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BIOGENIC SYNTHESIS OF SILVER NANOPARTICLES BY SEDILITIZIA ROSMARINUS BOISS SHOOT EXTRACT AND ITS ANTIBACTERIAL ACTIVITY AGAINST MDR BACTERIA

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ABSTRACT:

In the current study, water was used as a solvent to extract biomolecules of the shoot of Sedilitizia rosmarinus plant to reduce silver nitrate to silver nanoparticles (AgNPs). The extract was filtered and left for 24 hours, two distinguished layers were formed, a green top layer called supernatant (sup) and a white precipitate layer named sediment (sed) layer, both layers were used separately in the biogenic synthesis of AgNPs. GCMS analysis was performed to find out the content of biomolecule compounds present in both layers (sup and sed). To characterize of AgNPs, various spectroscopic methods have been used: Ultraviolet-Visible spectroscopy (UV-vis), Fourier transform infrared (FTIR), powder X-ray diffraction (XRD), scanning electron microscope (SEM). Uv-vis results of AgNPs (supAgNPs and sedAgNPs) showed peaks at 420 and 425 nm respectively. FTIR results proved that the biomolecules present in both layers contributed to the reduction and capping of silver nitrate to AgNPs. XRD study demonstrated the crystalline nature of AgNPs manufactured in the current study with a face center cubic (FCC) structure. Spherical AgNPs shape with size ranging from 45-99, and 58-96 nm for supAgNPs and sedAgNPs respectively done by SEM microscope. Antibacterial activity of AgNPs shows a significant inhibition against multidrug- resistant bacteria (MDR) and concentrations. Staphylococcus aureus was recorded as the most susceptible bacteria with an inhibition zone diameter of 22 mm with a mean rank (3.45), followed by E.coli, Staphylococcus hominus, and Proteous mirabilis with mean rank 2.96, 2.21, and 1.39 respectively.

Keywords: Silver Nanoparticles, S. rosmarinus Aqueous Extract, Antibacterial Activity, MDR Bacteria.

I. INTRODUCTION

Nanotechnology defines as the science that deals with nanomaterial characterized with nanoscale, and it has many applications in different fields of science, like chemistry, biology, and biotechnology, etc. Nanomaterials acquired their role in many fields of science because it has numerous features make it significant due to it is large surface area compared to volume higher than in their bulk materials (Yagoob *et al*., 2020). Silver ion was recognized for a long time for about 4000 B.C.E., and it existed in nature, also it has economic importance such as gold and copper. Metallic silver possesses many applications in industry such as textiles, makeup, water purification, currency, food industry, and jewelry. In medicine, it has significance due to it is antibacterial properties allowing it used in burns and wound treatment. (Liang, *et al*., 2013, Barillo *et al*., 2014). Nanoparticles especially silver nanoparticles were synthesized nowadays for their characters as a result of it is large surface area to volume ratio and localized surface plasmon resonance. Moreover, different methods were used to synthesis silver nanoparticles were recorded including photochemical, Physical, and chemical, but these methods were expensive and harmful to humans and the environment. So biogenic methods preferred according to it is low cost and ecofriendly. Nanoparticles can be classified into:(1) 0 zero-dimensional (0D) measured in nanoscale in all dimensions example nanoshell, (2) one-dimensional (1D) characterized by 1-100 nm in size with one layer, it has different uses like solar cells and fiber optic system, (3) two-dimensional (2D) as in carbon nanotubes, (4) Three-dimensional (3D) as in cells fullerenes and quantum dots (Yaqoob et al., 2020). Biogenic synthesis can be defined as a method used in the synthesis of

nanoparticles such as silver nanoparticles using living organisms or their extracts like plants, fungi, bacteria, and algae, This method had gained attention in different fields of biology, microbiology, and biotechnology (Siddiqi *et al*., 2018).

From the time that antibiotics were discovered by Alexander Fleming in 1928 when his experiments revealed that fungus Penicillium notatum inhibits the growth of bacteria. Later bioactive compound was isolated and named penicillin, this antibiotic was still in laboratory experiments until the Second World War, there was an increasing need for antibiotics to treat wounded soldiers to protect them from death with gangrene. This discovery prompted many researchers to find new sources of antibiotics such as Selman Waksman, who obtained many new antibiotics from filamentous bacteria Streptomyces (Alani, 1990). But these antibiotics were still few compared with the development of bacterial resistance mechanisms against antibiotics, for example, only seven types of antibiotics were discovered for the period from 1936 to 1962, and nearly thirty years later, no new antibiotics were discovered, while the resistance of bacteria increased day by day. In addition, the incorrect use of antibiotics has led to the emergence of bacterial strains that are resistant to many antibiotics called Multi-Drug resistant bacteria (MDR) which resistant to many types of antibiotics. And here the idea of conflict between humans and antibiotics was established, as whenever humans developed new antibiotics, bacteria developed a new way to obstruct the action of these antibiotics. These bacteria can resist antibiotics in several ways including damage of electron transport chain, tear cell membrane, protein disruption DNA damage, prevent of proton efflux pump, inhibit biofilm formation, block protein synthesis, and reactive oxygen species (ROS) production (Baptista, *et al.,* 2018).

