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RESEARCH ARTICLE

Silver Nanoparticles Synthesized from Citrus aurantium L. & Citrus sinensis L. leaves and Evaluation the Antimicrobial Activity

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Abstract

The increases in incidence of drug resistance among pathogenic fungi have made the search for new anti-microorganism inevitable. Nowadays, the synthesis of metal nanoparticles by using green methods is increased rapidly. We synthesized Silver nanoparticles from Citrus aurantium L. and Citrus sinensis L. leaves. AgNPs biosynthesized were characterized by particle analyzer, zeta potential, Fourier transform-infrared spectroscopy (FTIR), X-ray diffraction (XRD), and UV-Vis spectroscopy to study the diameter of particles, structure properties of samples, and optical spectroscopy properties respectively. The results obtained revealed that size of AgNPs were 60.5 and 31.72 nm for Citrus aurantium L. and Citrus sinensis L. leaves respectively, with crystallized face-centered cubic structure. The AgNPs biosynthesis showed effective inhibitory antifungal activity against Candida albicanns.

Keywords: *Biophysics, Antibacterial test, Physical properties.*

Introduction

Any Materials synthesis with Structure less than 100 nm will have unusual photo electrochemical, chemical, optical, catalytic, electronic, and particularly antimicrobial properties [1]. In the last years, the General ways to prepare the Nanoparticles are chemical and physical methods which are not environmentally friendly [2]. Nowadays, Plant extracts have been used to synthesis of Silver nanoparticles (Ag NPs) that are environmentally, non-toxicity, and low cost friendly materials. Silver nanoparticles that are prepared by using biological materials have the same properties of a high dispersion, smaller in size, and high surface area, because of the plant extracts work as reducing agents as well as capping agents [3-5].

Also, one of best source that constitute chemical compounds are Plants, which are nowadays have a potential use in medicine and other applications. Some parts of plants like fruits, root, bark, seeds, leaves, and flowers are containing flavonoids, volatile oils, fixed oils, resins, phenols, alkaloids, steroids, tannins, and glycosides which are called active compounds. So, these secondary products of plant materials are responsible on The beneficial medicinal effects [6]. Recent, many reports investigated the biosynthesis of silver nanoparticles by different plant parts like Punica granatum peel [7], Citrus sinensis peel [8], Annona squamosa peel [9], Lemon Peel [10], and Mango peel [11]. For the purpose of this report, we adopted strategy to synthesis AgNP via low-cost, eco-friendly, and simple approaches from peel extract to avoid disadvantages of the classical chemical methods. The physicochemical properties and antifungal activity were identified.

Material and Methods

Biosynthesis of Silver Nanoparticles (AgNPs)

Sterile deionized triple - distilled water was used to synthesis a stock solution from (1 x 10^{-3} M, $AgNO_3$), which were utilized in the next dilutions, later. AgNPs were made according to the method described by Gurunathan et al. [12]. First, it was taken 7 ml of 10 mg/ml of aqueous extract of P.

ostreatus that were filled with sterile distilled water to a total 10 ml. After that, the solution is added to 5 ml of 1 x 10^{-3} M $AgNO_3$ solution and then exposed for 7 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 nanoparticles were formatted [13].

Prepare Orange Bitter (*C.aurantium* L.) Orange (*C. sinensis* L.) Peel Extract

The method of Harborne et al. was used to prepare the peel extract [14]. First, it was taken 20 grams of peel plants and was washed repeatedly by distilled water to clean them by remove the dust and organic impurities which they present in it.

The dry samples for both plants were added to distilled water at temperature (20-25^0 C) and the final volume of solution was becoming 200 ml. After that, the solution left for 30 minutes to rocking horizontal (Horizontal shaker), the sample was left to settle for an hour, and then nominated three layers of muslin cloth to remove hard plankton. Finally, the solutions were filtered through filter papers many times.

Characterization

The particle size of Ag in the solutions was estimated by particles size analyzer (Nano Brook, model 90Plus USA). The surface charge of Ag nanoparticles was characterized using zeta potential (Nano Brook, model Zeta Plus). The crystallinity of nanoparticles was studied by using X-ray diffractometer (XRD-6000, Shimadzu, Japan).

FT-IR Fourier Transform Infrared (Fourier Transform Infrared Spectroscopy; FT-IR 8400S/ Shimadzu / Japan) was used to study the major changes in the surface composition and surface chemical bonding, and Absorbance UV-Vis spectra was obtained by using AA 6300 / Shimadzu /Japan.

