

# Environmental Friendly Synthesis of Silver Nanoparticles Using Leaf Extract of Mureira Tree (*Azadirachta indica*) Cultivated in Iraq and Efficacy the Antimicrobial Activity

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## Abstract

In the present study, environment friendly and cost effective silver nanoparticles were synthesized using the leaves extract of Mureira tree cultivated in Iraq as the reducing and capping agent. The nanoparticles were characterized using UV-visible, FT-IR, XRD, and SEM methods. The surface plasmon resonance peaks in absorption spectra for silver colloidal solution showed an absorption peak at 420 nm in a UV-visible spectrum. The functional biomolecules such as carboxyl groups present in the seaweed responsible for the silver nanoparticles formation were characterized by FT-IR. The XRD results suggested that the crystallization of the bio-organic phase occurs on the surface of the silver nanoparticles or vice versa. The broadening of peaks in the XRD patterns was attributed to particle size effects and the average particles size about 30 nm which was calculated by using the Dubai-Scherrer equation. The silver nanoparticles synthesized by the help of Mureira tree leaves extracts were scanned using SEM. It reveals that a silver nanoparticle seems to be spherical in morphology. The results shows that silver nanoparticles synthesized by Mureira leaves extract has effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. The inhibition zone was 17 mm for *Enterobacter cloacae*, 18 mm for *Escherichia coli* and *Klebsiella pneumonia*, 16 mm for *Proteus mirabilis* and *Pseudomonas aeruginosa*, 15 mm for *Bacillus sp.*, *Staphylococcus aureus* and *Streptococcus spp.* The antimicrobial activity of Mureira leaves extract has lowest effect against tested isolates. The inhibition zone was 7 mm for *Enterobacter cloacae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus sp.* and *Streptococcus spp.* 10 mm for *Klebsiella pneumonia*, and 8 mm for *Staphylococcus aureus*. The study revealed that the silver nanoparticles synthesis by using leaves extract of Mureira tree could be as a therapeutic agent for human microbial infections.

**Keywords:** Silver nanoparticles, pathogenic bacteria, Mureira tree, Leaf Extract, antimicrobial activity.

## 1. Introduction

Nanotechnology is a latest field of modern research dealing with synthesis, design and manipulation of nanosized particles. Remarkable development in nanotechnology has opened a novel and very wide frontier of application that includes the synthesis of nanoscale material, exploration of their unique physical chemical and optoelectronic properties. Nanotechnology has gained tremendous importance in state of the art techniques for health care, food technology, cosmetics, environmental health, mechanics, optics, chemical industries, biomedical, electronics, space engineering, energy science, drug-gene delivery, optoelectronics, catalysis, light emitters, nonlinear optical devices, single electron transistors, and photo electrochemical applications etc [1-4]. Currently, silver nanotechnology, is becoming popular due to its above extensive applications and distinctive properties (e.g. size and shape dependent several properties such as, optical, magnetic and electrical properties), which can be incorporated into biosensor materials, antimicrobial applications, composite fibres, cosmetic products, cryogenic superconducting materials, and electronic components. Nanomaterials have a long list of applicability in improving human life and its environment. The first relation between human life and nano scale was developed naturally in ayurveda, which is a 5000-year-old Indian system of medicine. It had some knowledge of nanoscience and technology before the term 'nano' was even formed. Modern science has just started exploring nanoscience in the 21st century [5]. The wide applications have attracted the attention of scientists to produce them by different methods. The above chemical methods are tedious, more time consuming and expensive. The scientists, therefore, have now recently found easier ways of biological methods to prepare Ag nanoparticles through green nano route [5, 6] using plant extract mediation for chemical reduction of AgNO<sub>3</sub>. The method allows to undergo highly controlled and hierarchical assembly. It also provides advantage of being cost effective, less time consuming and environment friendly. There is no need to use high temperature, high pressure or toxic chemicals [7]. Some examples are geranium leaf assisted biosynthesis of silver nanoparticles [8], synthesis of nanoparticles using fungus [9, 10], and soluble starch [11]. Silver nanoparticles are toxic to bacteria and can destroy antibiotic resistant bacteria such as methicillin resistant *S. aureus* [12, 13]. Infact bacteria are not able to develop resistance against silver like they do with antibiotics [14, 15]. Silver nanoparticles are better than silver based compounds and silver ions kill microbes effectively [16, 17]. Neem (*Azadirachta indica*) belongs to *Meliaceae* family, and is well known in India and its neighboring countries for

more than 200 years as one of the most versatile medicinal plant having a wide spectrum of biological activity. Every part of the tree has been used as a traditional medicine for household remedy against various human ailments, from antiquity [18, 19, 20]. Neem has various phytochemicals identified to be carbohydrates, alkaloids, steroids, phenols, saponins and flavonoids [21]. The advantage of using Neem leaves for bioreduction of metal ions is that it is easily available throughout the year. Studies have suggested that biomolecules like protein, phenols, flavonoids and some phytochemicals has ability to reduce the metallic ions to the nanosize and also play an important role in the capping of the nanoparticles for its stability [22].