Sedilitizia rosemarinus Boiss. belonging to the family Chenopodiacene, distinguished by succulent, shrubby shoot articulated stems with leaves accumulate salts in their bodies. Distributed in eastern, Mediterranean regions Sudia Arabia, Syria, Iraq, and Iran. In Iraq, it's distributed in - North-west and south desert. In Basra, it is recorded in Um-Qasr, Zubair, and other adjacent regions. It is named shnan in ours and used to clean clothes for their high content of saponin. (Chakravarty, 1976). Also, it is used to help cook pea's seeds instead of using soda.

In this study, environmentally-friendly silver nanoparticles (AgNPs) were synthesized using an aqueous extract of *S. rosmarinus* as a reductant and capping agent. AgNPs were characterized by spectroscopy methods (UV-Vis, FTIR, XRD, and SEM) and evaluate it is ability to inhibit MDR human pathogenic bacteria.

II. MATERIALS AND METHODS

Preparation of Plant Extracts

The aerial part (shoot) of Sedilitiza rosmarinus plant was used to prepare an aqueous extract. The plant was obtained from the local market in a plastic sac washed with distilled water several times to remove dust particles. Grind into fine Particles using an electronic grinder. Fifty grams of plant powder were used to prepare aqueous extract using Soxhlet apparatus and 500 ml of DW as solvent. Extraction was done for six hours. Left to cool down at room temperature. The solution was filtered with No.1 filter paper. The filtrate was left in the refrigerator at 4^0C until the next day. GCMS was carried out to detect the composition of aqueous extract of biomolecules.

Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized by mixing 50 ml of Plant extracts with 450 ml of (1mM) silver nitrate in an Erlenmeyer flask. Heated to 80 ^{0}C for 2 hours in the water bath. Silver nanoparticles synthesized indicated by a color change of the solution from white or green to dark brown. Let cool down at room temperature in dark. Centrifuged at 3000 rpm for10 minutes. Pellets washed twice with double distilled water, left to dry. Kept at 4 ^{0}C in the refrigerator until use.

Characterization of Silver Nanoparticles

Characterization of silver nanoparticles (AgNPs) was done by spectroscopic methods include UV-visible spectrophotometer (Uv-vis), Fourier transform infrared (FTIR) to know the biomolecules included in reducing silver nitrate to AgNPs. X-ray diffraction (XRD) analysis was conducted to prove the crystalline structure of biogenic synthesized AgNPs. The size and shapes of AgNPs were accomplished using a scanning electron microscope (SEM).

Antibacterial Activity of Biogenic Silver Nanoparticles

Silver nanoparticles (AgNPs) were used as antimicrobial agents against Gram-positive and Gram-negative MDR human pathogenic bacteria by using the agar well diffusion method, Briefly, Petri dishes containing Mueller-Hinton agar were incubated with the bacterial inoculum (1 X $l0^8$) CFU spread on the plates using a cotton swab. Pits 6mm were done using a cork borer filled with 50µl of *S.rosmarinus* sup and sed aqueous extract (0.1g/ml) and AgNPs solutions in different concentrations (1000, 500, 250, 125, 63) µg/ml., plates were incubated at 37^0C for 24 hours, examined to detect antibacterial activity by measuring the diameter of the inhibition zone in (mm).

III. RESULTS AND DISCUSSION

Preparation of Plant Extract

Aqueous extract of *S. rosmarinus* was left in the refrigerator at 4^0C until the next day. The aqueous extract was distinguished into two separated layers, the upper layer was green supernatant and the lower layer was white sediment layer. The supernatant layer was named **sup** whereas **sed** refers to the second white sediment layer as shown in figure (1).

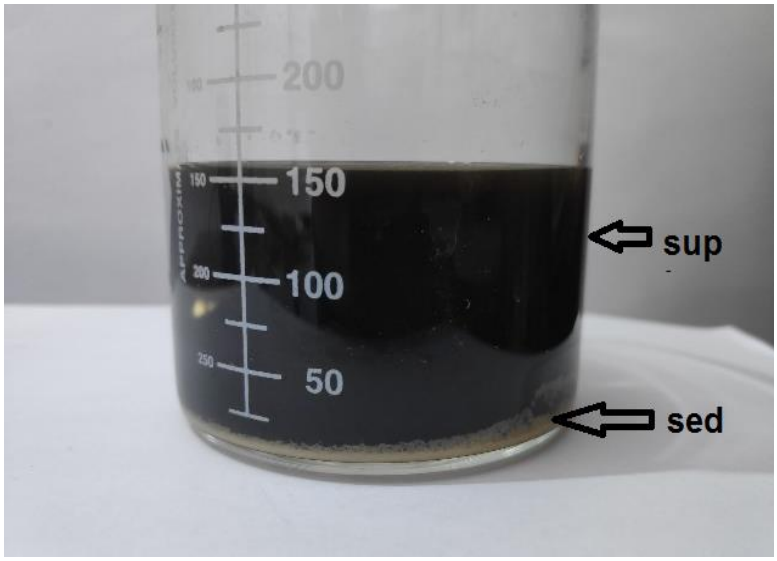
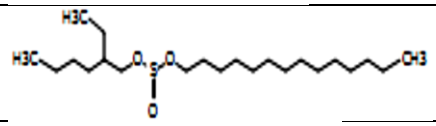
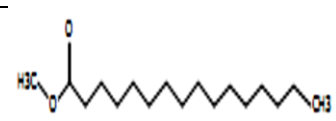


Figure 1. Aqueous extract of *S. rosmarinus*; sup (Supernatant layer); sed (sediment layer).