Antimicrobial Activity

The antimicrobial activity of biosynthesis AgNPs against pathogenic microorganisms like Fungi (Candida) by using agar disk diffusion method were test.

Result and Discussion

Characterization of Biosynthesis Ag Nanoparticles

The distribution of particles diameter and zeta potential of the biosynthesis AgNPs were determined by the technique of dynamic light scattering (DLS) as shown in fig. 1. The distribution curve for particle size vs. Intensity, which was obtained from biosynthesis AgNPs reveals that main diameter is 60.5 and 31.72 nm for the Citrus aurantium L. & Citrus sinensis L. leaves respectively *(*Fig.1-a & b*).*

We believe that the satiated sources of citric acid and ascorbic acid in the citrus fruit peel extract may possibly obligation to efficient stabilization and reduction of metal ions of obtained Ag nano particles [14]. Fig.1-c&d shows the zeta potential of biosynthesis AgNPS from the Citrus aurantium L. & Citrus sinensis L. leaves respectively. The value of it was around -2.91 and 3, 13 mV and the width was 12.3 & 12.52 mV for the Citrus aurantium L. & Citrus sinensis L. leaves respectively.

The zeta potential curve is point out the degree of repulsion between similarly charged and adjacent particles in dispersion. The value of it can be related to the stability of colloidal dispersions. So, the measurement value indicated that the presence of low repulsion and much stability among the biosynthesized nanoparticles [15].

X-ray diffraction for colloid that contained AgNPS was carried out to emphasize the nature of crystalline nanoparticles and the X-Ray Diffractions pattern was shown in fig.2. The diffracted spectrum of 2Θ were recorded from 20^{o}to 80^o.From the pattern. Four strong reflections at 38.46, 46.34, 64.73 and 78.06 were observed. When obtained XRD spectrum was compared with the standard, it confirmed that corresponds to the planes of (1 1 1), (2 0 0), (2 2 0) and (3 1 1) respectively.

The interplanar spacing (d_{hkl}) values are estimated 2.337, 1.954, 1.437 and 1.223Å for (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes respectively with face-centered-cubic structure (FCC) [16].

The crystallite size of nanoparticles was calculated by using Debye-Scherer's formula [$D = K\lambda/\beta \cos\theta$]. The calculated crystalline size was around 34 nm. The large different of value of particle size from observed by DLS and XRD results is due to the measured size in DLS technique also encompass the bioorganic compounds surround the core of the nanoparticles [15]. Fig.3 shows FTIR spectrum which implemented in order to set apart the existence of various functional groups in solutions responsible for the bio-reduction of Ag^+ and capping/stabilization of Ag nanoparticles. The transmission bands were found at the 3423, 2922, 2855, 1744, 1633, 1452, 1377, 1239, 1045 and 597 cm^{-1} pointed out the existence the capping agent with the nanoparticles [16].

Fig. 4 shows the UV-Vis spectrum. Only single broad peak was observed at 417 nm. Previous reports named it as broad Surface plasmon resonance (SPR) peak which are confirmed the biosynthesis of AgNPs. Past articles indicated that the SPR peak has been observed for Ag-nano particles and might be referred to spherical shape of nanoparticles [17].

