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Peel extract mediated synthesis, characterization and antimicrobial activity of silver nanoparticles using pumpkin

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ABSTRACT

Green synthesis of nanoparticles using plant extract is a simple, rapid, eco-friendly and reliable method for the production of nanoparticles. The present study indicates the formation of silver nanoparticles (AgNPs) using pumpkin peel extract. The aqueous peel extract is added to 1 mM silver nitrate solution and the formation of silver nanoparticles is primarily detected by the change of colour from colourless to brown. The reduction of Ag^+ to Ag^0 is confirmed by UV-Visible spectrum. Silver nanoparticles show a surface plasmon resonance peak at 451 nm. Fourier transform infra-red spectroscopy is performed to detect the bioactive molecules liable for reduction and capping of biogenic silver nanoparticles. The nature of the particle is determined from the X-ray diffraction analysis. The antimicrobial activity of the synthesised nanoparticles is tested and shows toxic effects on Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Pseudomonas aerogenisa, Aspergillus and Candida albicans.

***Keywords**: Green synthesis, pumpkin peel extract, silver nanoparticles, characterization, antimicrobial activity*

Introduction

Nanobiotechnology is presently one of the most dynamic disciplines of research in contemporary material science whereby plants and different plant products are finding an imperative use in the synthesis of nanoparticles. Nanoparticles are usually ≤100 nm in each spatial dimension and are commonly synthesized using top-down and bottom-up strategies [1]. Owing to their high surface-to-volume ratio, surface energy, spatial confinement and reduced imperfections, metal nanoparticles have characteristic physical, chemical, electronic, electrical, mechanical, magnetic, thermal, dielectric, optical and biological properties as opposed to bulk materials [2]. Hazardous substances have been used for the synthesis of nanoparticles in the traditional wet methods. Other dry methods such as UV irradiation, aerosol and lithography are also not considered environment-friendly. The use of such toxic chemicals is still the subject of paramount concern because toxic chemicals on the surface of nanoparticles and non-polar solvents in the synthesis limit their applications in clinical fields. Therefore, the green synthesis of clean, bio-compatible, non-toxic and environment-friendly nanoparticles of low cost, produced both extracellularly and intracellularly deserves merit [3].

Among noble metal nanoparticles, silver nanoparticles (AgNPs) have received considerable attention owing to their attractive physiochemical properties. Green synthesis of AgNPs by various plants has been reported [4-7]. Investigations on the green synthesis of AgNPs with plant extracts have been made so far and the present study concentrates on the green synthesis of AgNPs using pumpkin peel extract. The major constituents of pumpkin peel are anti-oxidants, vitamins, amino acids, carotenes, fatty acids, pectin and carbohydrates. [8]. The AgNPs synthesised from pumpkin peel extract is characterized by UV-Visible and FTIR spectroscopy. The nature of the AgNPs is determined from XRD analysis. The antimicrobial activity of AgNPs against toxic human pathogens is evaluated.

2. Experimental Section

Pumpkin used for the synthesis of AgNPs was bought from the local market. Silver nitrate ($AgNO_3$) used for the synthesis of silver nanoparticles was procured from Merck. Double-distilled deionized water was used to perform the experiments.

2.1 Preparation of pumpkin peel extract

20 g of pumpkin peel was thoroughly washed with double distilled water. This was taken in a 250 ml Erlenmeyer flask with 100 ml of double distilled water and then boiled the mixture for 5 min. The extract was filtered using Whatmann filter paper. The filtrate was collected and then centrifuged for about 8,000 rpm for about 10 minutes. The supernatant extract was collected and used as reducing agent for the synthesis of AgNPs.

