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Green way genesis of silver nanoparticles using multiple fruit peels waste and its antimicrobial, anti-oxidant and anti-tumor cell line studies

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Abstract. Green synthesis of silver nanoparticles (SNP) opens a new path to kill and prevent various infectious diseases and also tumor. In this study, we have synthesized silver nanoparticles using multiple fruit peel waste (pomegranate, orange, banana and apple (POBA)). The primarily nanoparticles formation has been confirmed by the color change. The synthesized SNP were analyzed by various physicochemical techniques such as UV- Visible spectroscopy, x-ray diffraction (XRD), fourier transform infra red (FT-IR) spectroscopy and transmission electron microscope (TEM). The formation of SNP was confirmed by its absorbance peak observed at 430 nm in UV-Visible spectrum. Further, the obtained SNP were identified by XRD and TEM, respectively to know the crystalline nature and size and shape of the particles. The activities of SNP were checked with human pathogens (Salmonella, E.coli and Pseudomonas), plant pathogen (Fusarium) and marine pathogen (Aeromonas hydrophila) and also studied the scavenging effect and anticancer properties against MCF-7 cell lines. This studies proves that the SNP prepared from fruit waste peel extract approach appears extremely fast, cost efficient, eco-friendly and alternative for conventional methods of SNP synthesis to promote the usage of these nanoparticles in medicinal application.

1. Introduction

Nanotechnology is an important field of modern research dealing with design, synthesis, and manipulation of particles structure ranging from approximately 1-100 nm. Metal nanoparticles have a high specific surface area to volume ratio. Because of the unique properties of nanoparticles, including optical properties, electronic properties, medicinal properties and magnetic properties [1-5]. These metal nanoparticles pulls in an expanding interest as a result of novel elements and alluring application in the therapeutic field [6]. Several processes physical and chemical [7] were developed including chemical reduction using a variety of organic and inorganic reducing agents, electrochemical techniques, physicochemical reduction, and radiolysis are widely used for the synthesis of silver nanoparticles and considering the real life application of nanoparticles in the area of medicine [8], catalysis [9], detection [10], etc. Recently, nanoparticle synthesis is most interesting scientific areas for attention to produce nanoparticles using environmentally friendly methods (green chemistry) [11] for the search of benign methods for the development nanoparticles and searching antibacterial, antioxidant, and antitumor activity of natural products.

Most of the reported research concentrate on single fruit peel waste extract for the preparation of SNP and they have also used thermal decomposition method [12]. But in this study, we have proven that SNP prepared from multiple fruit waste peel extract approach appears extremely fast, cost efficient, eco-friendly and alternative for conventional methods. Fruits peel wastes of POBA are

assembling more consideration for choosing a full range of pharmacological activities. It is experienced for its general tonic, anticancer, hostile toleprotic, against hyperglycemias, hostile to unfavorably susceptible against diabetic properties [13]. It enhances the phagocytic and bactericidal limit of polymorphs, ensures against gastric mucosal harm and searches free radicals. Since this plant has in like manner been depicted to have against fibrotic, hostile to oxidant, mitigating, resistant module as nontoxic [14]. Among numerous metal nanoparticles, SNP plays crucial role as a drug as it is profoundly had antimicrobial [15, 16] anti-inflammatory [17] and hostile to tumor activity. In this study, we have synthesized silver nanoparticles using multiple fruit peel waste of POBA. The SNP formation were confirmed and analyzed by various physicochemical techniques such as UV-Visible spectroscopy, x-ray diffraction (XRD), fourier transform infra-red (FT- IR) spectroscopy and transmission electron microscope (TEM). The activities of SNP were checked with human pathogens (Salmonella, E.coli and Pseudomonas), plant pathogen (Fusarium) and marine pathogen (Aeromonas hydrophila) and also studied the scavenging effect and anticancer properties against MCF-7 cell lines.

2. Materials and method

2.1. Preparation of fruit peel extract

The multiple fruit peel waste of POBA used in this experiment was collected from a local market in Chennai Koyambedu. Each fruit peel waste of 2g were cut into smaller pieces and dried under direct sunlight for 48 hrs. Then the dried materials were grid into powder form and collected in a sample bottle. About 0.1g of the POBA powder dissolved in 100 ml distilled water and stirred for 30 min to obtain the POBA peel solution. This aqueous peel solution is used for the preparation of SNP.