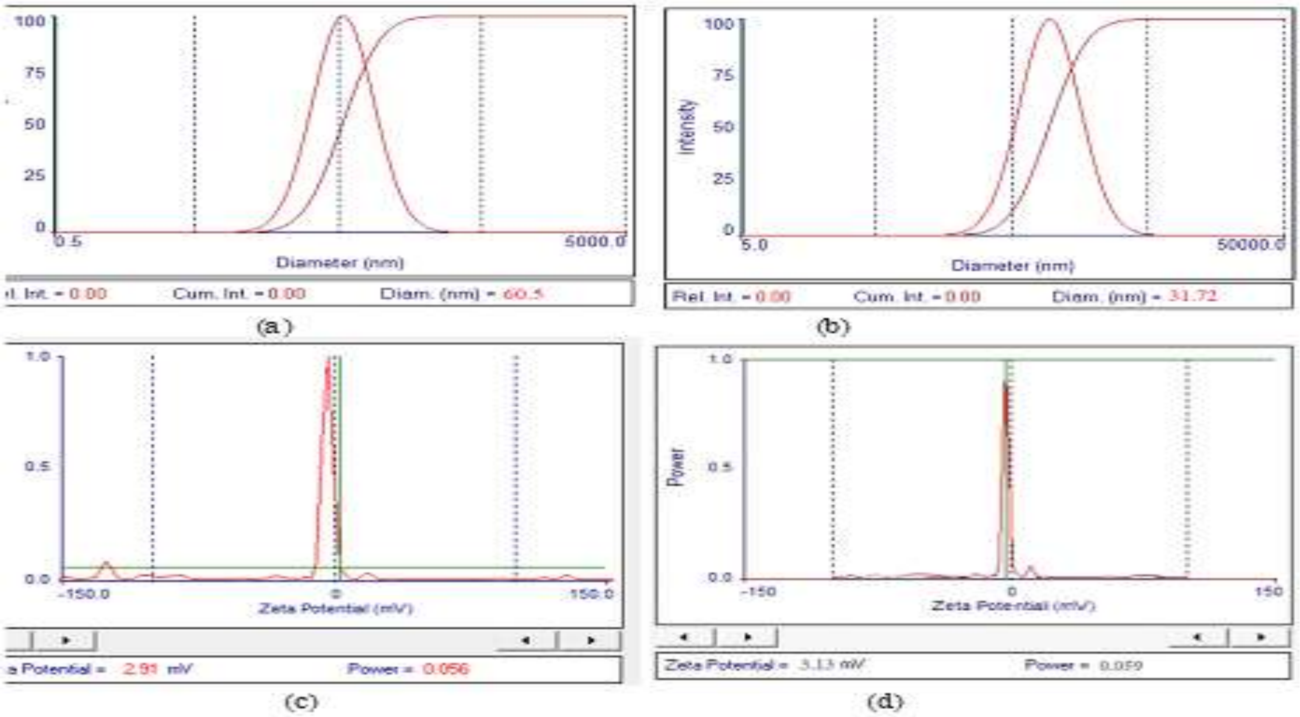

Fig. 1: (a & b) Particle size and (c& d) Zeta potential of AgNPS by the Citrus aurantium L. & Citrus sinensis L. leaves respectively