2.2 Preparation of silver nanoparticles

Aqueous solution of 1 mM $AgNO_3$ was prepared and used for the synthesis of silver nanoparticles. 10 ml of pumpkin peel extract was added to 90 ml of 1 mM aqueous $AgNO_3$ solution in a 250 ml Erlenmeyer flask and incubated at room temperature. The sample colour changes from colourless to brown after 30 minutes indicate the formation of AgNPs. Ninety-five percent of the bio reduction of Ag^+ ions to Ag^0 occurred within 2 hours. The AgNPs were centrifuged at 15,000 rpm for 10 min and subsequently dispersed in sterile distilled water to get rid of any uncoordinated biological materials. The pellet of AgNPs collected at the bottom of the centrifuge tube was collected, dried and stored at -4° C.

2.3 Characterization Techniques

The absorption spectral measurement of AgNPs was carried out using Shimadzu UV-1800 spectrophotometer. FTIR analysis of the dried AgNPs was carried out through the potassium bromide (KBr) pellet (FTIR grade) method in 1:100 ratios and spectrum was recorded using Shimadzu IR Affinity-1. Phase formation of the synthesized nanoparticles was characterized by X-ray diffraction. Diffraction data for thin thoroughly dried nanoparticle films on glass slides were recorded on an X-ray diffractometer (Ultima III, Rigaku, Tokyo, Japan) with Cu Kα radiation (λ = 1.5406 Å) source in the 2θ range of 10°–80° with 4°/minute scanning rate.

2.4 Antimicrobial activity

Antimicrobial activities of synthesized silver nanoparticles against four bacteria and two fungi cultures of *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aerogenisa*, *Aspergillus* and *Candida albicans* were assayed by Kirby-Bauer discs diffusion method. These antimicrobials were grown in LB broth for 24 h. Approximately 20 ml of molten and cooled Muller Hinton agar was poured into the Petri dishes. The tested organisms were swapped over the agar medium and the AgNPs containing disks were kept over the medium using sterile forceps. Antimicrobial activity was evaluated by measuring the zone of inhibition for the test organisms. The diameters of zones were measured to the nearest millimetre with vernier calipers.

3. Results and Discussion

An inexpensive, versatile and very reproducible method for large scale synthesis and characterization of AgNPs by reduction process using peel extract of pumpkin is reported in this section. This peel extract can act both as reducing and stabilizing agents for the formation of AgNPs.

3.1 Absorption spectral analysis of AgNPs

The formation of AgNPs is preliminary confirmed by colour change from colourless to brown, followed by UV-Visible spectrophotometric analysis. The colour of AgNPs depends on the intensity and the size of nanoparticles. The brown colour of AgNPs in aqueous solution is due to the surface plasmon resonance phenomenon which results from the collective oscillations of their conduction band electrons in response to electromagnetic waves. Absorption spectrum of AgNPs formed in the reaction media after 30 minutes has an absorbance at 451 nm, broadening of peak indicated that the particles are poly dispersed (**Fig.1**).

