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# Green Synthesis of Silver Nanoparticles Using Leaf Extract of Al-Rawag tree (*Moringa oleifera* Lamarck) 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 Al-Rawag tree cultivated in Iraq as the reducing and capping agent. The nanoparticless were characterized using UV-visble, FT-IR, XRD, and SEM methods. The surface plasmon resonance peaks in absorption spectra for silver colloidal solution showed an absorption peak at 430 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 Al-Rawag 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 leavesextract of Al-Rawag tree has effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. The inhibition zone was 14 mm for *Enterobactercloacae* and *Escherichia coli*, 20 mm for *Klebsiella pneumonia*, 16 mm for *Proteus mirabilis*, *Bacillus sp.* and *Streptococcus spp.*12 mm for *Pseudomonas aeruginosa*, 18 mm for *Staphylococcus aureus*. The antimicrobial activity of leavesextract of Al-Rawag tree has effect against tested isolates less than Silver nanoparticles synthesis by it.

The study revealed that the silver nanoparticles synthesis by using leaves extract of Al-Rawag tree could be as a therapeutic agent for human microbial infections.

**Keywords**: Silver nanoparticles, pathogenic bacteria,Al-Rawag tree, Leaf Extract, antimicrobial activity.

## Introduction

Nano silver one of the highly commercialized nanomaterials produced about 320 tons for year [1]. Due to the strong antimicrobial activity, AgNPs are also used in consumer products, household water filters, clothing, cosmetics, respirators, antibacterial sprays, detergents, cutting boards, socks, shoes, cell phones, laptop key boards, and children's toys. These

retail products exploit the antimicrobial properties of silver nanomaterials [2]. Nanomaterials have a long list of applicability inimproving human life and its environment. The firstrelation between human life and nano scale was developednaturally in ayurveda, which is a 5 000-year-old Indiansystem of medicine. It had some knowledge of nanoscienceand technology before the term 'nano' was even formed.Modern science has just started exploring nanoscience inthe 21st century [3]. 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 [4] using plant extract mediation for chemical reduction of $AgNO_3$. 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 [5]. Some examples are geranium leaf assisted biosynthesis of silver nanoparticles [6], synthesis of nanoparticles using fungus [7, 8],soluble starch [9], (*Azadirachta indica*) neem and leaf broth [10]. Silver nanoparticles are toxic to bacteria and can destroy antibiotic resistant bacteria such as methicillin resistant *S. aureus* [11, 12]. Infact bacteria are not able to develop resistance against silver like they do with antibiotics [13, 14]. Silver nanoparticles are better than silver based compounds and silver ions kill microbes effectively [15]. *Moringa oleifera* L., a wild herbaceous plant is very common in all tropical countries, including India. The stems are slender and often reddish in color, covered with yellowish bristly hairs especially in the younger parts. The leaves are oppositely arranged, lanceolate and are usually greenish or reddish; underneath measuring about 5 cm long *Moringa oleifera* (Moringaceae) is a small to medium evergreen tree widely distributed in Asia, Africa, and America. The plant is not only well known for high nutritional contents but also recognized for its therapeutic values [16]. The leaves of *M. oleifera* have been indigenously used for various medicinal purposes such as treating bronchitis, controlling glucose level, and reducing glandular swelling [16, 17]. Numerous pharmacological investigations of *M. oleifera* leaves have been reported on anti-inflammation, anti-infection, antidiabetic, antioxidant, and antihyperlipidemic activities [18-23]. Recently, isoquercetin, astragalin, and crypto-chlorogenic acid were reported to be major active components in *M. oleifera* leaves [24]. Isoquercetin is a powerful natural antioxidant which possesses several potential therapeutic effects including antiasthma and antihypertension [25-27]. Astragalin is also reported as a natural antioxidant agent exhibiting some biological properties such as attenuation of inflammation, inhibition of dermatitis, and cellular protective effect [28, 29]. Chlorogenic acid and its isomers are esters of quinic and caffeic acids that have abilities to inhibit oxidation and also promote various pharmacological activities such as antiobesity, reduction of plasma and liver lipids, and inhibition of acute lung injury [30]. Standardization of herbal extracts is essential to ensure their quality and biological activities. Some analytical techniques including high performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LCMS) were previously developed for the quantitative analysis of the *M. oleifera* leaf extract [31].

