



EXTRACT MANGIFERA INDICA PARTS TO STUDY THE GREEN SYNTHESIS OF SILVER NANOPARTICLES

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Abstract

The green synthesis silver nanoparticles has main significance in the modern research evolution due to the antimicrobial ability of biosynthesized silver nanoparticles to compared to using of plant extract alone . In recent study, the silver nanoparticles were green synthesized using *Mangifera indica* leaves and peels extracts separately and the biological inhibitory effect of biosynthesized silver nanoparticles were investigated using agar diffusion methods against (*Staphylococcus aureus*, *proteus sp.*, *Pseudomonas aeruginosa*, *E. coli*, *Serriatia sp.*, and *Candida albicans*), also the ability of biosynthesized silver nanoparticles against the selective bacterial and fungal isolates were studied and compared with amoxicillin. The characterization of biosynthesized silver nanoparticles were indicated by changing the color of solution to reddish brown. UV-vis spectroscopy conformed the formation of the nanoparticles due to present the significant beak at 450 nm. The absorption beak was detected by FT-IR spectrum at 685.46 cm^{-1} . While the Scan Electron Microscope analysis (SEM) was fixing the size at 35nm to biosynthesized silver nanoparticles by leaves extract and 29nm to silver nanoparticles by peel extract.

The outcomes measured significant effect at different concentrations to biosynthesized silver nanoparticles, plants extracts. The results showed potent effect to silver nanoparticles by peels extract with inhibition zone ranged between 15-45 mm. The silver nanoparticles by leaves extract reveled remarkable inhibitory effect with 11-42mm inhibition zone, while the amoxicillin fail to inhabit the growth of *Staphylococcus aureus*, *Serriatia sp.* and *Candida*



albicans. The findings of the present study focused on the ability of using biosynthesized Ag-NPs as altar choice in many medical and biomedical application particularly in treating many bacterial and fungal infection.

Keywords: Nanotechnology, Green synthesis silver nanoparticles, *Mangifera Indica* extracts, biomedical, Antibacterial and Antifungal.

1. Introduction

The nanoparticles synthesis have synthesis of nanoparticles (NPs) have a special area in the recent investigation due to their unique characters and ability to applied in many fields such as medicals , pharmaceuticals and industrial sites. Many obstacles were faced the chemical and physical approaches nanotechnology, which include the chemicals toxicity, high energy expenditure, and high temperature [1]. The evolution the biological ways to synthesis nanoparticles led to rising the sustainability and ecofriendly methods to fabricate the nanoparticles by bioprecursors that easier, cheaper less energy consumption, and non-toxic with minimal harmful wastes to environment [2]. The plant extracts contain a variety of bioactive and phytochemical compounds such as alkaloids, phenolics, terpenoids, flavonoids, and proteins [3]. The green synthesis using plant extracts is a common option that act as capping, reducing, stabilizing factor to prepare nanoparticles with adding advanced therapeutic and unique biological properties can used in medical and environmental application [4].

NPs are distrustful a bridge in the bulk size and structure of atomic materials. In addition, the inorganic nanoparticles have noteworthy features due to their small size and highly surface area [5]. Ibrahim et al. was noted the novelty of using colicin isolated from of *E. coli* to create silver nanoparticles, and highlighted that some results have confirmed the toxicity of silver nanoparticles to bacterial, cancer cells [6]. The resistance of microbial infection to antibiotics is one of the important reasons to increase the trend toward adopting modern studies that seek to investigate a new agents for better, economically and free side effect agents to treat microbial infection in worldwide with no adverse effect since a long time [7].



Mangifera indica is a tropical ever green tree, contain active phytochemicals compounds (alkaloid, flavonoid, tannin and phenolic compounds) that are inextricably connected to reduce many diseases due to their role as anti-inflammatory, anti-diabetic, analgesic, and immunoprotective [8].

Consequently, so as to fight he microbial resistance, it is essential to look for environmentally friendly alternatives. Therefore, the present work demonstrates the role of leaves and peels ethanolic extracts of *Mangifera indica* in biosynthesized of silver nanoparticles and focusing on Invitro antimicrobial efficiency to improve a biocompatible products using in many applications to human benefits.

2. Materials and Methods

2.1. Chemicals

Muller Hinton media, Muller Hinton Agar, nutrient agar were obtained from Hi-Media, Mumbai, India. Silver nitrate (AgNO_3) purchased from Hi Media Laboratories Pvt. Mumbai, India.