In Iraq the *Azadirachta indica* tree called Mureira tree because of its bitter taste which is used as medicinal plant having a wide spectrum of biological activity beside that it's used in repelling Insects and its extract is used as a safe and natural pesticide. The present study aims to biosynthesis of the silver nanoparticles using Mureira tree leaves extract and evaluation of their antibacterial activity against various human multi drug resistant pathogenic bacteria.

## 2. Methods and materials

### 2.1 Collection of pathogens

The multiple antibiotic-resistant isolates collected from microbiology diagnosis laboratory, Al-Numan hospital. Which included *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Streptococcus sp.*, *Enterobacter cloacae*, *Bacillus sp.*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* used for the antimicrobial activity.

### 2.2 Plant Material Collection

Mureira tree leaves are shown in (Figure 1). Its purchased from Regional Botany Garden Gherai'at, Baghdad, Iraq.



Figure 1. Mureira tree leaves.

### 2.3 Synthesis of silver nanoparticles

*Silver nitrate (AgNO<sub>3</sub>)* purchased from Merck limited, India. Mureira leaves were used in the present study, rinsed with sterile distilled water to remove any associated debris. These clean fresh materials were cut into fine pieces and grinded in a pestle and mortar (20 g of the sample in 100 ml of distilled water). The resulted infusion was filtered thoroughly using Whatmann No.1 filter paper. For the reduction of Ag<sup>+</sup> ions, 10ml of Mureira tree leaves extract was mixed to 90 ml of 0.1mM aqueous of AgNO<sub>3</sub> solution drop wise with constant stirring at 50-60°C until the colour change [23].

### 2.4 Characterization of silver nanoparticles

#### 1- UV-Vis Spectra analysis

The reduction of pure Ag<sup>+</sup> ions was monitored by measuring the UV-Vis spectrum of the reaction medium after 30 min. UV-Vis spectrophotometer is procured from Shimadzu. A small aliquot of the sample was taken for UV-Vis spectrum analysis (350-750 nm). The maximum absorbance spectrum of As-Ag nanoparticles was observed



at 455 nm.

## 2- Fourier Transform Infra Red Spectroscopy (FT-IR)

FT-IR measurements were carried out using (8300 FT-IR Shimadzu Spectrophotometer) the range from  $4000\text{ cm}^{-1}$  to  $400\text{ cm}^{-1}$ . After complete reduction of  $\text{AgNO}_3$  ions by Naringe leaf extract, the mixture was centrifuged at 10000 rpm for 10 min to remove protein or other bioorganic compounds that were present in the solution. The silver nanoparticles pellet obtained was air dried. The dried nanoparticles were mixed with the potassium bromide (KBr) to made thin pellets and were used for FT-IR analysis in transmittance mode.

## 3- X-Ray Diffraction (XRD) analysis

Resulting solution of the developed nanoparticles of silver was centrifuged at 10,000 rpm for 30 min. The solid residues of Ag NPs were washed twice with deionized distilled water and then dried at  $80^\circ\text{C}$  to obtain powder Ag NPs used for X-ray powder diffraction measurements. The powder X-ray diffraction (XRD) patterns were recorded on (Shimadzu XRD-6000) with copper radiation ( $\text{Cu K}\alpha$ ,  $1.5406\text{ \AA}$ ) at 40 kV and 30 mA [24].

## 3- SEM Analysis of Silver Nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using (Inspect S 50) SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid.

## 2.5 Determination of antimicrobial activity

The antibacterial activity of silver nanoparticles was tested by standard agar well diffusion method [25]. Wells were made using sterile cork-borer under aseptic conditions. The inocula were prepared by diluting the overnight cultures with 0.9 % sodium chloride to 0.5 McFarland standards and were swabbed onto the plate. Synthesized solutions were loaded on marked wells with the help of micropipette under aseptic conditions and incubated at  $37^\circ\text{C}$  for 24 h. The zone of inhibition was measured and expressed in millimeters.