The present work has focused on the development of the easy synthesis of silver nanoparticles by an environmentally friendly procedure. In Iraq the *Moringa oleifera* Lamarck tree called Al-Rawag tree which is used in boiled food preparation and used for diabetes and high pressure syndrome; because they thinks the different parts of this plant played important roles in regulating glucose and lipid metabolic disorders.Al-Rawag leavesextract was used for the silver nanoparticles synthesis, and evaluation of their antibacterial activity against various human multi drug resistant pathogenic bacteria.

## Materials and Methods

**Collection of pathogens**

The multiple antibiotic-resistant isolatescollectedfrom 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.

**Plant Material Collection**

Al-Rawag tree (*Moringa oleifera* Lam.) leaves are shown in (Figure 1). Its purchased from Regional Botany Garden Gherai'at, Baghdad, Iraq.

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Figure 1. Al-Rawag tree leaves.

**Synthesis of silver nanoparticles**

Silver nitrate ($AgNO_3$) purchasedfrom Merck limited, India.Al-Rawag 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+ ions, 10ml of Al-Rawag leaves extract was mixed to 90 ml of 0.1mM aqueous of $AgNO_3$ solution drop wise with constantstirring at 50-60ºC until the colour change [32].

**Characterization of silver nanoparticles**

**1- UV-Vis Spectra analysis:**

Thereduction of pure Ag+ 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 $cm^{-1}$ to 400 $cm^{-1}$. After complete reduction of $AgNO_3$ ions by Naringeleaf 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 80ºC 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 (Cu $K_\alpha$, 1.5406 Å) at 40 kV and 30 mA [33].

**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.

**Determination of antimicrobial activity**

The antibacterial activity of silver nanoparticles was tested by standard agar well diffusion method [34]. 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 ºC for 24 h. The zone of inhibition was measured and expressed in millimeters.

## Results and dissection

The reduction of $Ag^+$ into Ag-NPs during exposure to Al-Rawag leaves extractwas able to be followed by the color change. The fresh suspension ofAl-Rawag leaves extractwas greenish yellow. However, after the addition of $AgNO_3$ and stirring for one hour at 60ºC, 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.

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Figure 2:Synthesis of silver nanoparticles usingAl-Rawag leaves extract: A - silver nitrate before addition of leaf extract. B- Fresh leaves extract, and C - After addition of leaf extract.

**UV-Vis Spectrophotometry**

The Formation of metal nanoparticles by reduction of the aqueous metal ions during exposure of Al-Rawag leaf extractmay be easily followed by UV–Vis spectroscopy (UV- shimadzu spectrophotometer). UV-Vis absorption spectrum of silver nanoparticles in the presence of Al-Rawag leaf extractis shown in figure (3). The surface plasmon resonance peaks in absorption spectra for silver colloidal solution showed an absorption peak at 430 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).