2.2. Preparation of SNP

According to our previous investigation [18], we have synthesized SNP using about 3ml of POBA was added to 47 ml of 0.1mM solution of silver nitrate and mix the solution well, rapidly the color of the solution has turned which implies that the reduction was completed within a short period (2 min) at room temperature with the appearance of yellowish brown color which confirms the formation of SNP (Scheme 1). The obtained SNPs were analysed by using UV-Visible spectroscopy, XRD, FT-IR and TEM.

2.3. Antimicrobial activity

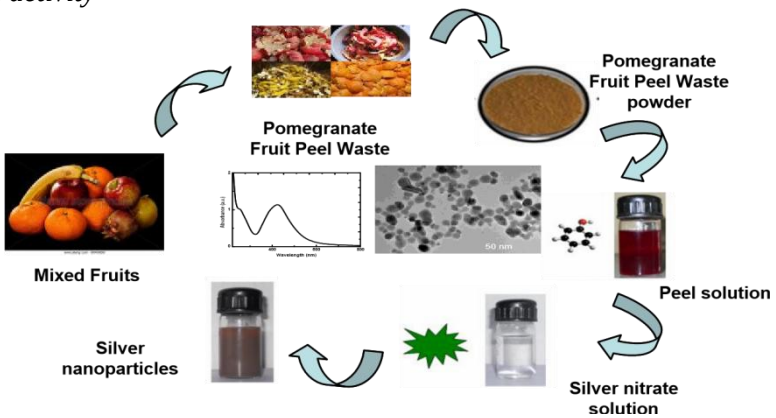

Figure 1. Schematic representation of SNP from fruit peel waste.

The antimicrobial activity has tested by using the agar well diffusion method. The isolated human pathogens (***Salmonella, Pseudomonas, E.*** **coli)**, plant pathogen **(Fusarium *soaps*)**, marine pathogen (***Aeromonas hydrophila***). A pure culture has sub cultured in respective broth and the strain is spread on the plates. Four circular wells were made and loaded with the SNP as well as

control. Then the plates are incubated to observe zone of inhibition.

2.4. Antioxidant activity based on DPPH assay

The scavenging activity towards 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical was studied with slight modification an aliquot (1000 - 100µL) of SNP or control and of (1000 - 1600µL) H2O was mixed with 1ml of10µm in absolute methanol. The mixture was shaken vigorously and left to stand for 30 min in the dark before measuring the absorbance at 517 nm against a control and the free radical scavenging activity was calculated using following equation.

% scavenging = OD control – OD test / OD control x 100

2.5. Cell viability test (cell line study)

The assay is based on the reduction of soluble yellow tetrazolium salt to insoluble purple formazan crystals by metabolically active cells. Only live cells are able to take up the tetrazolium salt. The enzyme (mitochondrial dehydrogenase) present in the mitochondria of the liver cells is able to convert internalized tetrazolium salt to formazan crystals, which are purple in color. Then the cells are lysed and dissolved in DMSO solution. The color development is then determined in an ELISA reader at 570 nm. The cell lines were plated separately in 96 well plates at a concentration of 1 × 104 cells/well. After 24 h, cells were washed twice with 100 µl of serum-free medium and starved for an hour at 37 °C. After starvation, cells were treated with different concentrations of test compound (5-20µg/ml) for 24 h. At the end of the treatment period the medium was aspirated and serum free medium containing MTT (0.5 mg/ml) was added and incubated for 4 h at 37ºC in a CO2 incubator. The 50% inhibitory concentration value (IC50) of the treated drug was identified in normal (Untreated) cell line. The MTT containing medium was then discarded and the cells were washed with PBS (200 µl). The crystals were then dissolved by adding 100 µl of DMSO and this was mixed properly by putting up and down. Spectrophotometrical observance of the purple, blue formazan dye was measured in a microplate reader at 570 nm (Beograd 680). Cytotoxicity was determined using Graph pad prism5 software.

3. Results and discussion

3.1. UV and FTIR

Figure 2 shows the UV–vis spectra of SNP formation using POBA at room temperature. The surface plasmon resonance (SPR) band appeared at 430 nm which clearly indicates the formation of SNP and revealed that the size of 25 nm [19]. Figure 3 illustrates the FTIR spectrum of SNP formed using POBA. The bands located at 3433.41 cm^{-1} were related to O-H extending and 2067.76 cm^{-1} was allocated to aromatic stretch, individually 1633.76 cm^{-1} has considered as basic and amine bunches extending.