## 3. Results

The reduction of  $\text{Ag}^+$  into Ag-NPs during exposure to Moreira leaves extract was able to be followed by the color change. The fresh suspension of Al-Rawag leaves extract was greenish yellow. However, after the addition of  $\text{AgNO}_3$  and stirring for one hour at  $60^\circ\text{C}$ , the emulsion turned Reddish brown. The color changes in aqueous solutions are due to the surface-plasmon resonance (SPR) phenomenon Figure 2 (B and C). The result obtained in this investigation is interesting because it can serve as a foundation in terms of identification of potential forest plants for synthesizing Ag-NPs.



Figure 2: Synthesis of silver nanoparticles using Moreira leaves extract: A - silver nitrate solution. B- leaves extract, and C - silver nanoparticles colloidal.

### UV-Vis Spectrophotometry

The Formation of metal nanoparticles by reduction of the aqueous metal ions during exposure of Moreira leaves extract may be easily followed by UV-Vis spectroscopy (UV- shimadzu spectrophotometer). UV-Vis absorption spectrum of silver nanoparticles in the presence of Al-Rawag leaf extract is shown in figure (3). The surface plasmon resonance peaks in absorption spectra for silver colloidal solution showed an absorption peak at 420 nm in a UV-visible spectrum, suggesting that the nanoparticles were dispersed in the aqueous solution with no evidence for aggregation in UV-Vis absorption spectrum Figure (3).

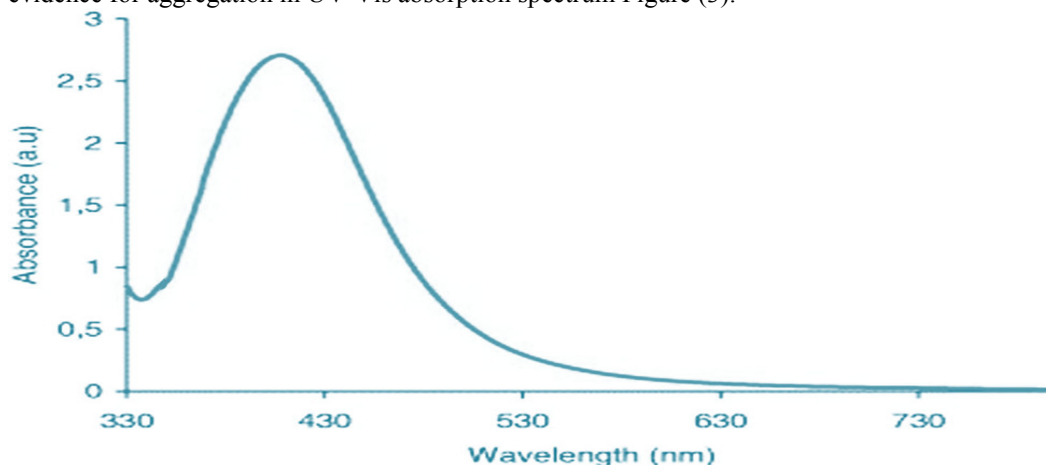


Figure 3. UV-Vis absorption spectra of Silver nanoparticles synthesized by exposure of Al-Rawag leaf extract with 0.1mM silver nitrate.

### Fourier Transform Infra Red Spectroscopy

The results of FTIR analysis of this study show different stretches of bonds shown at different peaks; 3327.21—N—H stretch, 1641.42 —C=C, and 1211.30—C=O. Figure 4 shows the peaks near 3440 $\text{cm}^{-1}$ , and 2968  $\text{cm}^{-1}$  assigned to OH stretching and aldehydic C—H stretching, respectively. The weaker band at 1629 $\text{cm}^{-1}$  corresponds to amide I arising due to carbonyl stretch in proteins. The peak at 1051  $\text{cm}^{-1}$  corresponds to C—N stretching vibration of the amine. The peak near 1743  $\text{cm}^{-1}$  corresponds to C=C stretching (non conjugated). The peak near 866  $\text{cm}^{-1}$  assigned to C=CH<sub>2</sub> and the peaks near 678  $\text{cm}^{-1}$  and 638  $\text{cm}^{-1}$  assigned to CH out of plane bending vibrations are substituted ethylene systems —CH=CH. FTIR spectra of silver nanoparticles exhibited prominent peaks at 1641, and 1382  $\text{cm}^{-1}$ . The spectra showed sharp and strong absorption band at 1641  $\text{cm}^{-1}$  assigned to the stretching vibration of (NH) C=O group [26].