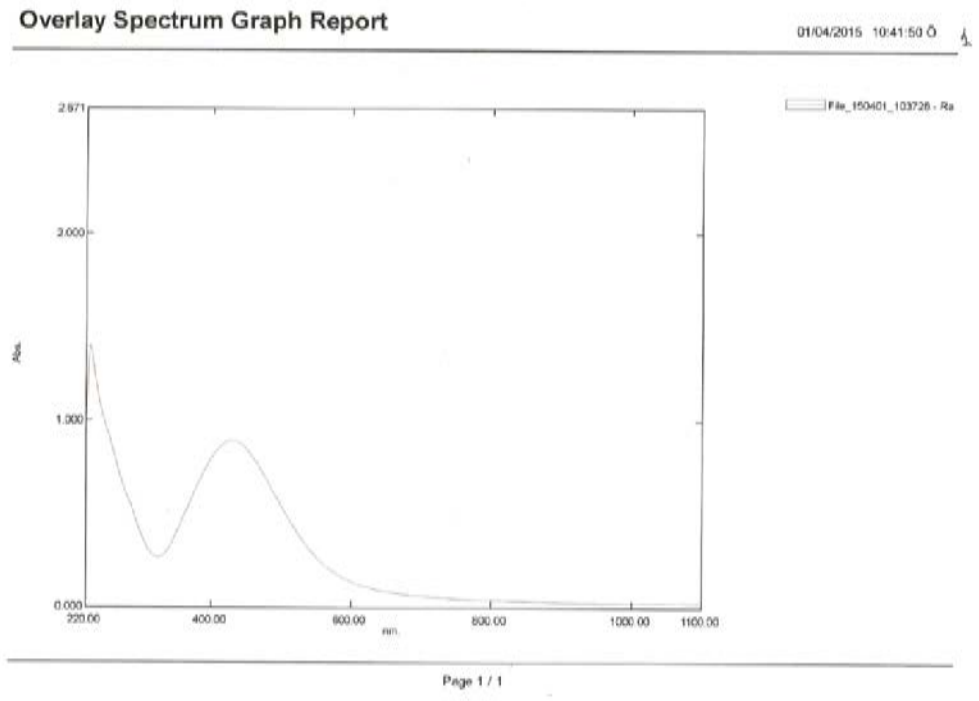


Figure 3. UV-Vis absorption spectra of Silver nanoparticles synthesized by exposure of Al-Rawag leaf extractwith 0.1mM silver nitrate.

**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 3440cm$^{-1}$, and 2968 cm$^{-1}$ assigned to

OH stretching and aldehydic C–H stretching, respectively. The weaker band at 1629cm$^{-1}$corresponds to amide I arising due to carbonyl stretch in proteins. The peak at 1051 cm$^{-1}$corresponds to C–N stretching vibration of the amine. The peak near 1743 cm$^{-1}$corresponds to C=C stretching (non conjugated). The peak near 866 cm$^{-1}$assigned to C=CH2 and the peaks near 678 cm$^{-1}$and 638 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 cm−1. The spectra showed sharp and strong absorption band at 1641 cm−1 assigned to the stretching vibration of (NH) C=O group[35].


Figure 4. Fourier Transform Infra Red Spectroscopy image of Silver nanoparticles synthesized by exposure of Al-Rawag leaf extractwith 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 $AgNO_3$ and using the Al-Rawag leaf extractas 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[36].

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

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).

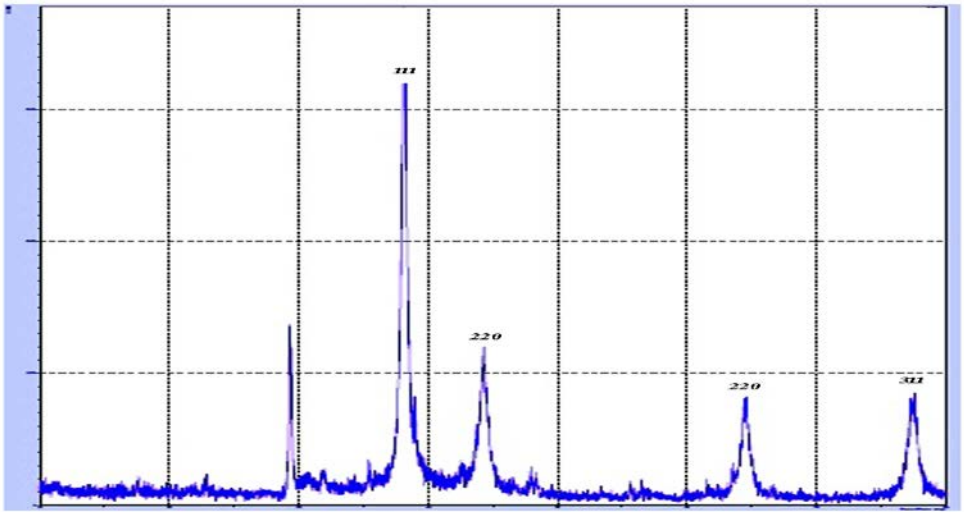


Figure 5. X ray diffraction of Silver nanoparticles synthesized by exposure of Al-Rawag leaf extract with 0.1mM silver nitrate.

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**SEM analysis of Silver nanoparticles**

The silver nanoparticles synthesized by the help of Al-Rawag leaf extractwere 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 clearlycomes from carbon. Quantitative analysis proved high silver contents (100%) in the examined samples the result shown in table (1).