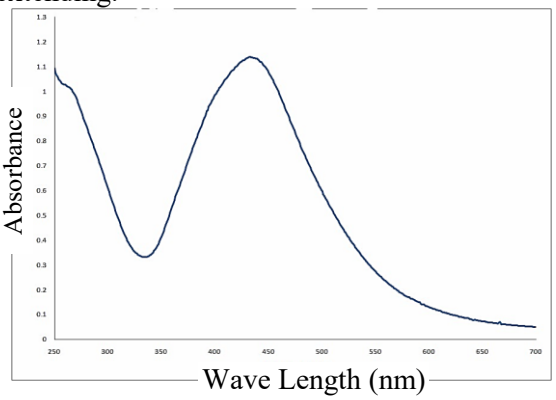

Figure 2. Visible Spectra of SNP at 430nm.

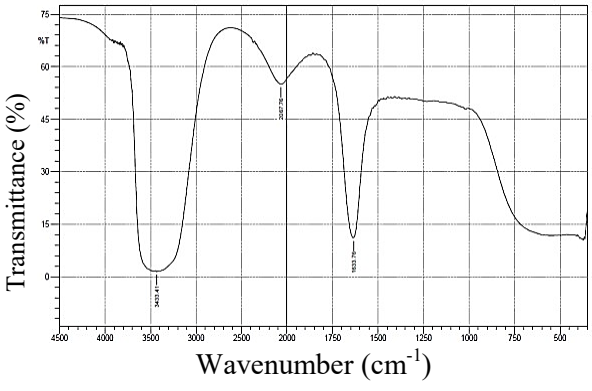

Figure 3. FT-IR of SNP from POBA.

3.2. XRD Pattern and TEM of SNP from POBA

Figure 4 illustrate the XRD pattern of biosynthesized AgNPs, shows the diffraction peaks at 2θ = 38.13°, 44.21°, 64.47° and 77.37° corresponding to the (111), (200), (220) and (311) can be assigned to face-centered cubic (fcc) lattice of metallic silver. Similar results were reported by previous investigator [20, 21]. Figure 5 shows the size and shape of SNP by using TEM analysis, the results have observed nanoparticles size in 25 nm which indicates well dispersion and spherical in shape.

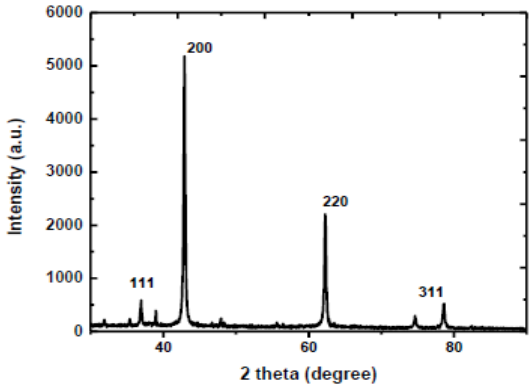

Figure 4. XRD pattern of the SNP.

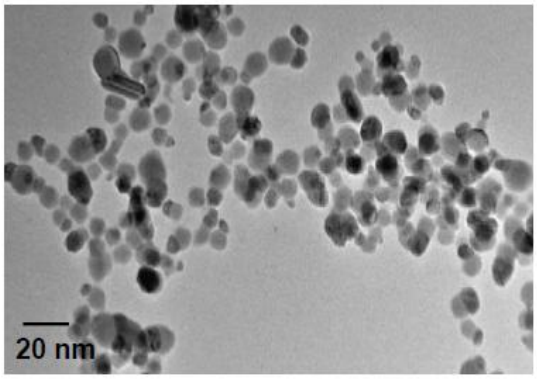

Figure 5. TEM Image of the SNP.

3.3. General Mechanism of SNP

Fruit extract is primarily composed of alkaloids and polyphenols. The active constituent that appears to be responsible for its multiple health benefits is Ellagic acid and it is naturally occurring phenolic compound found in several fruits and nuts and also it effectively protects cells from damaging free radicals. Phenolics possess hydroxyl and carboxyl groups, able to bind to heavy metals. They may inactivate metal ions by chelating. When metal salts come in contact with the ellagic acid present in the peel extract the ester oxygen atom and the ortho- phenolic hydroxyl of the ellagic acid form p track conjugation effect. The esterification of the carboxyl and hydroxyl groups of ellagic acid make the ortho-phenolic hydroxyl loose the hydrogen much easily, forming a steadier semi-quinone structure. Thus, ellagic acid has an easy electron loosing capacity which results in the formation of H+ radical, which reduces the size of silver to nanosize Punicalagins, tannin, along with Ellagic acid is a major antioxidant component of POBA and have free radical scavenging capacity. They are water soluble and have high bioavailability [11].

