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Green Synthesis of Silver Nanoparticles Using three Leaf Extracts of Ficus carica L. and evaluate its antibacterial activity against MDR Escherichia coli isolates.

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Abstract:

In this study, three *Ficus carica* L. leaf extracts were used to reduce silver nitrate to silver nanoparticles (aqueous, ethanol and methanol) extracts. These nanoparticles were then characterized by different spectroscopic and microscopic analyses Uv-Vis spectroscopy, Fourier Transform-Infrared (FTIR) analyses, and Scanning Electron Microscope (SEM). These synthesized silver nanoparticles were tested as an antibacterial agent against multidrug resistant (MDR) of *Escherichia coli* strains. The results showed that prepared AgNPs inhibited all (MDR) of *Escherichia coli* strains at most concentrations used in the study**.**

Key words: Silver nanoparticles; *Ficus carica* L. leaf extracts; Antibacterial activity; *Eschricia coli*, MDR.

Introduction

Nanoscience is the Science that study materials at the nanometer level, is interested in studying their size and composition, and compare the emergence of atoms, molecules, or bulk materials. Whereas nanoparticles: Nano objects in three dimensions measured in nanometers (Jeevanandam, *et al*.2018). Green synthesis of nanoparticles using plant extracts have

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attention in the last years over than chemical methods which is simple and performed in one step reaction, easy to scale up, increasing biocompability of the resulting biosynthesized nanoparticles with normal tissues due to lower toxicity, these plant extracts when they using information of nanoparticles enhancing silver nanoparticles (AgNPs) to colloidal stability, cost effective, and ecofriendly (Park,2014). According to Munger *et al*. (2014) when they test nanoparticles on human with two doses (100µg/day and 480 µg/day) they did not found any toxicity or inflammatory problems. Plants contain many of secondary metabolites such as protein, phenols, saponins and, flavonoids acts as a good source in synthesis of silver nanoparticles because they can reduce silver nitrate to silver nanoparticles, capping and stabilizing these nanoparticles instead of using chemical methods which it uses hazardous and high coast chemicals (Masum *et al*.,2019).

Unprogramed using of antibiotics represent a growing problem which induces bacteria to moderate it is antibiotics resistance lead in the emergence of multidrug resistance bacteria (MDR), which is resistant to more than one antibiotic resulting in an increase the morbidity and mortality of the most pathogenic bacteria (Franci *et al*.,2015).

Nanoparticles were founded to be alternative to antibiotics to control of pathogenic bacteria because it has a high potential ability to inhibit MDR bacteria (Ankanna,*et al*.2010). Silver has always used as an antimicrobial agent in the past due to low toxicity. Therefore, it used against both Gram- negative and Gram- positive bacteria (Prabhu and Poulose ,2012).

Ficus carica L. belong to the family Moraceae. The part eaten is the soft fruits which are rich in nutrients such as vitamins, carbohydrates, minerals, organic acids and phenolic compounds. (Jeong and Lachance.,2001; Veberic *et al.*,2013). Also, it has an important role in traditional medicine, especially leaves, which it used in the treatment of cough, colic treatment, indigestion, loss of appetite, prevention of nutritional anemia, anthelmintic, irritant potential, and in treatment of tuberculosis (Mawa *et al.,* 2013).

In this study, three solvents (water, ethanol, and methanol) were used to extract the active compounds in *F. carica* leaves and then used to reduce silver nitrate to silver nanoparticles. Furthermore, synthesizes AgNPs were analyzed with different analytical ways, and evaluate their antibacterial activity against some of MDR *Escherichia coli* strains.

Materials and methods

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Preparation of plant extracts

The plant dried leaves of *Ficus carica* L. were washes with distilled water, and left to dry at room temperature. Leaves grounded to fine powder and extracted in three ways:

1- Aqueous extract

Fifty grams of leaves powder were boiled in 500 ml of distilled water in reflux condenser for 6 hours; the extract was cooled to room temperature, then filtered with No.1 whatman filter paper. Extract was stored at 4°C until used in reducing silver nitrate.

2- Ethanol extract

Grounded leaves were put in thimble and extracted with 250 ml of ethanol as a solvent in soxhlet apparatus for 24h. The extract was evaporated at room temperature to yield ethanol extract.

3- Methanol extract

The same procedure in the previous paragraph was followed but replaced ethanol 95%with absolute methanol.

Synthesis of silver nanoparticles

Synthesis of silver nanoparticles was done according to Shankar *et al*., (2004) with some modifications. Briefly, fifty ml of each plant extracts (aqueous, ethanol, and methanol) were added to 450 ml of 1mM of AgNO3 solution. Mixed in Erlenmeyer flask and heated to 90°C for two hours. The color changed from pale yellow to dark brown indicate the formation of silver nanoparticles. The solutions left to cool at room temperature and were centrifuged at 3000 rpm. Then washed three times with double distilled water.