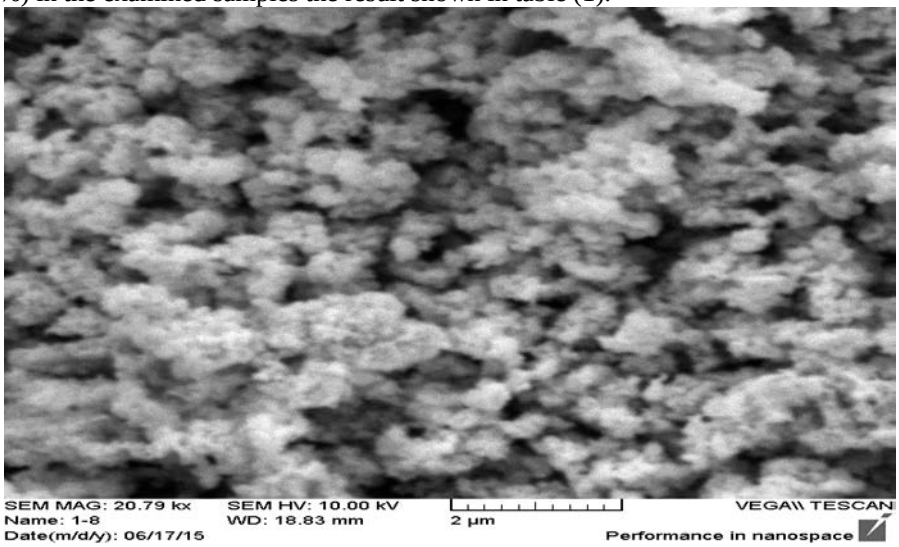


Figure 6.SEM micrographs of silver nanoparticles synthesised by Al-Rawag leaf extract.

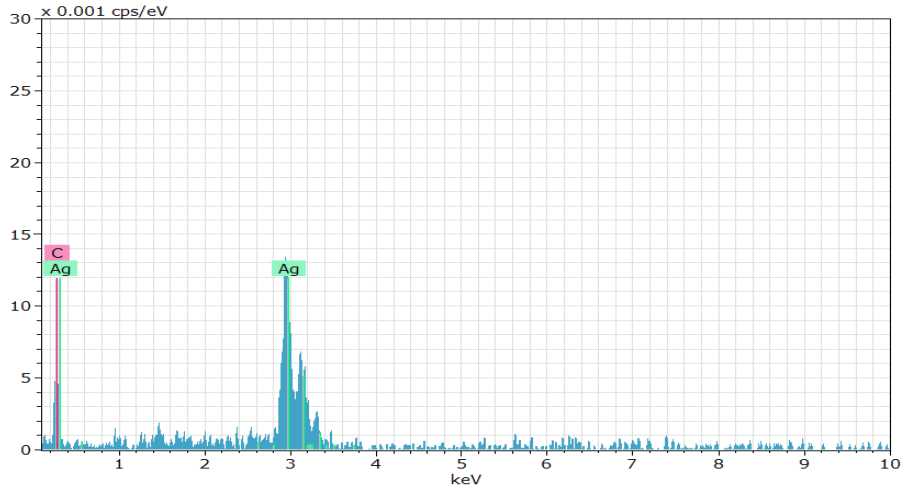


Figure (7).EDX characteristic spectrum obtained for silver powder.

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 Al-Rawag leaf extracthas effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. The inhibition zone was 14 mm for *Enterobactercloacae*and*Escherichia coli*,20 mm for *Klebsiella pneumonia*, 16 mm for*Proteus mirabilis*, *Bacillus sp.* and*Streptococcus spp.*12mm for*Pseudomonas aeruginosa*, 18 mm for *Staphylococcus aureus*. The antimicrobial activity of Al-Rawag leaves extract has no effect against tested isolates.

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

| Isolated bacteria | Zone of Inhibition | |
|---|---|---|
| | Al-Rawag leaves extract | Al-Rawag leaves extract /Ag-NPs |
| *Enterobacter cloacae* | 0 | 14 |
| *Escherichia coli* | 0 | 14 |
| *Klebsiella pneumonia* | 0 | 20 |
| *Proteus mirabilis* | 0 | 14 |
| *Pseudomonas aeruginosa* | 0 | 10 |
| *Bacillus sp.* | 0 | 14 |
| *Staphylococcus aureus* | 0 | 15 |
| *Streptococcus spp.* | 0 | 14 |

