits the strong toxicity in various chemical forms to a wide range of microorganism that is very well known and AgNPs have recently been shown to be a promising antimicrobial material [46]. Silver ions have been known to bind with the negatively charged cell wall resulting in the rupture and consequent denaturation of proteins which leads to cell death [52]. The synthesized AgNPs with smaller size can act drastically on cell membrane and further interact with DNA and causes damage [53]. Other proposed mechanisms include the AgNPs causing depletion of intracellular ATP by rupture of plasma membrane or by blocking respiration in association with oxygen and sulfhydryl (–S-H) groups on the cell wall to form R-S-S-R bonds thereby leading to cell death [54].

Conclusion

Silver nanoparticles synthesized by the green chemistry approach reported in this study using lemon peels extract can be used as an antimicrobial agent in biomedicine and pharmaceutics. This study demonstrated that synthesized AgNPs have spherical shape within nanorange of 9.3 nm to 20.3 nm with the main diameter of 15.76 nm and have a promising antibacterial activity, with maximal growth inhibition against both *P. aeruginosa* and *E. coli* of 21.5 ± 1.3 and 19.0 ± 0.20 mm, respectively, as well as antifungal activity against both *A. flavus* and *C. albicans* with mean diameter of inhibition zone of 14.0 ± 0.15 and 12.5 ± 0.45 mm, respectively. Biogenic method used in this study is non-toxic, environmentally friendly, simple, and low-cost.

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