2.2. Activation the Selective isolates

The microbial isolates were used in antimicrobial assay (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas sp.*, *Proteus sp.*, *Serratia sp.* and *Candida albicans*) were isolated in microbiology labs of biotechnology department/ University of technology-Baghdad. All isolates activated by culturing in Brain Heart Infusion broth and incubated under aerobic condition at 37°C for 24 hrs. Then, assessing the bacterial turbidity by comparing with (0.4-0.5) McFarland standard [9].

2.3. Extraction of Mango leaves and peels

All the fresh parts (leaves and peels) of *Mangifera indica* were collected from local farms in Baghdad /Iraq separately and washed under running clean water supply then a triple times washed with D.W. and leave it to dry in eared, a way from sun light.



Fifty grams of leaves powder extracted in Soxhlet apparatus with 300 mL of 70% ethanol for 6 hrs. The extract evaporated and collect the leaves extract powder till use. The peels extract prepared under same conditions and procedure [10].

2.4. Preparation of silver nanoparticles

The silver nanoparticles was prepared in following procedure: 10^{-3} M of AgNO_3 was prepared using D.W and kept in sterile amber color container, at room temperature for 24hrs. Plant extracts prepared by dissolve 1 gm of plant powder (leaves, peels) separately in 10 mL of D.W., and mixed with 90 mL of AgNO_3 with stir under dark condition and kept till notice the changing in color of solution [11].

2.5. Detect the characterization of biosynthesized silver nanoparticles

The characterization of biosynthesized silver nanoparticles were detected by determine the resonance of surface plasmon with UV-Vis Spectroscopy at wave length (350-650) nm, FT-IR analysis (Bruker instrument in Billerica, MA, USA) was covering the range 400–4000 cm^{-1} to investigate the functional groups covering the range of to identify various functional groups and the Scan Electron Microscope (SEM) [12].

2.6. The sensitivity of isolates to Amoxicillin

The ability of Amoxicillin to inhibit the selective isolates was determined using a diffusion method. The amoxicillin solution prepared by dissolve 1000 μg of Amoxicillin nitrate in 10ml of distilled water.

100 μl of microbial culture was spread on plates and leaves to dry for 20 min and then the agar surface poured by cork borer to create 5 mm well in each plate, filled with various concentrations of prepared amoxicillin (control, 0.5%, 1%, 1.5%, 2% and 2.5%). All the plate incubated aerobically at 37°C for 24 hrs. The sensitivity of isolate to amoxicillin was detected by measured the size of inhibition zone [13].

2.7. Antimicrobial effect of Silver nanoparticles.



The activity of silver nanoparticles against selective pathogenic strains were assessed by using agar diffusion method. 100 µl of microbial culture was spread on plates and leaves to dry for 20 min and then the agar surface poured by cork porer and filled with various concentrations of prepared silver nanoparticles (control, 0.5%, 1%, 1.5%, 2% and 2.5%). The inhibition zone measured by mm after 24 hours of incubation represented the antimicrobial effect of nanoparticles [14].

3. Results and Discussion

Numerous methods have been used in preparation of silver nanoparticles such as physical, chemical, and biological methods. Preparing silver nanoparticles using plant extracts is among the most prominent methods for creating non-toxic, low-cost and high-efficiency compounds. In this work, the silver nanoparticles prepared in three steps. The silver ions (Ag^+) are reduced in first stage to metal atoms (Ag^0). In next stage, the accumulation of small silver NPs start to form bigger particles led to increase the stability of thermodynamic reaction. During the last stage, the final shape of compound is determined. adding *Mangifera indica* (leave extract and peels extract) to a silver nitrate solution cause changing the color of solution from colorless to reddish brown which indicate the formation of silver nanoparticles (Fig. 1), due to a phenomenon known as surface plasmon resonance, that include the ability of conducting electron to oscillate at the surface of nanoparticles in resonance with incident light. The forming of silver nanoparticles led to interact the positive and negative charges on the surfaces of nanoparticles. SPR involves the oscillation of conduction electrons at the surface of the nanoparticles in resonance with the incident light. When Ag nanoparticles are formed, the interaction between the positive and negative charges on their surface resulting in distinctive of absorption bands in area of visible light, and causing visibly change in color of solution [15]. Also the results of the UV-visible spectroscopy were signify the synthesis of nanoparticles by apparent broadening beak at 450 nm.[16].The extracellular reduction in Ag^+ ions indicated the production of silver nanoparticles.