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

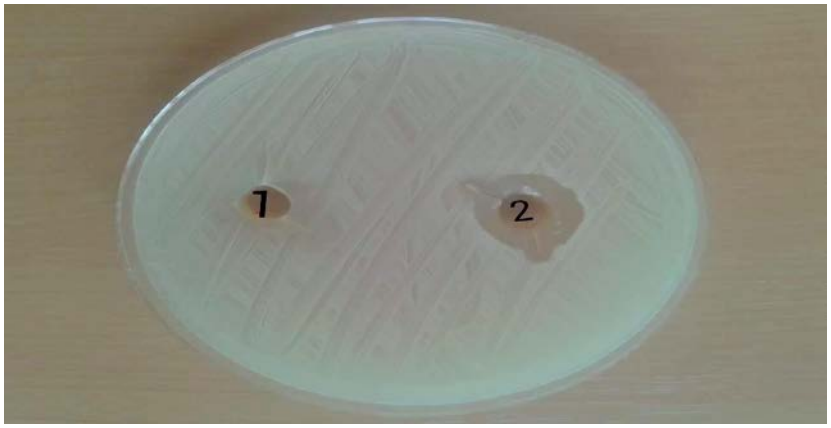


Figure 8. The antibacterial effect ofAl-Rawag leaves extract (1), Silver nanoparticles synthesized by Al-Rawag leaves extract(2) using the test bacterium*Staphylococcus aureus*.

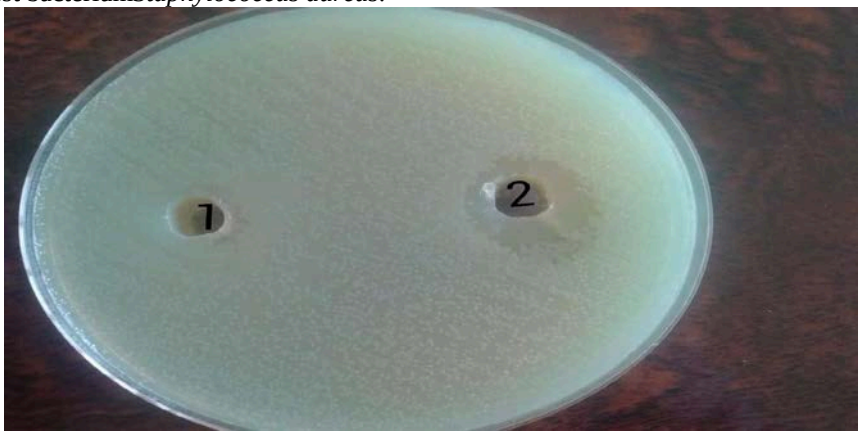


Figure 9. The antibacterial effect of Al-Rawag leaves extract (1), Silver nanoparticles synthesized by Al-Rawag leaves extract (2) using the test bacterium*Escherichia coli*.

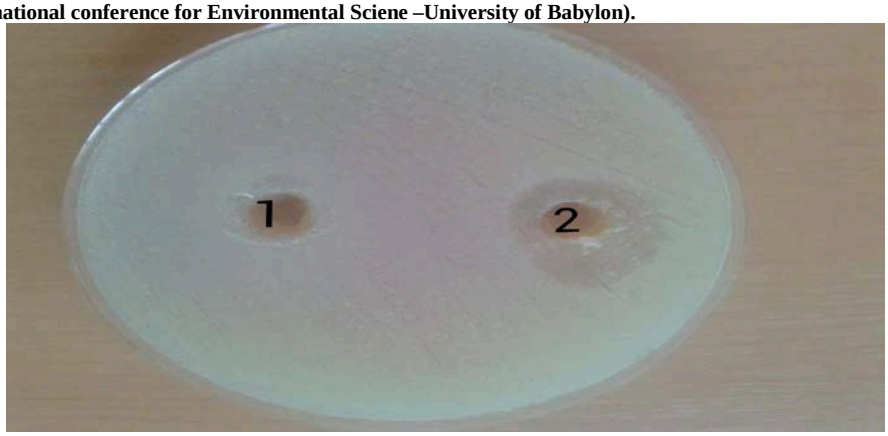


Figure 10. The antibacterial effect of Al-Rawag leaves extract (1), Silver nanoparticles synthesized by Al-Rawag leaves extract (2) using the test bacterium*Klebsiella pneumonia*.