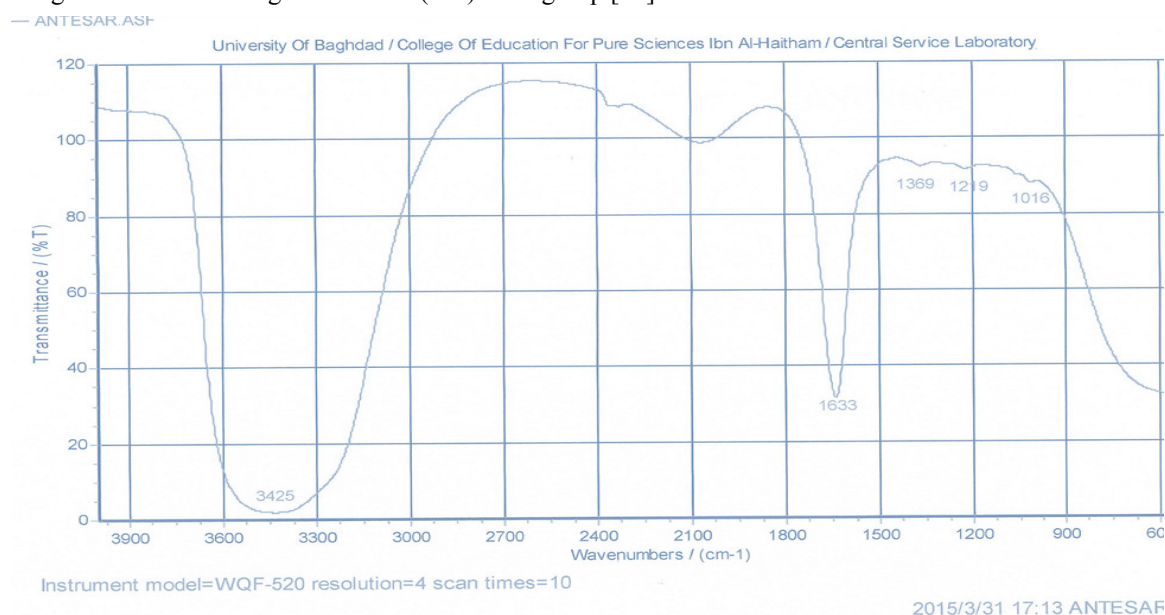


Figure 4. Fourier Transform Infra Red Spectroscopy image of Silver nanoparticles synthesized by exposure of Moreira leaves extract with 0.1mM silver nitrate.

### Powder X-ray diffraction

Figure (5) shows the XRD patterns of Ag-NPs. The X-ray diffraction patterns of the Ag-NPs synthesized by using AgNO<sub>3</sub> and using the Moreira leaves extract as the reducing and capping agent are shown in Fig. 5. All the reflections correspond to pure silver metal with face centered cubic symmetry. The reflections were indexed as (111), (200), (220) and (311) with the corresponding 2θ values of 38.12, 44.31, 64.46 and 76.98 respectively (JCPDS 04-0783). The intensity of peaks reflected the high degree of crystallinity of the silver nanoparticles. However, the diffraction peaks were broad indicating that the crystallite size is very small. The average particle size of Ag-NPs can be calculated using the Debye - Scherrer equation [27].

$$D = K \lambda / \beta \cos \theta$$

where K is the Scherrer constant with value from 0.9 to 1 (shape factor), λ is the X-ray wavelength (1.5418 Å), β is the width of the XRD peak at half-height and θ is the Bragg angle and D is the grain size. From the Scherrer equation, the average crystallite size of Ag-NPs is (30 nm).