3.4. Antimicrobial activity

The fruits peel waste interceded SNP has demonstrated better antibacterial movement against human pathogen. This outcome may get cell demise brought on by the SNP in the particular pathogens [22]. Figure 6(a) obviously shows that the SNP has solid antimicrobial movement towards human pathogens contrasted with plant and marine pathogens. Refer Figure 6(b) Fusarium and Figure 6(c) Aeromonas Hydrophila respectively. Minimal zone of restraint has thought about separately in Table 1.

Table 1. Zone of Inhibition

Organisms	5µl	10µl	15µl	Control
Salmonella	8	8.2	9	9
E.coli	6.5	6.8	7	7.2
Pseudmonas	6.4	6.8	6.9	7
Fusarium	6.3	6.5	6.7	7.3
	5.2	5.7	6	6.2

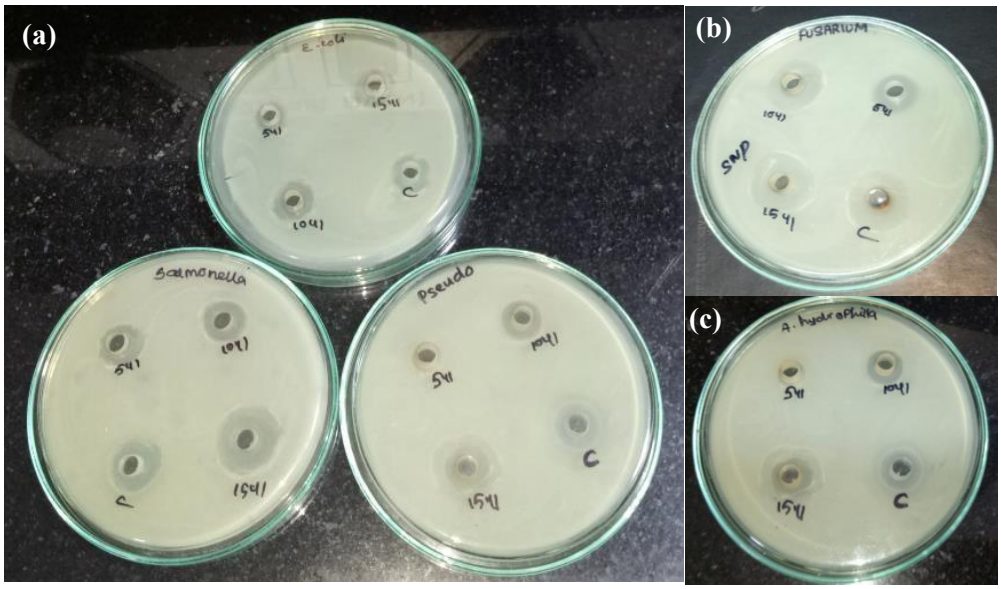

Figure 6. Antimicrobial activity.

3.5. Antioxidant activity

DPPH scavenging activity was studied using POBA mediated SNP by varying the concentrations 100, 200, 300, 400, 500 µg/ml was found in 25%, 37%, 40%, 60% and 80% at the different concentrations (Figure 7). The 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical was widely used as the model system to investigate the scavenging activities of several natural compounds. DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH which can be quantified by its decrease of absorbance at wavelength 517 nm. Radical scavenging activity increased with increasing percentage of the free radical inhibition. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH which reacts with a suitable reducing agent.