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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. We have reported a fast, convenient and extracellular method for the synthesis of silver nanoparticles by reducing silver nitrate with the help of Al-Rawag leaves extract. The nanoparticless were characterized using UV-visble, 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 430 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 synthesised by Al-Rawag leaves extract has effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. The inhibition zone was 14 mm for *Enterobacter cloacae* and*Escherichia coli*, 20 mm for *Klebsiella pneumonia*, 16 mm for *Proteus mirabilis*, *Bacillus sp*. and *Streptococcus spp*. 12 mm for *Pseudomonas aeruginosa*, 18 mm for *Staphylococcus aureus*. The antimicrobial activity of Al-Rawag leaves extract has no effect against tested isolates;manystudies [16-28] referred to Al-Rawag leaves extract by the number of solvents such as ethyl alcohol, methyl alcohol, chloroform and ether, haves the effectiveness of antibacterial. The solvent used in the present study and the amount of Al-Rawag leaves used it not possess the effectiveness of antibacterial Table (2) and Figure (8-10). Our interpretation of these results, the silver nanoparticles synthesised by Al-Rawag leaves extract has another mechanism to kill bacteria not found in Al-Rawag leaves extract. This finding agreement with the several studies [29, 33, 34, 35].

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 [37, 38, 39]. 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 [41]. 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 [39]. Although their inference involved some sort of binding mechanism, still unclear is the mechanism of the interaction between Ag nanoparticles and component(s) 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.

**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 Al-Rawag 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.

## References

**[1] Gottschalk, F., R. W. Scholz, and B. Nowack.** Probabilistic material flow modeling for assessing the environmental exposure to compounds: Methodology and an application to engineered nano-TiO2 particles.*Environ. Modeling Software*; 25: 320-332. 2010.

**[2] Kaegi, R., B. Sinnet, S. Zuleeg, H. Hagendorfer, E. Muller, R. Vonbank, M. Boller, and M. Burkhardt.** Release of silver nanoparticles from outdoor facades. *Environ Pollut*; 158: 2900-2905. 2010.

**[3] Dubeya, S. P., M. Lahtinenb, and M. Sillanpaa.** Tansy fruit mediated greener synthesis of silver and gold nanoparticles. *Process Biochem*; 45: 1065-71. 2010.

4- Song, J. Y. and B. S. Kim, (2009). Rapid biological synthesis of silver nanoparticles using plant leaf extracts.*Bioprocess Biosyst Eng*; 32(**1**):79-84.

**[5] Sondi, I. and B. Salopek-Sondi.** Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *Journal of Colloid and Interface Science*; 275(1): 177-182. 2004.

**[6] Shankar, S.S., A. Ahmad, and M. Sastry.** Geranium leaf assisted biosynthesis of silver nanoparticles. *Biotechnol Prog*; 19(6):1627-31. 2003.

**[7] Mukherjee, P., A. Ahmad, D. Mandal, S. Senapati, S. R. Sainkar, M. I. Khan, R. Ramani, R. Parischa, P. V. Ajaykumar, M. Alam, M. Sastry, and R. Kumar.** Bioreduction of AuCl4- ions by the fungus, *Verticillium sp*. and surface trapping of the gold nanoparticles formed.*Angew Chem Int Ed*; 40: 3585-3588. 2001.

**[8] Ahmad, A., P. Mukherjee, S. Senapati, D. Mandal, M. I. Khan, R. Kumar, and M. Sastry.** Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. *Colloids Surf B Biointerfaces*; 28:313-318. 2003.

**[9] Vigneshwaran, N., R. P. Nachane, R. H. Balasubramanya, and P.V. Varadarajan**A novel one-pot 'green' synthesis of stable silver nanoparticles using soluble starch.*Carbohydrate research*; 341(12): 2012-2018. 2006.

**[10] Shankar, S. S., A. Rai, A. Ahmad, M. Sastry.** Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. *Journal of colloid and interface science*; 275(2): 496-502. 2004.

**[11] Edwards-Jones, V.** Antimicrobial and barrier effects of silver against methicillin-resistant Staphylococcus aureus. *Journal of Wound Care*; **15**: 285-290. 2006.

**[12] Strohal, R., M. Schelling, M. Takacs, W. Jurecka, U. Gruber, and F. Offner.** Nanocrystalline silver dressings as an efficient anti-MRSA barrier: a new solution to an increasing problem. *J Hosp Infect*; 60(3): 226-30. 2005.