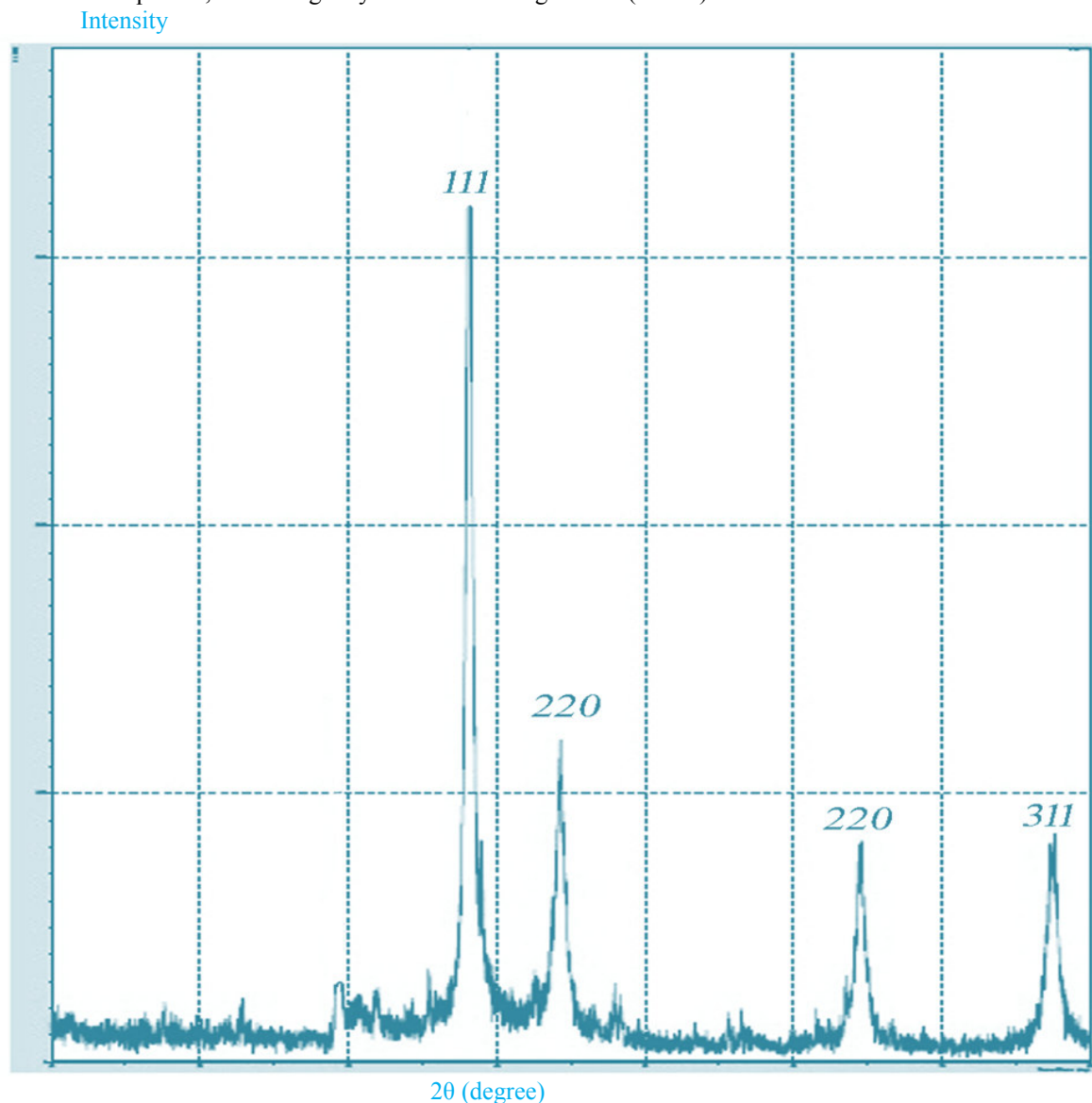


Figure 5. X ray diffraction of Silver nanoparticles synthesized by exposure of Moreira leaves extract with 0.1mM silver nitrate.

### SEM analysis of Silver nanoparticles

The silver nanoparticles synthesized by the help of Moreira leaves extract were scanned by SEM as shown in figure (6). It reveals that silver nanoparticles seem to be spherical in morphology and particles form cluster. It is easy to notice that the examined particles consist of a number of smaller objects of a few micrometers in size. However, we did not manage to examine the structure of the observed nanoparticles because of difficulties connected with getting higher magnification. In Figure (7), a standard EDX spectrum recorded on the examined



sample is shown. In the middle part of the presented spectrum a strong peak located at 3 KV. This maxima is directly related to the silver characteristic line L. The maximum located on the left part of the spectrum at 0.2 kV clearly comes from carbon. Quantitative analysis proved high silver contents (100%) in the examined samples the result shown in table (1).