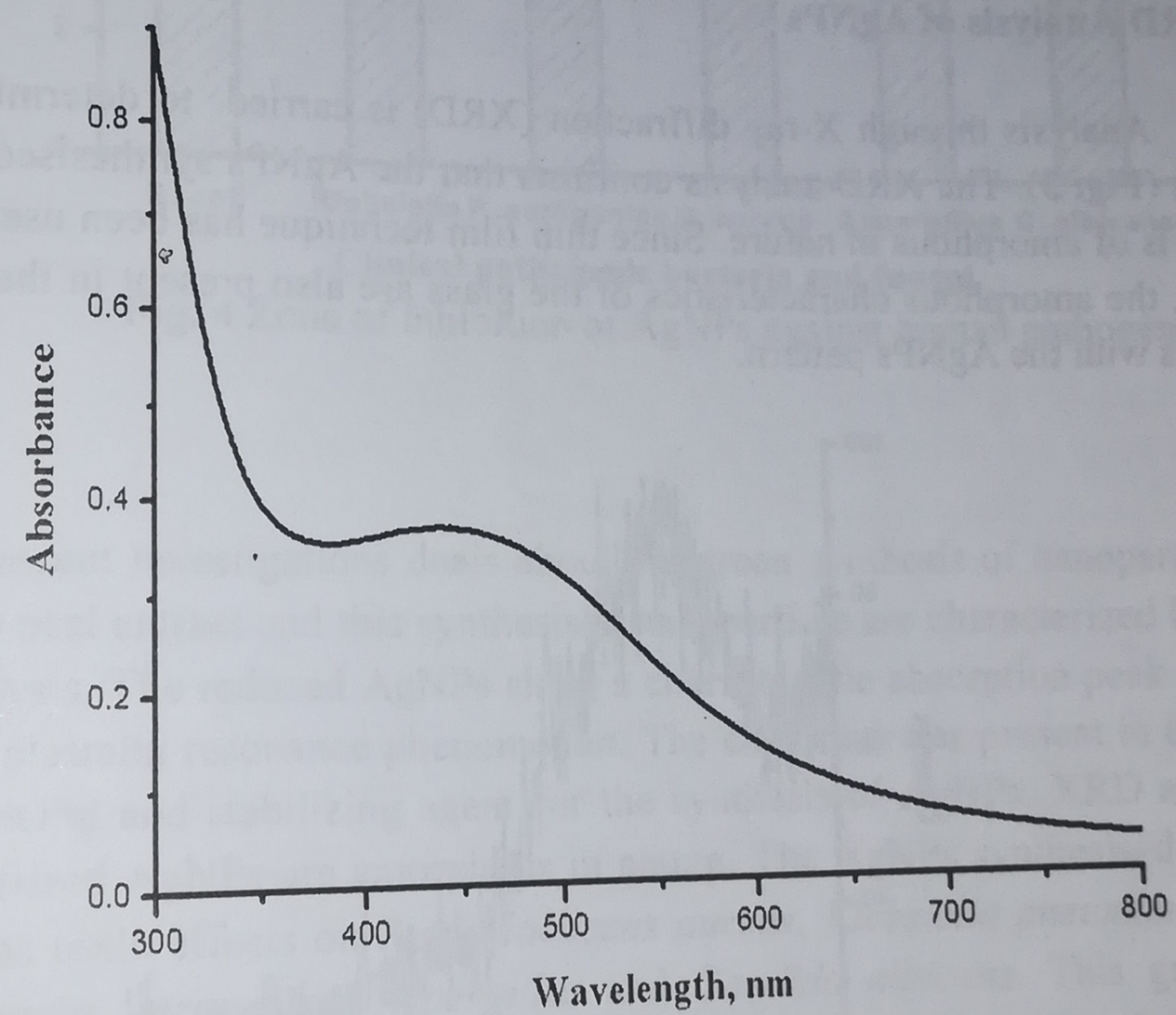

Fig. 1 Absorption spectrum of AgNPs synthesised from the peel extract of pumpkin

3.2 FTIR analysis of AgNPs

The FT-IR spectrum (**Fig. 2**) shows band at 3462, 2928, 2852, 1628, 1462, 1024 and 548 cm^{-1} respectively. The presence of weak band at 2928 cm^{-1}, 2852 cm^{-1} and 1462 cm^{-1} corresponds to the C-H stretching of methyl and methylene group. A broad band at 548 cm^{-1} indicates the stretching vibrations of alkyl halides. The weak band at 1024 cm^{-1} is assigned for the C-O stretching of primary alcohols. The broad band at 3462 cm^{-1} corresponds to the O-H stretching vibration of H-bonded alcohols. The relatively strong absorption peak around 1628 cm^{-1} indicates C=O stretching of carbohydrates. FTIR analysis confirms that the above functional groups present in the peel extract of pumpkin act as both reducing and stabilizing agent at room temperature for the formation of AgNPs.