**[13] Singh, R.P., S. Magesh, and C. Rakkiyappan.** Ginger (Zingiber officinale) Root Extract: A Source of Silver Nanoparticles and Their Application. IJBEST; 2(3): 75-80. 2011.

**[14] Baker, C., A. Pradhan, L. Pakstis, D. J. Pochan, and S. I. Shah.** Synthesis and antibacterial properties of silver nanoparticles. *J Nanosci Nanotechnol*; 5(2): 244-9. 2005.

**[15] Pal, S., Y. K. Tak, and J. M. Song.** Does the Antibacterial Activity of Silver Nanoparticles Depend on the Shape of the Nanoparticle? A Study of the Gram-Negative Bacterium *Escherichia coli.Appl. Environ. Microbiol*; 73(6): 1712-1720. 2007.

**[16] Anwar, F., S. Latif, M. Ashraf, and A. H. Gilani.** *Moringa oleifera*: a food plant with multiple medicinal uses. *Phytotherapy Research*; 21(1): 17-25. 2007.

**[17] Pandey, A., K. Pradheep, R. Gupta, E. R. Nayar, and D. C. Bhandari.** Drumstick tree' (*Moringa oleifera* Lam.): a multipurpose potential species in India. *Genetic Resources and Crop Evolution*; 58(**3**): 453-460. 2011.

**[18] Ou´edraogo, M., A. Lamien-Sanou, and N. Ramde.** Protective effect of *Moringa oleifera* leaves against gentamicin-induced nephrotoxicity in rabbits. *Experimental and Toxicologic Pathology*; 65(3): 335-339. 2013.

**[19] Singh, B. N., and R. L. Singhet**. Oxidative DNA damage protective activity, antioxidant and anti-quorum sensing potentials of *Moringa oleifera*. *Food and Chemical Toxicology*; 47(6): 1109-1116**.** 2009.

**[20] Ndong, M., M. Uehara, S. I. Katsumata, and K. Suzuki.** Effects of oral administration of *Moringa oleifera* Lam on glucose tolerance in Goto-Kakizaki and wistar rats. *Journal of Clinical Biochemistry and Nutrition*; 40 (**3**): 229-233. 2007.