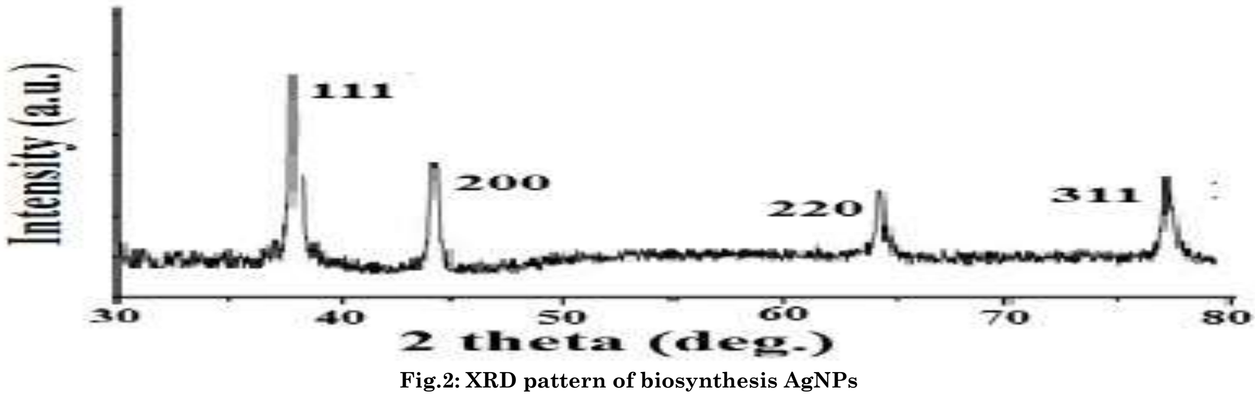

Fig.2: XRD pattern of biosynthesis AgNPs

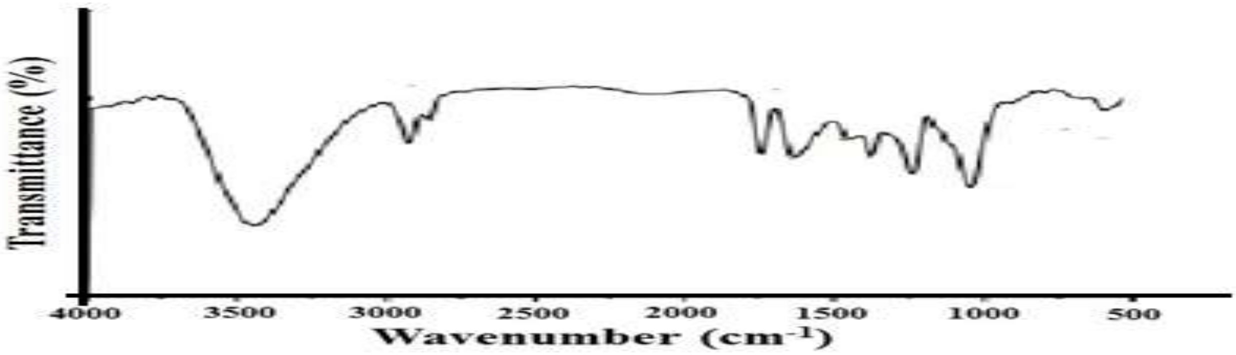

Fig.3: FTIR spectrum of Ag nanoparticles

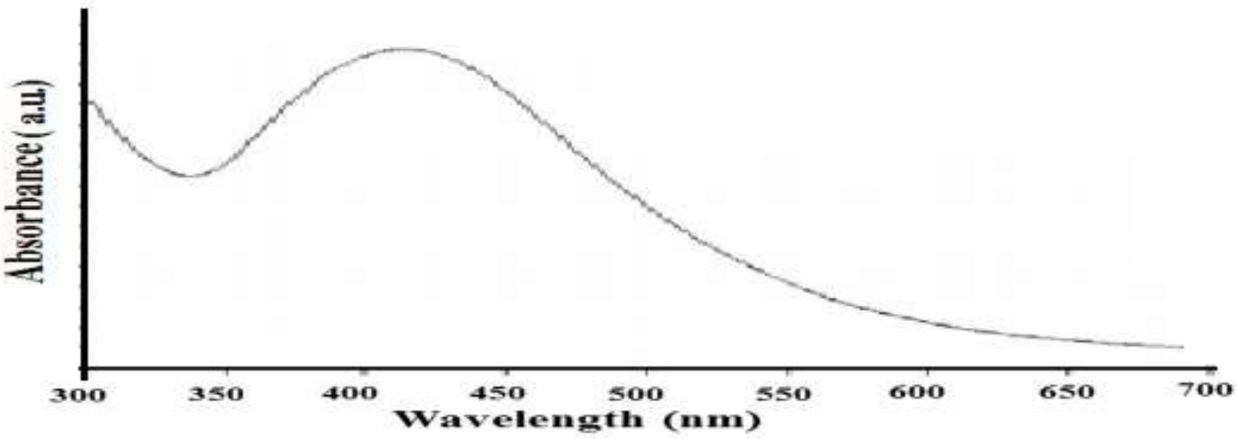

Fig.4 UV-Vis Spectrum of biosynthesis AgNPS

Screening of Antifungal Activity

Fig.5 (a & b) showed the nanoparticles have activity against fungi because the antimicrobial activity is concerned with compounds that make locally killed microbial or decreased slowly their growth. Nowadays, most of current antimicrobial agents are produced from chemically modified natural compounds [18]. A ruler was used to measure Zone of inhibition, which is defined as the average diameter of the area around the discs that stops the growth of bacteria. It was observed that the Nanoparticles were synthesized from leaf of C. sinensis L. leaves, have antifungungal activities more than the Nanoparticles were synthesized from leaf of Citrus aurantium leaves. In our result cannot measure exactly the diameter of inhabitation zone, because the shape of it not like circle. So, the diameter of zone Inhibition around the disk containing biosynthesis AgNPs in medium with C. sinensis L. leaves and Citrus aurantium leaves were 82 and 50 percent effective respectively. Thus, the greener-synthesized silver nanoparticles can be used as an antifungal agent against fungal pathogens.

Figure 5: Antifungal Activities of Nanoparticles synthesized from (a) Citrus sinensis L. leaves, and (b) Citrus aurantium leaves

Conclusion

A novel approach for biosynthesis of AgNPs from Citrus sinensis L. leaves and Citrus aurantium L. were investigated in this work, which provide simple, low cost, and efficient ways for the synthesis of nanomaterials. AgNPs have face-centered cubic structure in shape with particle size around 60.5 &31.72 for Citrus aurantium L. and Citrus sinensis L. leaves respectively. The spectrum of UV-vis and XRD shows that Ag ions were exposed to these plant extracts, which confirmed that silver ions were reduction to silver nanoparticles. AgNPS biosynthesis from plant extracts shows good antifungal activities against Candida albicann.

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