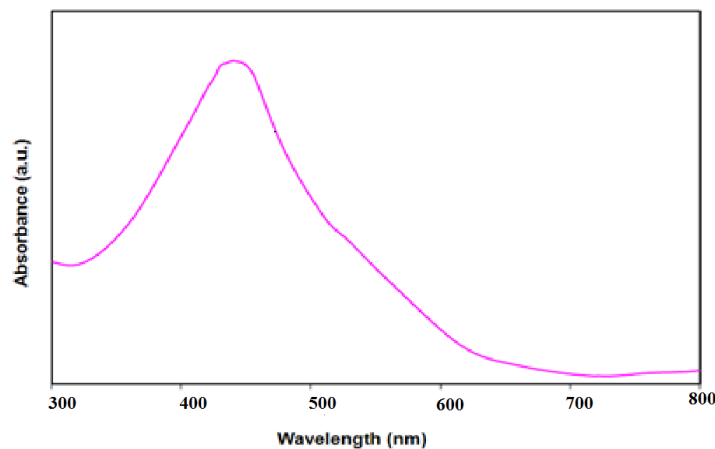


Figure 1: UV-Vis spectrophotometer indicate the formation of biosynthesized Silver nanoparticles.

The results of FT-IR analysis to silver nanoparticles solution showed significant absorption peaks at 3527.97, 3197.39, 2049.41, 1624.73, 1358.81, and 685.48 cm^{-1} as presented in Figure 4. The appearance of absorption band at 3197.39 cm^{-1} refer to presence of alkanes (C-H), the band at 2049.41 cm^{-1} refer to alkynes. The appearance of band at 1624.7 cm^{-1} mention to presence C-N, C=N, and C-O group at 685.48 cm^{-1} . The analysis of FT-IR to biosynthesized Ag NPs FTIR analysis exposed presence of many functional groups such as alkane, alkyne and aromatic C-O group. Many be due to coating or capping the NPs by organic metabolites from extracts of *Mangifera indica* (leave extract and peels extract) during the synthesis of nanoparticles. These outcomes are depending with previous studies [16].

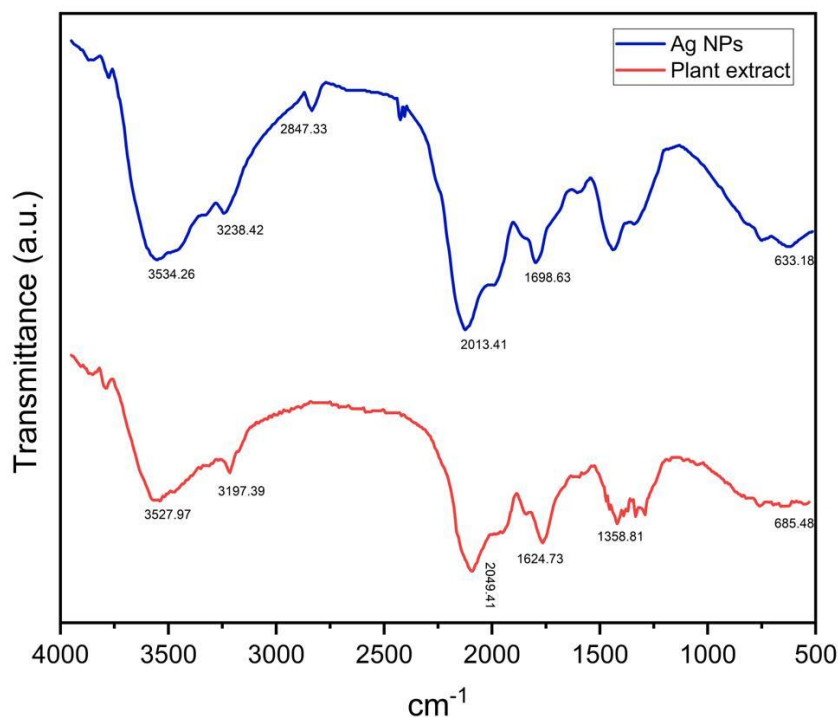


Figure 2: FT-IR of silver nanoparticles

The SEM assay was used to detect the shape and size of silver nanoparticles as noted in Figure 3 (A and B) noticed that the most particles look like spherical with size 29 nm for Ag NPs prepared by peels extract, and 35 nm for Ag NPs prepared by leaves extracts, these results settled with results of UV-Spectrophotometer [17].