In order to detect the chemical composition of the two (**sup** and **sed**) layers of aqueous extracts GC / MS analysis were performed as in Table (1) and (2).

Table 1. GC/MS results of aqueous extract of *S. rosmarinus*; sup (Supernatant layer).

NO	Phytochemical compound	RT (mi)	Area%	Molecular Weight	Formula	Chemical Strucrure Chemical structure
1-	Methane, isocyanato	4.668	0.235	57.0513	C_2H_3NO	
2-	(2)-Sulfurous acid, dodecyl 2-ethylhexyl ester	16.541	0.33	362.6	$C_2OH_{42}O_3S$	
3-	Pentadecane	17.002	1.0596	212.41	C15H32	
4-	Hexadecanoic acid, methyl ester	21.652	6.197	270.5	$C_{17}H_4O_2$	

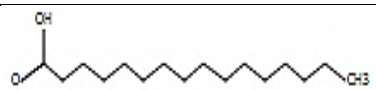
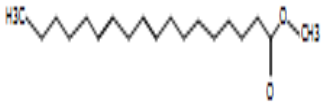
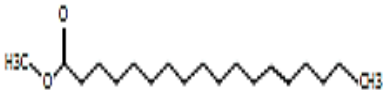
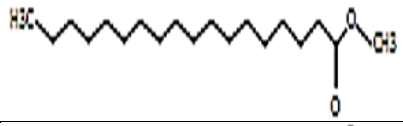
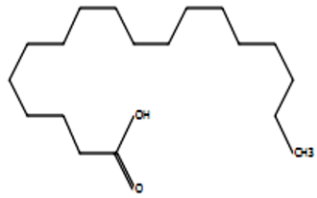
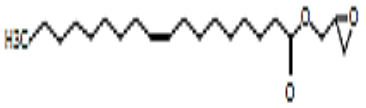
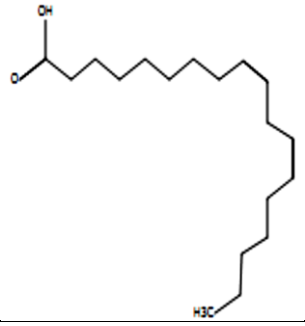
5-	n-Hexadecanoic acid	22.026	1.67	256.4241	$C_{16}H_{32}O_2$	
6-	9,12-Octadecadienoic acid, methyl ester	23.264	22.728	294.4721	$C_{16}H_{32}O_2$	
7-	13-Octadecenoic acid, methyl ester	23.322	15.62	296.5	$C_{19}H_{36}O_2$	
8-	Methyl stearate	23.541	3.15	298.5	$C_{19}H_{38}O_2$	
9-	Linoelaidic acid	23.773	19.64	280.4	$C_{18}H_{32}O_2$	
10-	Octadecanoic acid	23.94	2.62	284.5	$C_{18}H_{36}O_2$	
11-	Glycidyl palmitate	25.002	1.22	312.5	$C_{19}H_{36}O_3$	
12-	1,5,9,13-Tetradecatetraene	26.412	1.94	190.32	$C_{14}H_{22}$	
13-	Glycidyl oleate	26.448	1.443	338.5	$C_{21}H_{38}O_3$	
14-	9,12-Octadecadienoic acid	27.883	1.656	280.4455	$C_{18}H_{32}O_2$	