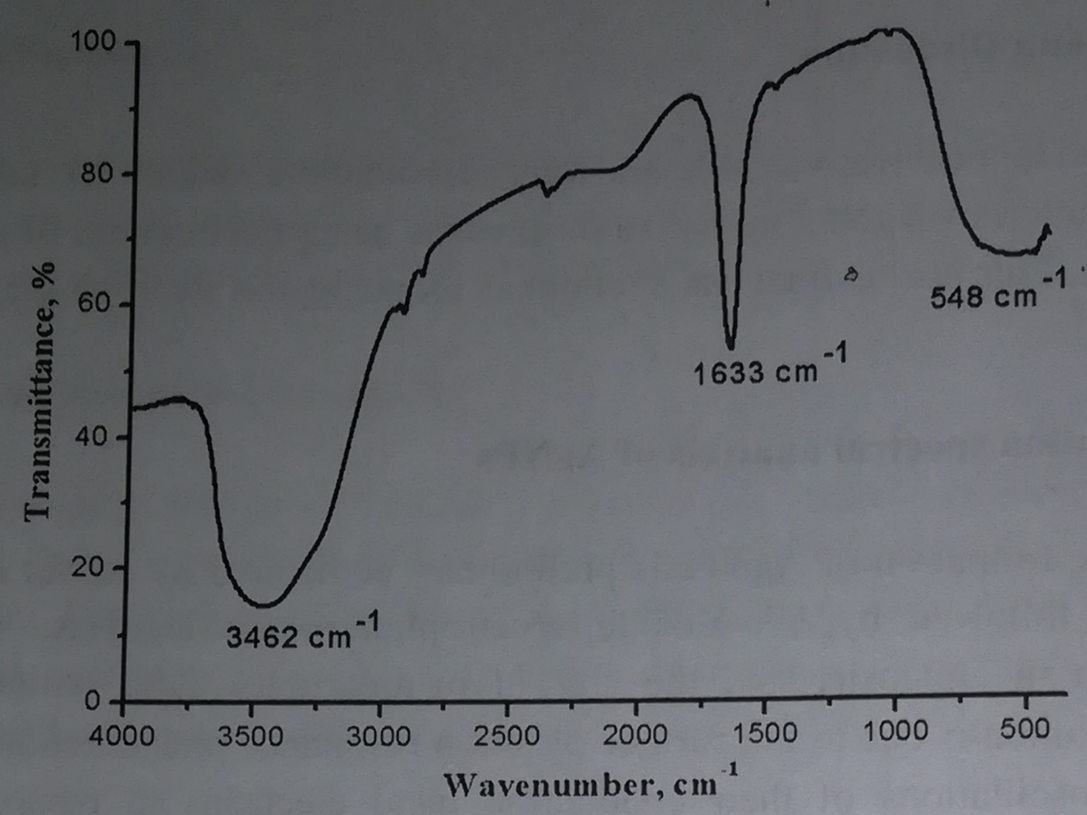

Fig. 2 FTIR spectrum of AgNPs

3.3 XRD Analysis of AgNPs

Analysis through X-ray diffraction (XRD) is carried to determine the nature of the AgNPs (**Fig. 3**). The XRD analysis confirms that the AgNPs synthesised from pumpkin peel extract is of amorphous in nature. Since thin film technique has been used to record the XRD pattern, the amorphous characteristics of the glass are also present in the XRD pattern and it overlaps with the AgNPs pattern.