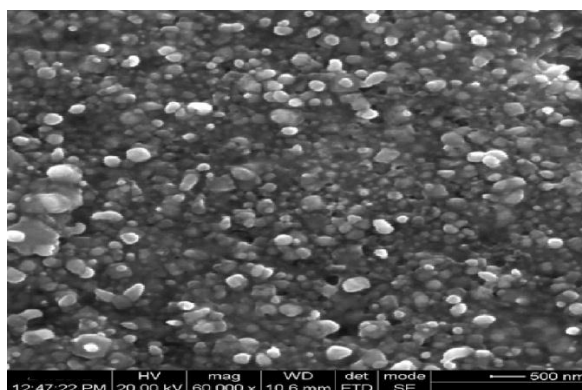


Figure 3a: SEM images of leaves extract- silver nanoparticles.

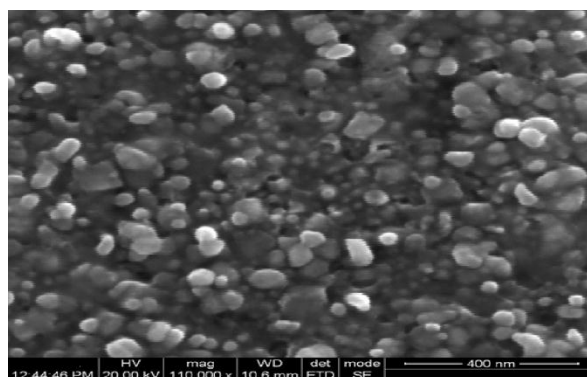


Figure 3b: SEM images of peel extract- silver nanoparticles.

The investigation focused on remarkable antimicrobial characters of biosynthesized silver nanoparticles whether it is prepared by *Mangifera indica* peel extract or leaves extract. The experiment include using (0.5%, 1%, 1.5%, 2% and 2.5%) concentrations of each extract against (*Staphylococcus aureus*, *proteus sp.*, *Pseudomonas aeruginosa*, *E. coli*, *Serriatia sp.*, and *Candida albicans*), the results showed that the efficiency of silver nanoparticles to inhabit the growth of microbial isolates were increased with creasing the concentration as presented in table 1 and Figure 4 Silver nanoparticles showed significant effect on gram positive bacteria, and *Candida*, while the gram negative bacteria look more resistance than other isolates. The highest inhibition on *Staphylococcus aureus* with (42 mm) with Ag NPs of leaves extract, and (45 mm)



with Ag NPs of peels extract, on the other hands, the inhibition zone measured (12, 18 mm) respectively to with Ag NPs of leaves extract and peels extract against *Pseudomonas aeruginosa*. Also the results showed good effect to *Mangifera indica* peel extract or leaves extract on selective isolates as seen in table 1. The results revealed abroad effect of both ethanolic extract of the mango leaves and ethanolic mango peels on all gram positive bacteria, however, the peels extract of *Mangifera indica* was more effective to inhibit the selective isolates with inhibition zones ranging from (0- up to 30) mm.

The sensitivity of gram-positive bacteria to the effect of antimicrobial agents including silver nanoparticles than gram negative bacteria due to the nature of gram-positive cell wall which consist of thin layers of peptidoglycan, thus forming rigid structure. The metal nanoparticles like Ag NPs [18, 19].

Metal nanoparticles like Ag-NPs has been researched extensively due its ability to inhibit the growth of microorganisms [20]. These ability may related to many reasons: the adhering of Ag-NPs on the cell surface effect on the integrity of microbial cell membrane that disturb the control of material and molecules flow through the cellular membrane and increase the permeability [21]. The presence of silver NPs in aqueous media like living cells led to release silver ions which has lethal properties, can bind to large molecules (DNA, RNA and proteins) beside their ability to effect on most cellular activities such as cellular respiration, replication and division [22]. Silver NPs can induce the release of ROS inside the cell [23].