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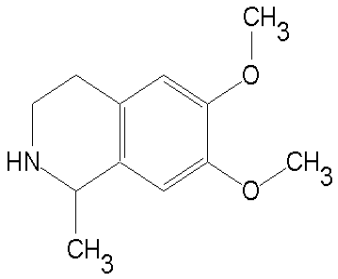
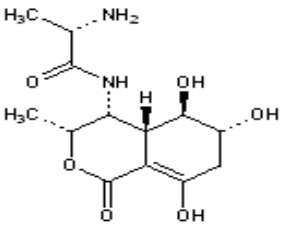
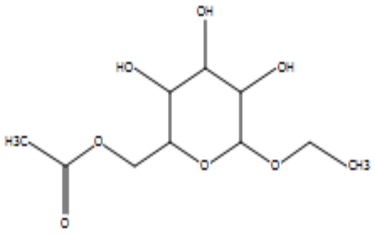
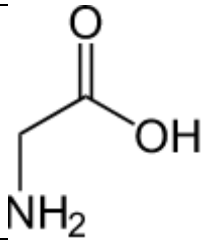
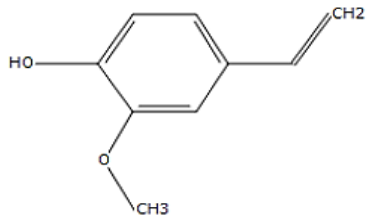
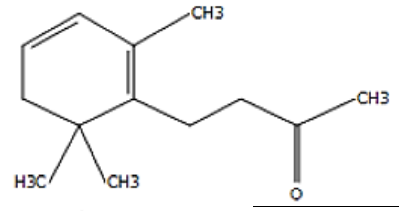
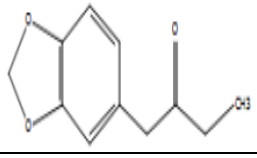
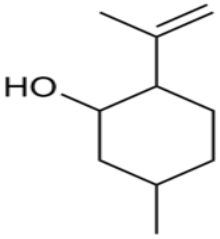
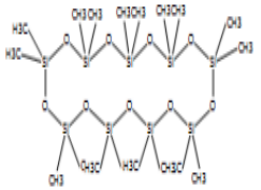
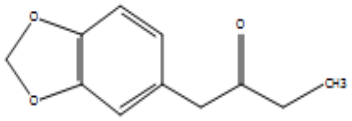
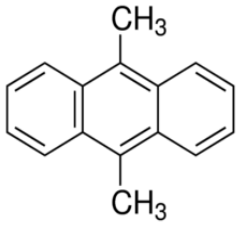
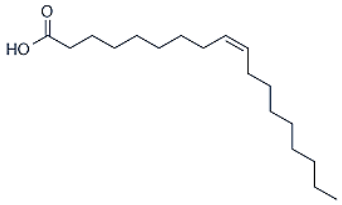
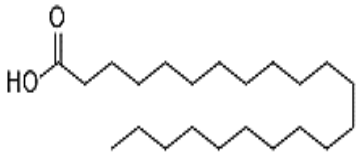
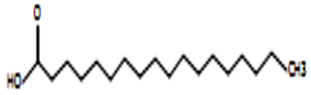
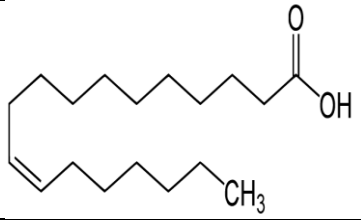
Table 2. GC/MS results of aqueous extract of *S. rosmarinus*; sed (Sediment layer)

NO	Phytochemical compound	RT (min)	Area %	Molecular Weight	Formula	Chemical Strucrure Chemical structure
1-	6,7-Dimethoxy-2- methyl-3,4-dihydro[1- D] isoquinolinium ion	5.223	11.84	206.26	$C_{12}H_{16}NO_2$	
2-	Actinobolin	5.531	93	300.31	$C_{13}H_{20}N_2O_6$	
3-	6-O-Acetyl-1-O-ethylmannopyranoside	5.903	2.96	250.25	$C_{10}H_{18}O_7$	
4-	Glycine	7.616	1.91	75.07	$C_2H_5NO_2$	
5-	2-Methoxy-4-vinylphenol $$ Phenol, 4-ethenyl-2-methoxy-	14.696	1.38	150.177	$C_9H_{10}O_2$	
6-	2-Butanone, 4-(2,6,6- trimethyl-1,3-cyclohexadien-1-yl)-	15.436	1.54	192.3	$C_{13}H_{20}O$	
7-	2-Butanone, 1-(1,3-benzodioxol-5-yl)-	15.707	1.41	72.11	C_4H_8O	

8-	Isopulegol	19.58	1.3	170833	$C_{10}H_{18}O$	
9-	Cyclononasiloxane	20.715	1.07	667.4	$C_{18}H_{54}O_9Si$	
10-	2-Butanone, 1-(1,3-benzodioxol-5-yl)-	22.077	17.13	192.21	$C_{11}H_{12}O_3$	
11-	2-methoxy-9,10-dimethylanthracene	23.102	4.24	236.31	$C_{17}H_{16}O$	
12-	Oleic Acid	23.754	24.13	282.5	$C_{18}H_{34}O_2$	
13-	Docosanoic acid	27.202	0.25	340.6	$C_{22}H_{44}O_2$	
14-	Stearic acid	23.9	9.2	284.5	$C_{17}H_{35}CO_2H$	
15-	cis-Vaccenic acid	24.998	1.43	282.461	$C_{18}H_{34}O_2$	