**[21] Verma, A. R., M. Vijayakumar, C. S. Mathela, and C. V. Rao**. In vitro and in vivo antioxidant properties of different fractions of *Moringa oleifera* leaves. *Food and Chemical Toxicology*; 47(9): 2196-2201. 2009.
**[22] Chumark, P., P. Khunawat, and Y. Sanvarinda.** The *in vitro* and *ex vivo* antioxidant properties, hypolipidaemic and antiathero-sclerotic activities of water extract of *Moringa oleifera* Lam. Leaves. *Journal of Ethnopharmacology*; 116(3): 439-446. 2008.
**[23] Vongsak, B., Sithisarn, P., & Gritsanapan, W.** HPLC quantitative analysis of three major antioxidative components of *Moringa oleifera* leaf extracts. *Planta Medica*; 78(11): 1252. 2012.
**[24] Bennett, R. N., Mellon, F. A., & Foidl, N.** Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees Moringa oleifera L. (Horseradish tree) and Moringa stenopetala L. Journal of Agricultural and Food Chemistry; 51(12): 3546-3553. 2003.
**[25] Vongsak, B., P. Sithisarn, and W. Gritsanapan**. Simultaneous Determination of Crypto-Chlorogenic Acid, Isoquercetin, and Astragalin Contents in Moringa oleifera Leaf Extracts by TLC-Densitometric Method. *Evidence-Based Complementary and Alternative Medicine*; 7:1-7. 2013.
**[26] Berkovich, L., G. Earon, I. Ron, A. Rimmon, A. Vexler and S. Lev-Ari.** *Moringa Oleifera* aqueous leaf extract down-regulates nuclear factor-kappaB and increases cytotoxic effect of chemotherapy in pancreatic cancer cells. *BMC Complementary and Alternative Medicine*; 13:212-217. 2013.
**[27] Okwari O. O., E. A. Alagwu, K. Dasofunjo, K. O. Okwari, and L. Obi.** Effect of Aqueous Leaf Extract of *Moringa oleifera* on Some Renal Function Indices of Rats. *International Journal of Pharma Sciences and Research*; 6(4): 777-782. 2015.
**[28] Leone, A., A. Spada, A. Battezzati, A. Schiraldi, J. Aristil and S. Bertoli.** Cultivation, Genetic, Ethnopharmacology, Phytochemistry and Pharma-cology of *Moringa oleifera* Leaves: An Overview.*Int. J. Mol. Sci*; 16: 12791-12835. 2015.
**[29] Vongsak, B., P. Sithisarn, and W. Gritsanapan**.Bioactive contents and free radical scavenging activity of *Moringa oleifera* leaf extract under different storage conditions. *Industrial Crops and Products*; 49: 419-421. 2013.
**[30] Coppin, J.** AStudy of the Nutritional and Medicinal Values of *Moringa oleifera* Leaves from Sub-saharan Africa: Ghana, Rwanda Senegal and Zambia. A thesis submitted to the Graduate School-New Brunswick Rutgers, The State University of New Jersey For the degree of Masters of Science. 2008.
**[31] Ganatra, T. H., U. H. Joshi, P. N. Bhalodia, T. R. Desai, and P. R. Tigar.** A Panoramic View on Pharmacognostic, Pharmacological, Nutritional, Therapeutic and Prophylactic Values of *Moringa oleifera* Lam. *IRJP*; 3(6): 1-7. 2012.
**[32] Tnvkv, P. and E. K. Elumalai.** Biofabrication of Ag nanoparticles using *Moringa oleifera* leaf extract and their antimicrobial activity. *Asian Pac J Trop Biomed*; 1(6): 439-442. 2011.
**[33] Sathyavathi, R., M. B. M. Krishna, and D. N. Rao**. Biosynthesis of Silver Nanoparticles Using *Moringa oleifera* Leaf Extract and Its Application to Optical Limiting. *J. of Nanoscience and Nanotechnology*; 10: 1-5. 2010.
**[34] Vibhute, S. K., V. S. Kasture, P. N. Kendre, and G. S. Wagh.** Synthesis of Silver Nanoparticles from *Moringa oleifera*: Formulation and Evaluation against *Cadidia albicans*. *Indo American Journal of Pharm Research*; 4(3): 1581-1587. 2014.
**[35] Kudle, M. R., K. R. Kudle, M. R. Donda, and M. P. Pratap Rudra.** Synthesis, characterization and antimicrobial activity of silver nanoparticles formed using *Moringa oleifera* lam gum. *Nanoscience and Nanotechnology*; 3(3): 45-48. 2013.
**[36] Langford, J.I. and A.J.C. Wilson.** Scherrer after sixty years: A survey and some new results in the determination of crystallite size. *J. Appl. Cryst*;**11**: 102-113. 1978.
[37] Hamouda, T., A. Myc, B. Donovan, A. Shih, J.D. Reuter, and Jr. JR. Baker, (2000). A novel surfactant nanoemulsion with a unique non-irritant topical antimicrobial activity against bacteria, enveloped viruses and fungi. *Microbiol Res*; 156: 1-7.
**[38] Dibrov, P., J. Dzioba, K. K. Gosink, and C. C. Häse.** Chemiosmotic mechanism of antimicrobial activity of Ag (+) in Vibrio cholerae. *Antimicrob Agents Chemother*; 46: 2668-70. 2002.
**[39] Dragieva, I., S. Stoeva, P. Stoimenov, E. Pavlikianov, and K. Klabunde.** Complex formation in solutions for chemical synthesis of nanoscaled particles prepared by borohydride reduction process. *Nanostruct Mater*; 12: 267-70.
[40] Sondi, I., and B. Salopek-Sondi, (2004). Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *J Colloid Interface Sci*; 275: 177-82. 1999.
**[41] Amro, N.A., L.P. Kotra, K. Wadu-Mesthrige, A. Bulychev, S. Mobashery, and G. Liu.** High-resolution atomic force microscopy studies of the *Escherichiacoli* outer membrane: structural basis for permeability. *Langmuir*; 16: 2789-96. 2000.
**[42] Danilczuk, M., A. Lund, J. Saldo, H. Yamada, and J. Michalik.** Conduction electron spin resonance of small silver particles. *Spectrochimaca Acta* Part a; 63: 189-91. 2006.