The ability of silver NPs to inhibit the growth of selective isolates compared to ability of Amoxicillin. The finding showed high effectiveness to Silver NPs exceed the ability of Amoxicillin. The outcomes showed ability of Amoxicillin to inhibit the growth of gram negative isolates and yeast with inhibition zone 50 mm with *Staphylococcus aureus*, 20 mm with *Candida sp.*, and 18 mm with *Serratia sp.*, also, the results showed the resistance of gram negative isolates to Amoxicillin as seen in Figure 4 and Table 1. The efficiency of Amoxicillin to effect on susceptible pathogens is through its ability to inhibit the biosynthesis of muco-peptide cell wall during the multiplication of cell which is necessary to divide of cell and this lead to cell lysis [24]. Besides that, it could be deduced that the activity of *M. indica* peels is more potent

antimicrobial agent than the activity of *M. indica* leaves rapprochement to Amoxicillin. The studies indicate the antimicrobial effectiveness of leave and peels of *M. indica* including *Staphylococcus aureus*, *Lactobacillus vulgaricus* *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis* and *Streptococcus agalactiae* and minimal activity against gram negative bacteria [25]. The recent investigation highlight on ability to prepare biosynthesized Ag NPs using *Mangifera indica* peels extract that observed remarkable activity against Gram negative and gram positive bacteria Further, the phytochemical research is required to distinguish the active essential responsible for more testing and studies to cover MDR infectious microorganisms.

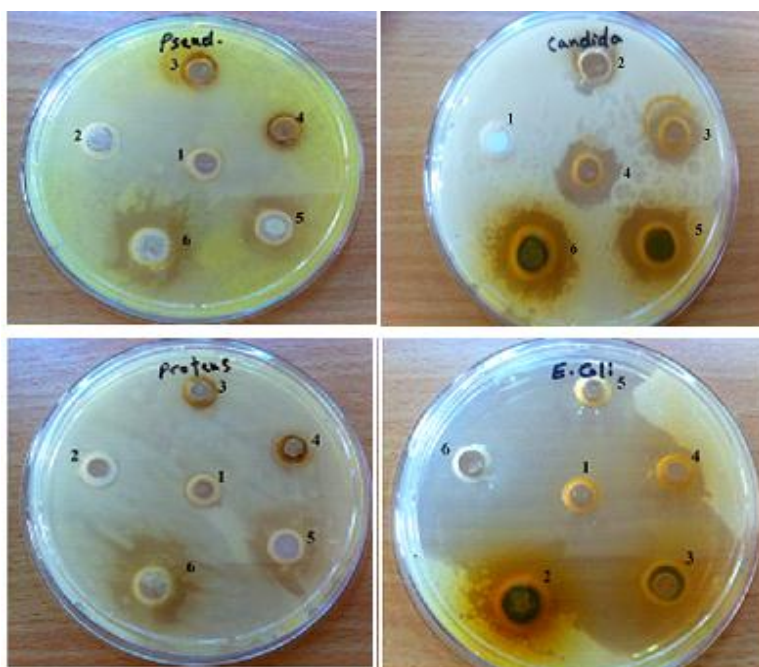


Figure 4: Antimicrobial activity of green synthesis nanoparticles on selective pathogens.



Table 1. Antimicrobial activity of green synthesis silver nanoparticles on selective pathogens.

| No | Microorganisms | inhibition zone (mm) | | | | |
|----|---------------------------|----------------------|----------------|---------|--------------|---------|
| | | Amoxicillin | Leaves Extract | Ag NP 1 | PealsExtract | Ag NP 2 |
| 1 | <i>Staph. aureus</i> | 50 | 15 | 42 | 16 | 45 |
| 2 | <i>Serratia sp</i> | 20 | 30 | 28 | 23 | 15 |
| 3 | <i>Candida albicans</i> | 18 | 23 | 21 | 23 | 20 |
| 4 | <i>E.coli</i> | 0 | 20 | 42 | 12 | 38 |
| 5 | <i>Proteus sp.</i> | 0 | 0 | 11 | 22 | 18 |
| 6 | <i>Pseudo. aeruginosa</i> | 0 | 0 | 12 | 21 | 18 |

4. Conclusions.

The search focused on successfully green synthesized silver nanoparticles using substances of *Mangifera indica* extracts. The characterization of biosynthesized silver nanoparticles were detected via various analytical assays that revealed the spherical shape with nano-scale size. The silver NPs confirmed the outstanding antimicrobial properties comparing to *Mangifera indica* extracts or Amoxicillin. Further, the research to use any various biologically active compounds in plants is required to distinguish the effectual principal responsible for these efficiency and ability to detect on broad type of microorganisms would be supported.

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