Tables (1,2) shows the results of the GC-MS analysis of the compounds present in both the sup and sed aqueous extracts of the S.rosmarinus plant and the highest area % compounds registered in the sup extract were as follows: 13-Octadecenoic acid, methyl ester; and Linoelaidic acid with area % was (15.62, 22.728)% respectively, whereas the lowest area % was methane, isocyanato (0.235)%. Also, the following chemical compounds were found in sed aqueous extract, the highest area % compounds were 6,7-dimethoxy-2-methyl-3,4-dihydroisoquinolin-2-ium; 2-Butanone, 1-(1,3-benzodioxol-5-yl); Oleic Acid; and Stearic acid represented by (11.4, 17.13, 24,13, 9.1) % respectively. Docosanoic acid was recorded as the lowest area with 0.25 %. In a similar study conducted in Iran by Davabi et al.(2020), they reported the chemical constituent of S.rosmarinus methanol extract were determined using GCMS analysis with compounds as follows: [N,N-Dimethylglycine; Benzenedicarboxylic acid; β-Sitosterol; 2-Methoxy-4-vinylphenol; n-hexanedioic acid; and 17-(5-Ethyl-6-methylheptan-2-yl)], and this partly in agreement with current study were also the same compounds were recorded such as (2-Methoxy-4-vinylphenol and n-*hexanedioic acid also recorded in this study*, but other compounds differed, and this may be due to the

geographical divergence, soil quality and the different environmental conditions in addition to the difference of the solvent used (Elsharkawy *et al.*, 2017).

Synthesis of Silver Nanoparticles

The results of the present study showed that the addition of both aqueous extracts sup and sed of S.rosmarinus to the silver nitrate solution led to a change in the color of the reaction mixture from green for sup and white for sed extract to brown indicates the formation of AgNPs as shown in figure (2). And this may be due to the action of a phenomenon that led to the formation of silver nanoparticles called surface plasmon resonance (Yu, *et al*., 2019).

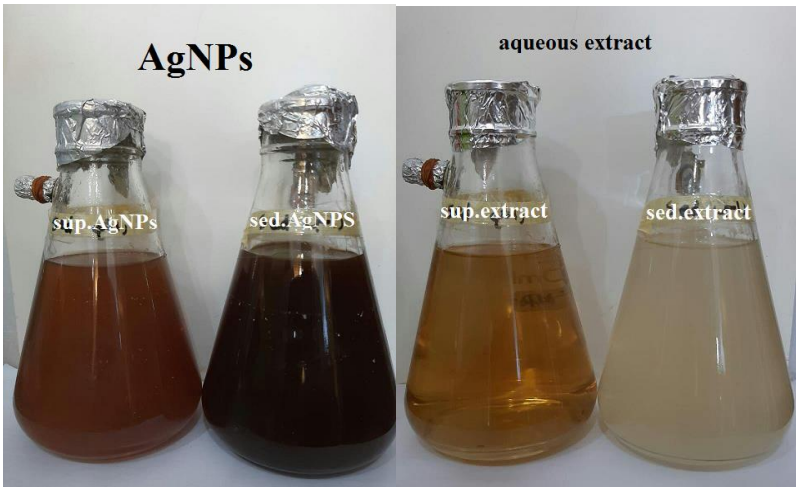


Figure 2. Color changed of sup and sed aqueous extracts of *S.rosmarinus* prove the formation of AgNPs.

Characterization of Silver Nanoparticles

For the purpose of characterization of synthesized AgNPs the following analyses were done.

UV-Visible Spectroscopy

Use a UV-vis analysis to confirm the production of AgNPs formed using *S.rosemarinus* sup and sed aqueous extract. Colloidal solution obtained from mixing the plant extract with a 1mM silver nitrate solution. Spectra were recorded it had the strong and sharp peaks for both types supAgNPS and sedAgNPs at 420 and 425 nm respectively compared with the spectra of sup and sed aqueous extracts only (without silver nitrate) as in figure (3). It is believed that colloidal solution formed due to the phenomenon of the plasmon resonance of silver quantum (Vanaja *et al.*, 2014). Furthermore some researchers tended to find a relationship between the wavelength of the UV-vis spectrum and the size of AgNPs like the study of Sengottaiyan *et al.* (2016) they found that the spectrum at 420 nm had AgNPs with size less than 25nm.

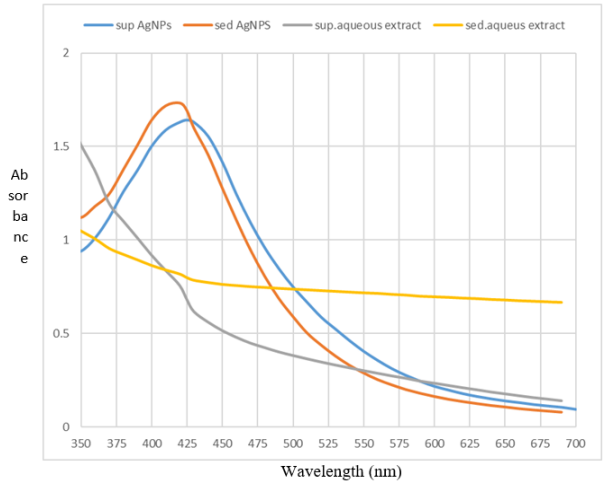


Figure 3. UV-visible of supAgNPs, sedAgNPs, sup, and sed of *S.rosmarinus* aqueous extract