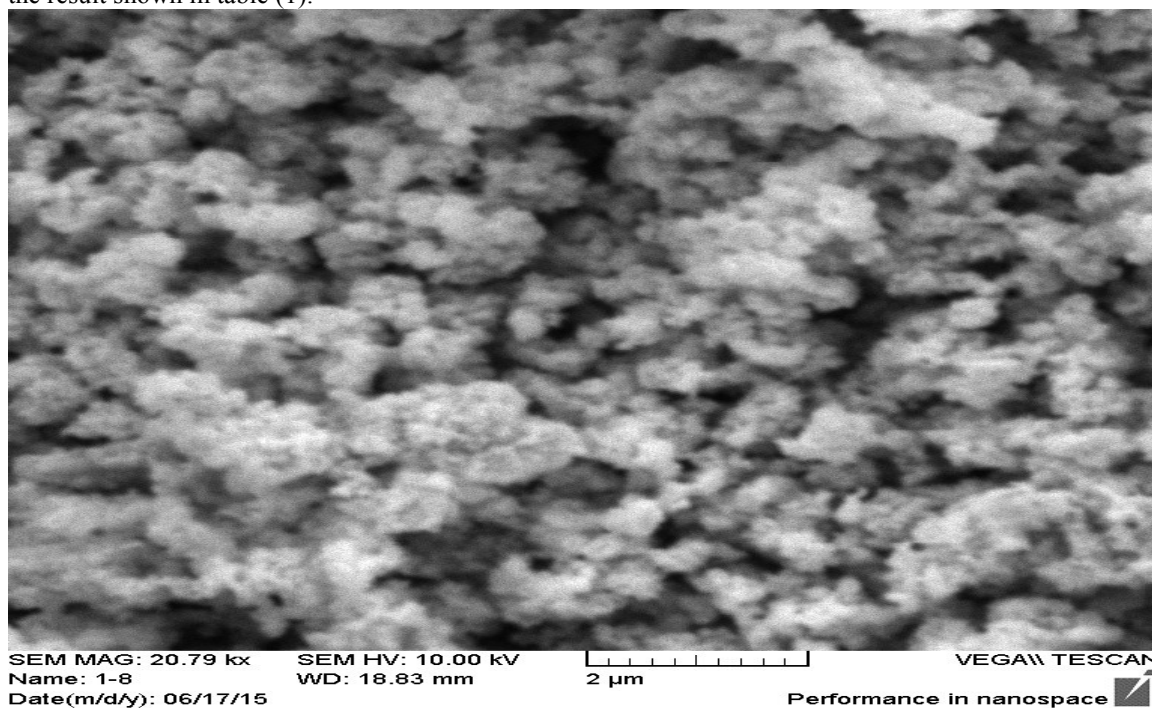


Figure 6. SEM micrographs of silver nanoparticles synthesised by Moreira leaves extract.

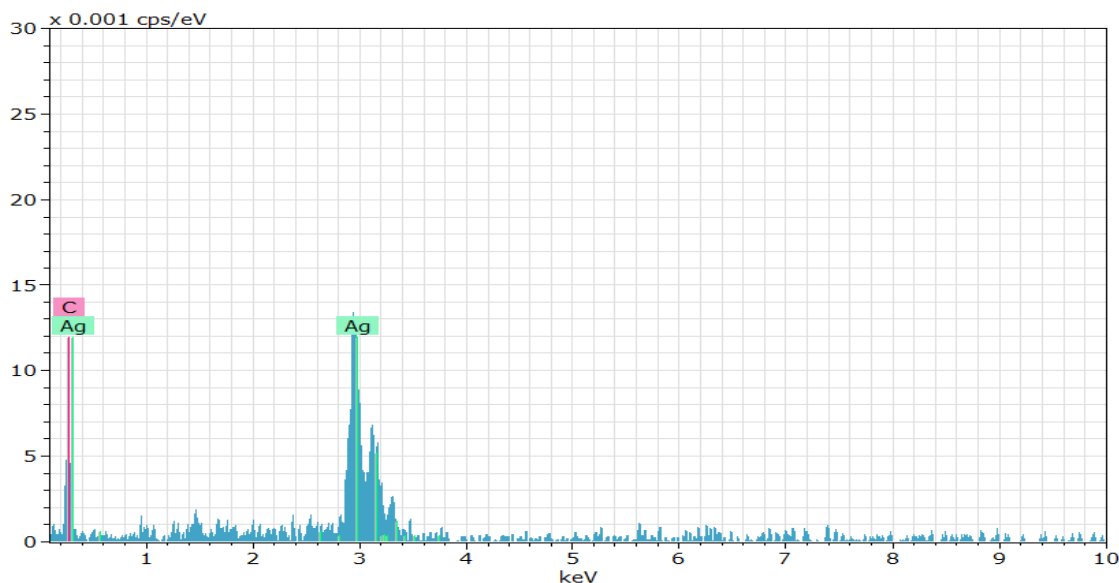


Figure (7).EDX characteristic spectrum obtained for silver powder.