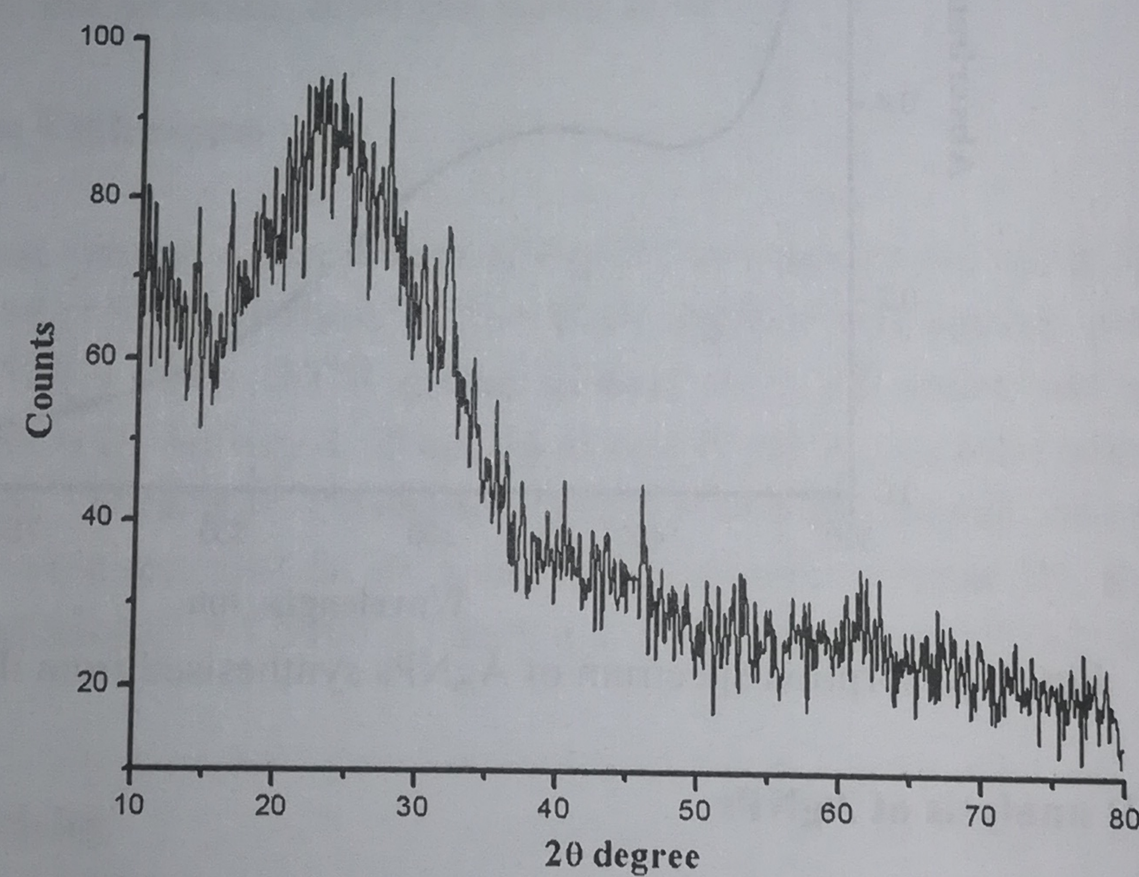

Fig. 3 XRD pattern of AgNPs

3.4 Antimicrobial activity

The antimicrobial activity of synthesised AgNPs is tested against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aerogenisa*, *Aspergillus* and *Candida albicans*. The AgNPs show a clear inhibition zone against the human pathogens taken in the present study (**Fig. 4**). Standard antibiotic disc Amikacin is used as the reference

drug for the evaluation of antimicrobial activity. The synthesised AgNPs show higher activity on *Pseudomonas aerogenisa, Klebsiella pneumonia* and *Staphylococcus aureus* than that of the other human pathogens. Surfaces of AgNPs affect / interact directly with the bacterial outer membrane, causing the membrane to rupture and killing bacteria.