Fourier-transform Infrared Spectroscopy (FTIR) Analysis of AgNPs

Fourier transform infrared (FTIR) analysis was used in the present study to identify biomolecules in sup and sed aqueous extract of *S.rosmarinus* that have a role in reducing, capping, and stabilizing of AgNPs synthesized by these extracts. The FTIR spectrum of sup extract recorded the following peaks at: 3468, 3439, 3417, 2939, 1631, 1471, 1404, 1409, 1338, and 1235 cm^{-1}. These spectra were shifted out to new peaks after used in the reduction of silver nitrate to silver nanoparticles as follows: 3487, 3477, 3477, 3446, 2920, 1618, 1467, 1367, and, 1317cm^{-1}. Some peks was recorded in supAgNPs spectrum only and these peaks were: 3263, 3057cm^{-1} bands. Likewise also aqueous sed extract was recorded peaks at: 3412, 2922, 1641, 1554, 1471, 1456, 1417, 1373, and 1323 cm^{-1}. Also these peaks were shifted out when used in AgNPs synthesis such as 3456, 3423, 2956, 2924, 1639, 1624, 1548, 1413, 1375, 1315, and 1219 cm^{-1}. Also, some peaks: 3091, and 3062 cm^{-1}were recorded in sedAgNPs spectrum only figure (4). The FTIR spectra recorded in AgNPs spectra only, and not found in the spectra of the sup and sed aqueous extract, these peaks were indicated that the formation of silver nanoparticles (Corciova and Ivanescu, 2018). In the case of AgNPs spectra, there was strong absorption peak about 3400 cm^{-1} shifted toward lower values at which is believed to be caused by the silver ions associated with hydroxyl or amine groups. The same is the case in the range of 1600 cm^{-1} for plant extract which decreased to about 1500 cm^{-1} in AgNPs spectra due to the binding of the type C=O and C-N of amide I protein which act as reductant, capping, and stabilization of AgNPs (Islam *et al*., 2019).

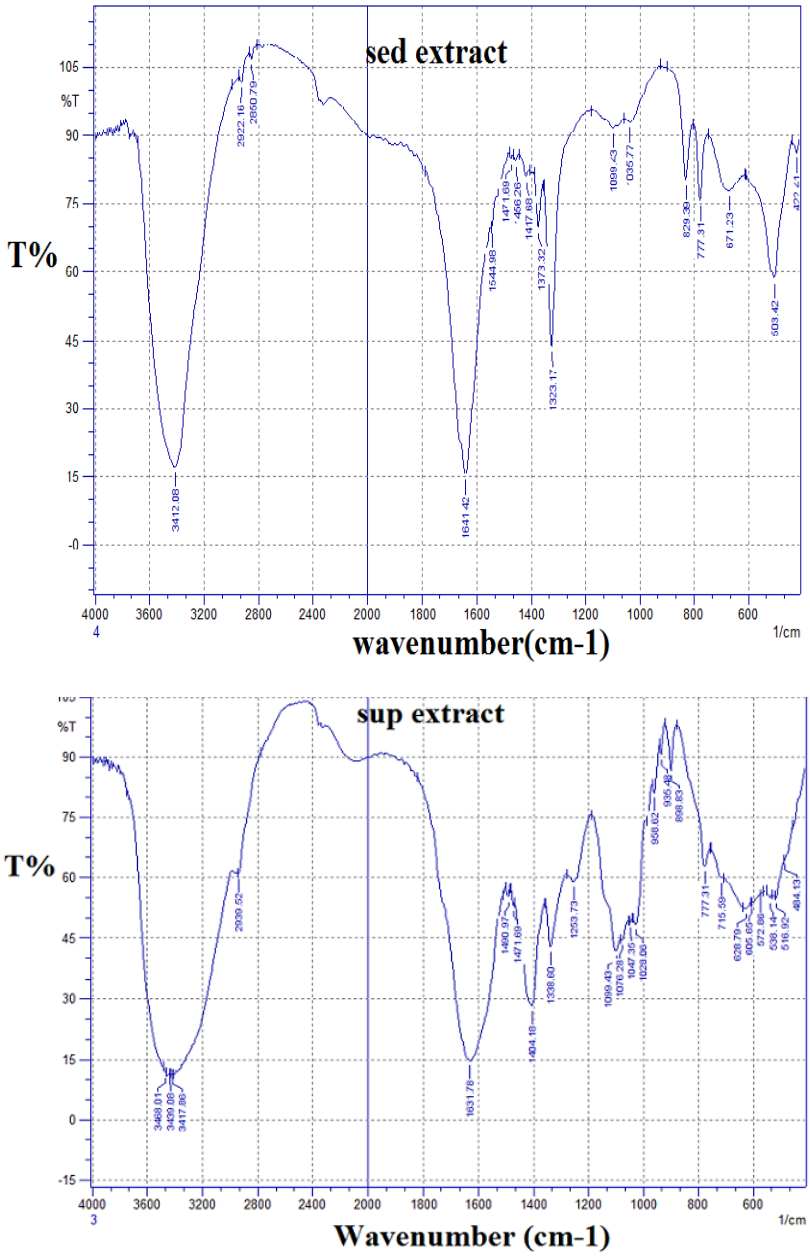


Figure 4. FTIR spectra of sup and sed extracts and supAgNPs and sedAgNPs

X-Ray Diffraction Analysis (XRD)