Table (1) shows the elements in silver nanoparticles

Element	AN	series	[wt.%]	[norm. wt.%]	[norm. at.%]
Carbon	6	K-series	0	0	0
Silver	47	L-series	75.13866	100	100
		Sum:	75.13866	100	100

The data in Table (2) and Figure (8-9) shows that silver nanoparticles synthesized by Moreira leaves extract has effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. The inhibition zone was 17 mm for *Enterobacter cloacae*, 18 mm for *Escherichia coli* and *Klebsiella pneumonia*, 16 mm for *Proteus mirabilis* and *Pseudomonas aeruginosa*, 15 mm for *Bacillus sp.*, *Staphylococcus aureus* and

*Streptococcus spp.* The antimicrobial activity of Moreira leaves extract has lowest effect against tested isolates. The inhibition zone was 7 mm for *Enterobacter cloacae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus sp.* and *Streptococcus spp.* 10 mm for *Klebsiella pneumonia*, and 8 mm for *Staphylococcus aureus*.

Table 2. The inhibitory activity of the Ag-NPs synthesized by Moreira leaves extract against the tested bacteria as demonstrated by diameters of the inhibition zone (mm)\*.

Isolated bacteria	Zone of Inhibition	
	Moreira leaves extract	Moreira leaves extract / Ag-NPs
<i>Enterobacter cloacae</i>	7	17
<i>Escherichia coli</i>	7	18
<i>Klebsiella pneumonia</i>	10	18
<i>Proteus mirabilis</i>	7	16
<i>Pseudomonas aeruginosa</i>	7	16
<i>Bacillus sp.</i>	7	15
<i>Staphylococcus aureus</i>	8	15
<i>Streptococcus spp.</i>	7	15

\* Zone of inhibition, including the diameter of the cup plate method (6.0 mm) .The recorded value is 1 mean value of 3 replicates.

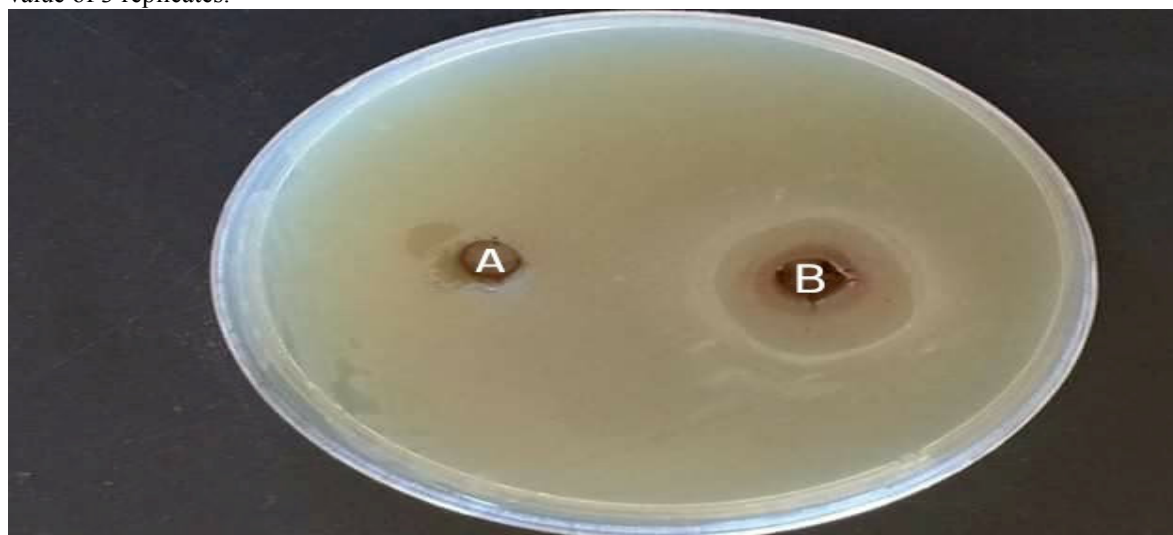


Figure 8. The antibacterial effect of Moreira leaf extracts (A), Silver nanoparticles synthesized by Moreira leaf extracts (B), using the test bacterium *Escherichia coli*.



Figure 9. The antibacterial effect of Moreira leaf extracts (A), Silver nanoparticles synthesized by Moreira leaf extracts (B), using the test bacterium *Klebsiella pneumonia*.



Figure 10. The antibacterial effect of Moreira leaf extracts (A), Silver nanoparticles synthesized by Moreira leaf extracts (B), using the test bacterium *Staphylococcus aureus*.