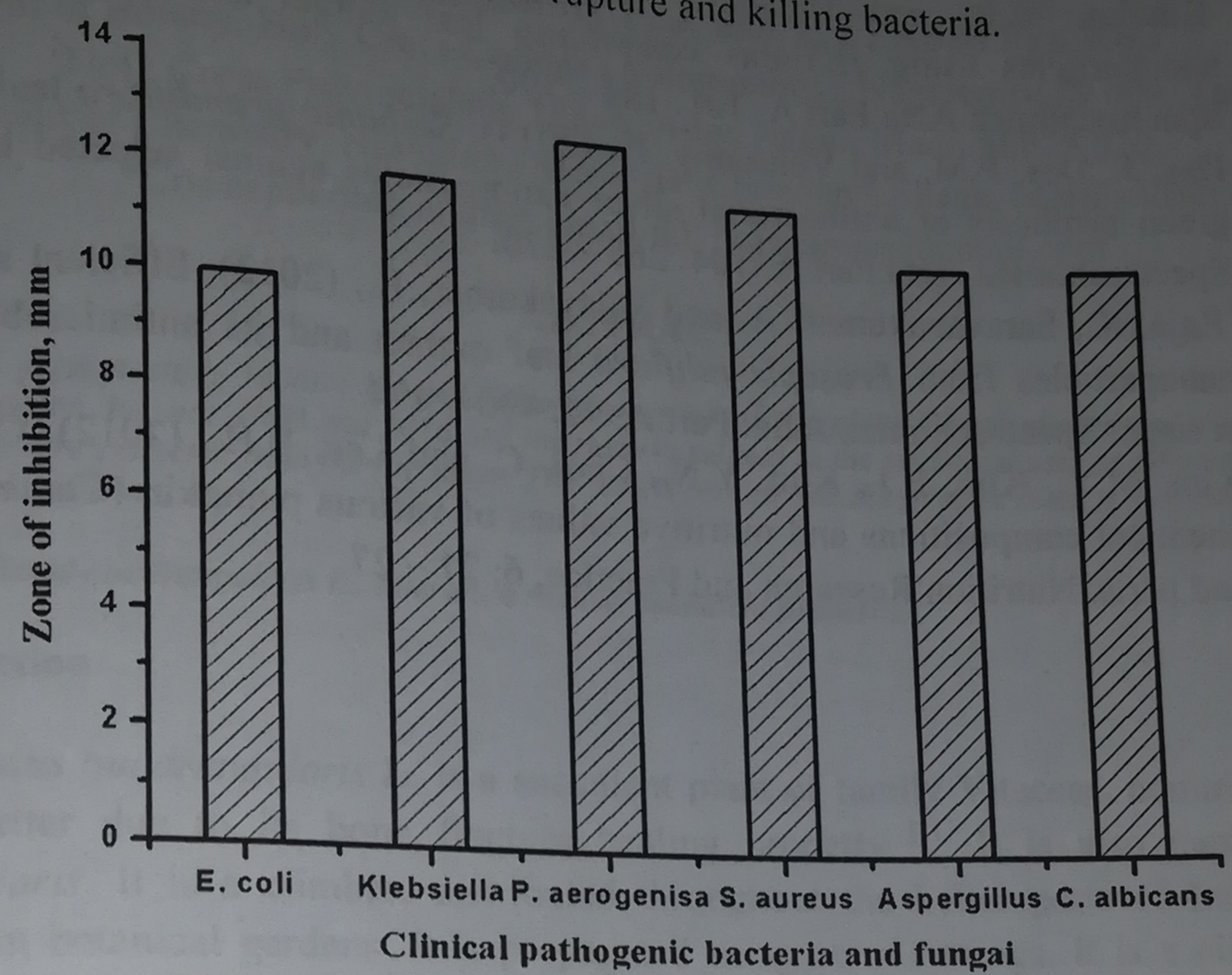

Fig. 4 Zone of inhibition of AgNPs against human pathogens

Conclusion

The present investigations deals about the green synthesis of nanoparticles of silver from pumpkin peel extract and this synthesised nanoparticle are characterized by UV-Visible and FTIR analysis. The reduced AgNPs show a characteristic absorption peak at 451 nm due to the surface plasmon resonance phenomenon. The carbohydrates present in the peel extract act as the reducing and stabilizing agent for the synthesis of AgNPs. XRD analysis reveals that the synthesised AgNPs are amorphous in nature. The AgNPs synthesised from pumpkin peel extract has toxic effects on *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Pseudomonas aerogenisa, Aspergillus* and *Candida albicans*. This green method is simple, rapid, eco-friendly and reliable, and it may have a potential use in the biomedical applications due to its high antimicrobial activity.

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