X-ray diffraction analysis (XRD) of AgNPs of both supAgNPs and sedAgNPs was used to prove the crystal nature of synthesized AgNPs. The XRD results of AgNPs recorded the main peaks of supAgNPs at (2Ɵ) 28.1616, 32.5174, 46.6273 identical to (110), (111), and (211) planes respectively. While sedAgNPs also listed highest peaks at (2Ɵ) 14.7501, 24.3972, 30.0037, 38.3521 conforming to (111), (220), (222), and (231) planes respectively, corresponding to the silver crystal as shown in figure (5). The stronger peaks prove that silver is the main component in the production of AgNPs, whereas lower peaks were recorded from the XRD results of both types of AgNPs, some researchers reported similar results such as Annadalakshmi et al., (2016) they believed that the presence of these lower peaks resulted from to the biomolecules found in the aqueous extract which used in the preparation of AgNPs that adhere to the surface of AgNPs

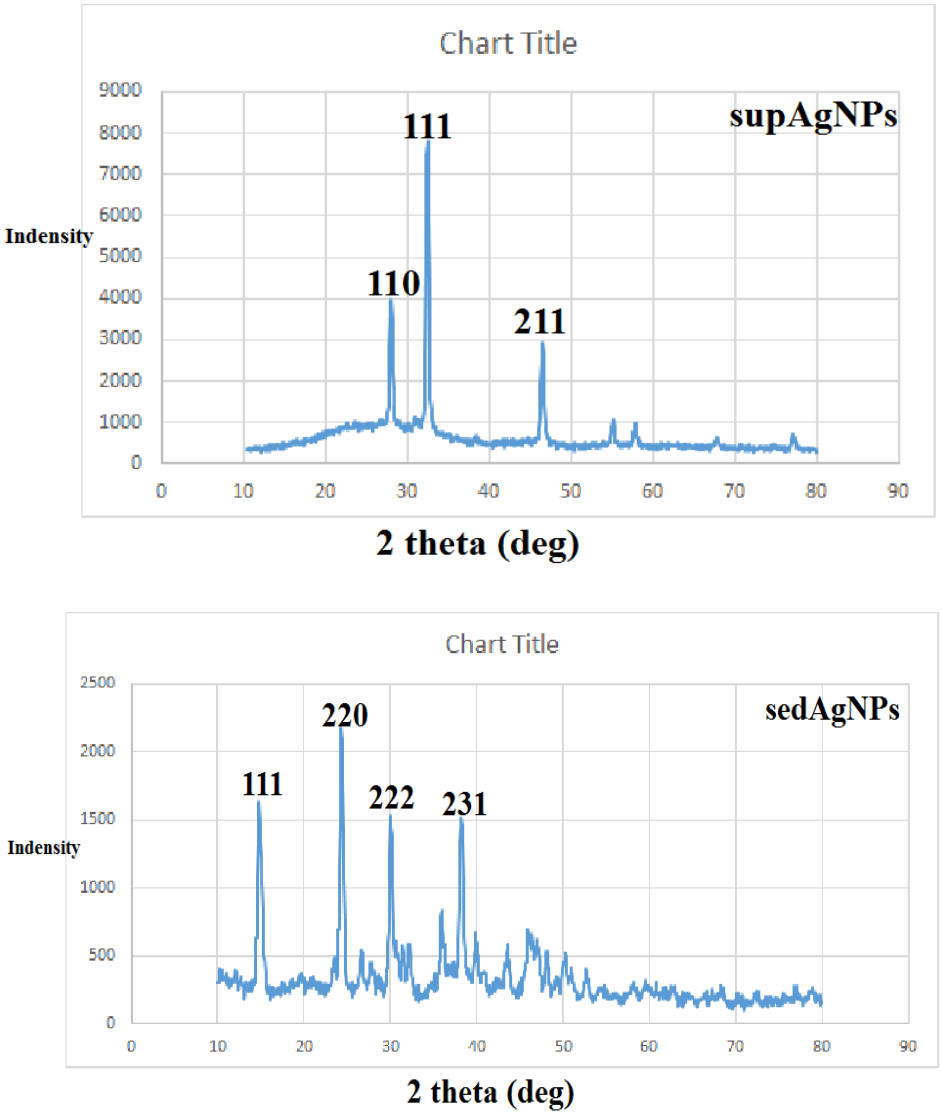


Figure 5: XRD results of supAgNPs and sedAgNPs.

Scanning Electron Microscope (SEM) Analysis

Scanning electron microscope (SEM) analysis was conducted to know the shape and size of AgNPs synthesized by two aqueous extracts (sup and sed) of S.rosmarinus shoot. The results showed that the shape of supAgNPs was spherical with dimensions ranging from 45-99nm. As for sedAgNPs were also spherical in shape and with size from 58-96nm figure (6). This is similar to many studies such as Garibo *et al*. (2020) when they tried to use the aqueous extract of Lysiloma acapulcensis in the synthesis of AgNPs. They recorded that the shape of nanoparticles was globose, and with a size ranging from 1.2-62nm.