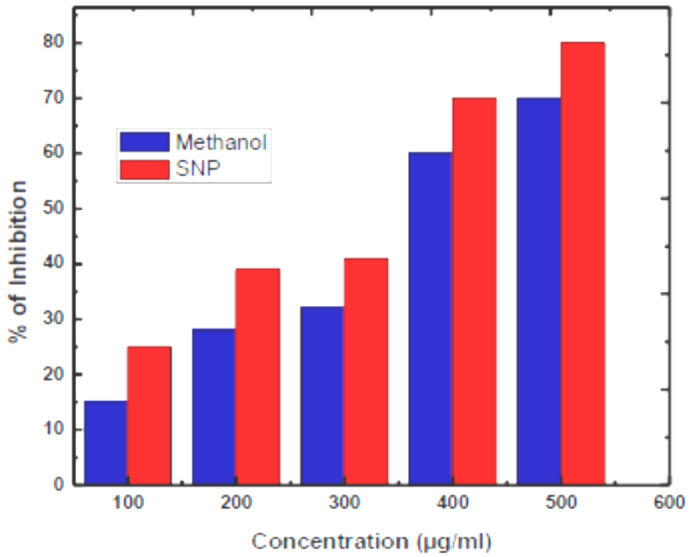

Figure 7. DDPH scavenging activity.

3.6. Cell line study

The cytotoxicity of cell line study has done with human breast cancer cells MCF-7. The effect of silver nanoparticle was studied at different concentrations (10 µg, 25 µg, 50 µg, 100 µg, 250 µg, 500 µg) against MCF7 cell lines by MTT assay which was shown in Figure 8. The IC50 value was calculated for silver nanoparticles and it was found to be 500 µg/ml. Figure 8 has illustrated that the bright field microscopic images of morphological characterization of MCF7 cell lines for normal cell (a) and treated cells with different concentrations of SNP (b) 10 µg, (c) 25 µg, (d) 50 µg, (e) 100 µg, (f) 250 µg, (g) 500 µg. Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. An increase in proliferation may be offset by cell death; evidence of cell death may be inferred from morphological changes. This study shows that the cytotoxicity has increased by dose dependant while the high concentration of the silver nanoparticle has better result comparing with minimal concentration. This is the first study to report the cytotoxicity of AgNPs synthesized using multiple fruit peel waste extract against MCF7 cell lines.

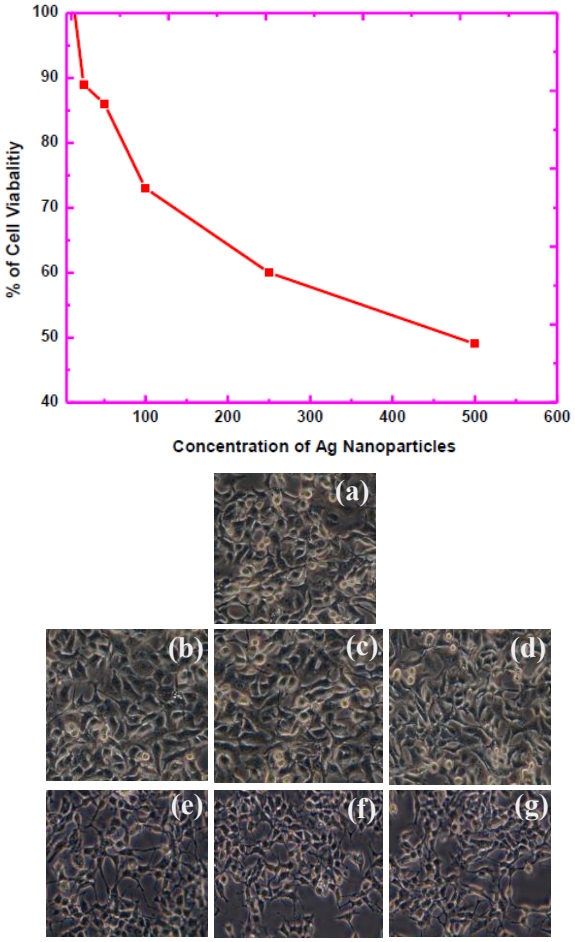

Figure 8. Cytotoxicity effect of SNP from POBA on MCF-7 Cell Lines.

4. Conclusion

The present study has shown the “green” synthesis route combination of silver nanoparticles from fruit peel waste materials has received increasing attention due to the development of eco-friendly technologies in material science. The characterization with UV- Visible spectroscopy, XRD, FT-IR and TEM is the evidence for the formation of nanoparticles. This study shows the successful synthesis of SNP with excellent morphological properties by a very facile, cost effective and green method. We have concluded that the SNP synthesized using fruit waste possess high antimicrobial activity against human, plant and marine pathogens and also the study show great attention of

anticancer activity against human breast cancer cell (MCF-7) lines which further suggest the potential use of this kind nanoparticles in various pharmacology field.

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