#### 4. Discussion

Nanotechnology has grown to be an important research field in all areas including medicinal chemistry. The size, orientation and physical properties of nanoparticles have reportedly shown to change the performance of any material. For several years, scientists have constantly explored different synthetic methods to synthesize nanoparticles. On the contrary, the green method of synthesis of nanoparticles is easy, efficient, and eco-friendly in comparison to chemical-mediated or microbe-mediated synthesis. The chemical synthesis involves toxic solvents, high pressure, energy and high temperature conversion and microbe involved synthesis is not feasible industrially due to its lab maintenance. Since, green synthesis is the best option to opt for the synthesis of nanoparticles.

The synthesis of metal and semiconductor nanoparticles is an expanding research area due to the potential applications for the development of novel technologies. Generally, nanoparticles are prepared by a variety of chemical methods which are not environmentally friendly. I have reported a fast, convenient and extracellular method for the synthesis of silver nanoparticles by reducing silver nitrate with the help of Moreira leaf extracts. The nanoparticles were characterized using UV-visible, FT-IR, XRD, and SEM methods. The surface plasmon resonance peaks in absorption spectra for silver colloidal solution showed that the absorption maximum was at 420 nm. The functional biomolecules such as carboxyl groups present in the seaweed responsible for the silver nanoparticles formation were characterized by FT-IR. The XRD results suggested that the crystallization of the bio-organic phase occurs on the surface of the silver nanoparticles or vice versa. The broadening of peaks in the XRD patterns was attributed to particle size effects and the average particles size about 30nm.

The results shows that silver nanoparticles synthesized by Moreira leaves extract has effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. The inhibition zone was 17 mm for *Enterobacter cloacae*, 18 mm for *Escherichia coli* and *Klebsiella pneumonia*, 16 mm for *Proteus mirabilis* and *Pseudomonas aeruginosa*, 15 mm for *Bacillus sp.*, *Staphylococcus aureus* and *Streptococcus spp.* The antimicrobial activity of Moreira leaves extract has lowest effect against tested isolates. The inhibition zone was between (7-10) mm Table (2) and Figures (8-10). Our interpretation of these results, the silver nanoparticles synthesized by Moreira leaves extract has another mechanism to kill bacteria more than Moreira leaves extract. The mechanism of the inhibitory effects of Ag ions on microorganisms is partially known. Some studies have reported that the positive charge on the Ag ion is crucial for its antimicrobial activity through the electrostatic attraction between negative charged cell membrane of microorganism and positive charged nanoparticles [28, 29, 30]. In contrast, Sondi and Salopek-Sondi, (2004) reported that the antimicrobial activity of silver nanoparticles on Gram-negative bacteria was dependent on the concentration of Ag nanoparticle, and was closely associated with the formation of 'pits' in the cell wall of bacteria. Then, Ag nanoparticles accumulated in the bacterial membrane caused the permeability, resulting in cell death. However, because those



studies included both positively charged Ag ions and negatively charged Ag nanoparticles, it is insufficient to explain the antimicrobial mechanism of positively charged Ag nanoparticles. Therefore, we expect that there is another possible mechanism. Amro *et al.* suggested that metal depletion may cause the formation of irregularly shaped pits in the outer membrane and change membrane permeability, which is caused by progressive release of lipopolysaccharide molecules and membrane proteins [32]. Also, Sondi and Salopek-Sondi speculate that a similar mechanism may cause the degradation of the membrane structure of *E. coli* during treatment with Ag nanoparticles [33]. Although their inference involved some sort of binding mechanism, still unclear is the mechanism of the interaction between Ag nanoparticles and components of the outer membrane. Recently, Danilczuk and co-workers (2006) reported Ag-generated free radicals through the ESR study of Ag nanoparticles. We suspect that the antimicrobial mechanism of Ag nanoparticles is related to the formation of free radicals and subsequent free radical induced membrane damage.

Our results support the hypothesis that Ag nanoparticles can be prepared in a simple and cost effective manner and are suitable for formulation of new types of bactericidal materials.

## 5. Conclusions

This investigation provides evidence that plant extract stabilized nanoparticles may be ideal candidates for future studies exploring their use in biomedical and pharmacy applications. This synthesis procedure offers a less cost-effective and green alternative to traditional protocols that may be readily scaled up for industry as a result of the low synthesis temperatures and time required. Since Moreira leaves are easily available throughout the nation, the active nano compound from this can be prepared and used as effective antibacterial reagents even against multidrug resistant bacteria, and home available, safe, cheap and with no side effect like the synthetic drugs.

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