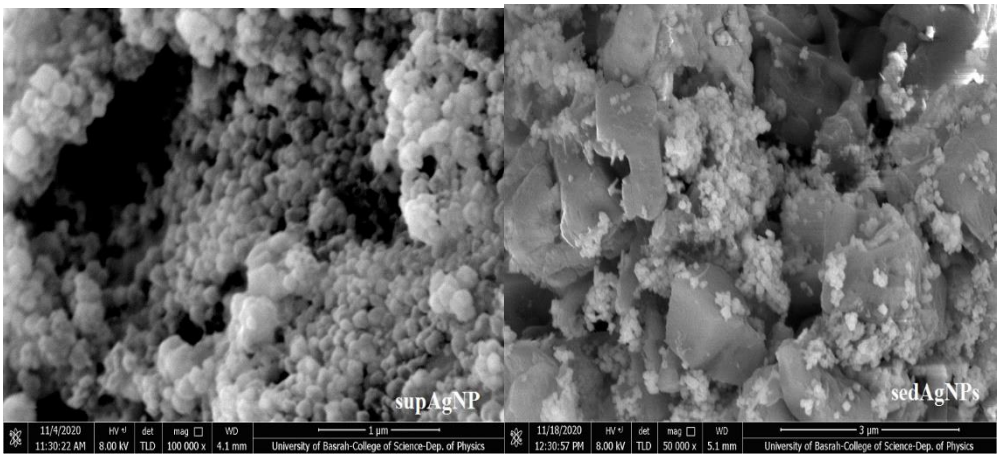


Figure 6. SEM image of silver nanoparticles synthesized by using sup and sed of *S.rosmarinus* aqueous extract

Antibacterial Activity of Biogenic Silver Nanoparticles

To determine the antibacterial activity of silver nanoparticles, inhibition zone diameter was measured around the wells containing AgNPs with concentrations (1000, 500, 250, 125, and 63 µg/ml), In addition to silver nitrate and aqueous extracts (sup and sed). The results recorded significant differences with chi-square with 119.146 when treating bacteria resistant to antibiotics (MDR). Furthermore there was a significant differences between the concentration of AgNPs, silver nitrate, and aqueous extract in inhibiting the bacteria, as well as the presence of a significant difference in the response of bacteria to the AgNPs concentrations, the results recorded that AgNPs with concentration of 1000µg/ml was more significant in inhibition of MDR *Staphylococcus aureus* bacteria with an inhibition zone diameter of 22 mm with mean rank (3.45), followed by *E.coli*, *staphylococcus hominus*, and *Proteous mirabilis* with mean rank 2.96, 2.21 and 1.39 respectively (Table 3). The mechanism of AgNPs' action in inhibiting bacterial growth is not well known and it is believed that cellular proteins become inactive after being treated with nanoparticles. After entering into the bacterial cell via cell walls, it will inhibit enzymes as well as may produce hydrogen peroxide, causing the death of the bacteria cell. Heavy elements are known to be interacting with proteins and then inhibiting cellular metabolism and then cell death. Silver nitrate sources only release Ag+ but the high potency of silver nanoparticles is attributable to the release Ag0 when dissolved in water (Das et al. 2011).

Also based on other studies the mechanism of action of silver nanoparticles in inhibiting the growth of bacteria by prevent it to form the biofilm. Also after AgNPs entering the cytoplasm of the bacterial cell it will work in two directions, the first is to inhibit cell wall synthesis and upsetting ribosome, thus block protein synthesis. The second trend in making silver nanoparticles is the formation of reactive oxygen species (ROS) which results in suppression of enzyme production, in addition to DNA degradation and cell death (Garg *et al*.,2020). The current study recorded that the shape of AgNPs synthesized by aqueous extract (sup and sed) of S.rosmarinus plant was spherical, according to Achary *et al*., (2018) this shape of nanoparticles can inhibit bacteria more than other shapes like rod nanoparticles.

Table 3. Antibacterial activity of AgNPs synthesized by *S.rosmarinus* aqueous extracts (sup and sed)

Bacteria		E.coli	Proteus mirabilis	Staphylococcus aureus	staphylococcus hominis
Antimicrobial agent	Concentration/mean rank	2.96	1.39	3.45	2.21
supAgNPS	1000 µg/ml	20	14	22	15
sedAgNPs		17	15	13	0.0
supAgNPS	500 µg/ml	19	13	19	14
sedAgNPs		15	14	11	0.0
supAgNPS	250 µg/ml	17	13	14	12
sedAgNPs		0.0	11	11	0.0
supAgNPS	125 µg/ml	16	11	13	11
sedAgNPs		0.0	0.0	11	0.0
supAgNPS	62.5 µg/ml	0.0	0.0	11	11
sedAgNPs		0.0	0.0	11	0.0

$AgNO_3$	1mM	0.0	0.0	11	11
Sup aqueous extract	150 mg/ml	0.0	0.0	0.0	0.0
Sed aqueous extract	150 mg/ml	0.0	0.0	0.0	0.0
Chi-Square	119.146				

Use chi-square analysis for nonparametric data (Friedman Test – Chi-square) below the probability level $P \leq 0.05$.

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