Antibacterial Activity of silver nanoparticles

Antibacterial Activity of synthesized silver nanoparticles were evaluated against human pathogenic MDR *Escherichia coli*, strains by agar well diffusion technique as described by Elbeshehy, *et al.* (2015) with little changes. Briefly, Petri plates containing Mueller – Hinton agar medium were swabbed with 10^6 cfu/ml of tested bacteria. Wells of 6 mm diameter were bored aseptically, 100µl of the silver nanoparticles from 1000 to 63 µg/ml were loaded on agar well, and plates were incubated at 37°C for 24 hrs. The inhibition zone was measured in mm.

Characterization of silver nanoparticles

Silver nanoparticles were characterized using Uv-Visible spectrophotometer analysis, Fourier Transform Spectroscopy (FTIR) analysis, and Scanning Electron Microscope (SEM) analysis.

a- Uv-Visible spectrophotometer analysis

Silver nitrate were reduced to AgNPs using three types of *F.carica* leaf extracts (aqueous, ethanol, and methanol) .The reduction was monitored using UV-VIS spectrophotometer , and their absorbance were recorded at 350-800nm.

b- Fourier Transform Spectroscopy (FTIR)

Infrared spectra of compounds prepared as KBr tablet were recorded using a device of Type FTIR- 84005 – SHIMADZU, Country of origin Germany and in the region (500−4000 cm-1) at room temperature; spectra were recorded at the University of Basra / College of Education for Pure Sciences.

c- Scanning Electron Microscope (SEM) analysis

SEM (Scanning Electron Microscope) analysis was done using Leo 1455vp (Germany) machine. Gold coating was used for sputtering to conduct the samples.

Results: -

Silver nanoparticles were done using leaf extracts of *Ficus carica* (aqueous, ethanol and methanol) extracts to reduce silver nitrate to silver nanoparticles were indicated by color changed of solution from pale yellow to dark brown for the previous extracts, and all of these extracts were found able to reduce silver nitrate to silver nanoparticles (fig.1) .

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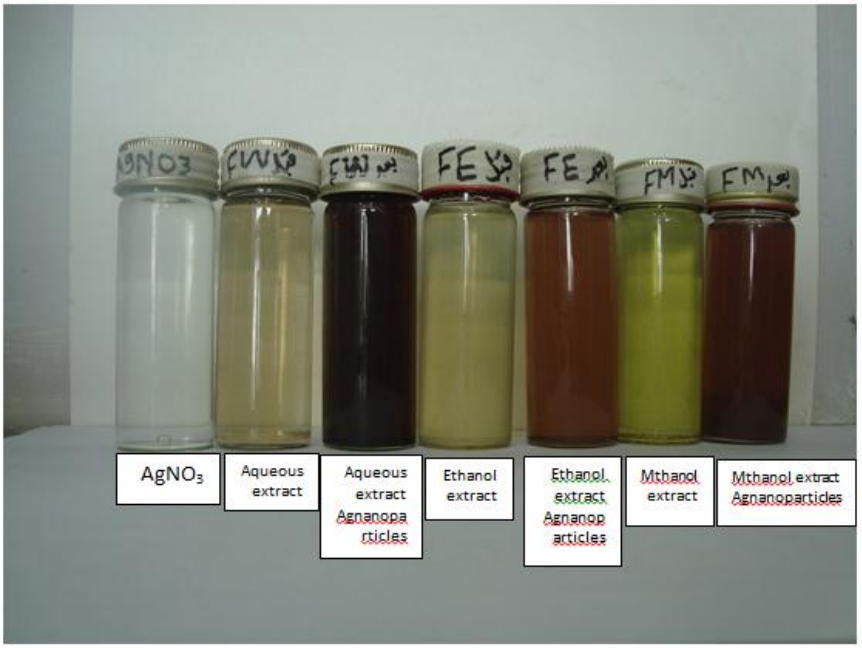

Fig. 1: Color change indicates the formation of silver nanoparticles.

Characterization of synthesized the formation of silver nanoparticles:

a-Uv-visible spectrophotometer analysis:

The aqueous, ethanol, and methanol extracts solutions changed from pale yellow color to dark brown (Fig. 1), when mixed with solution of silver nitrate .Color changed indicated the formation of AgNPs. These nanoparticles recorded (surface Plasmon resonance) peak at (425, 550) nm for aqueous, ethanol, and methanol AgNPs respectively (Fig 2).

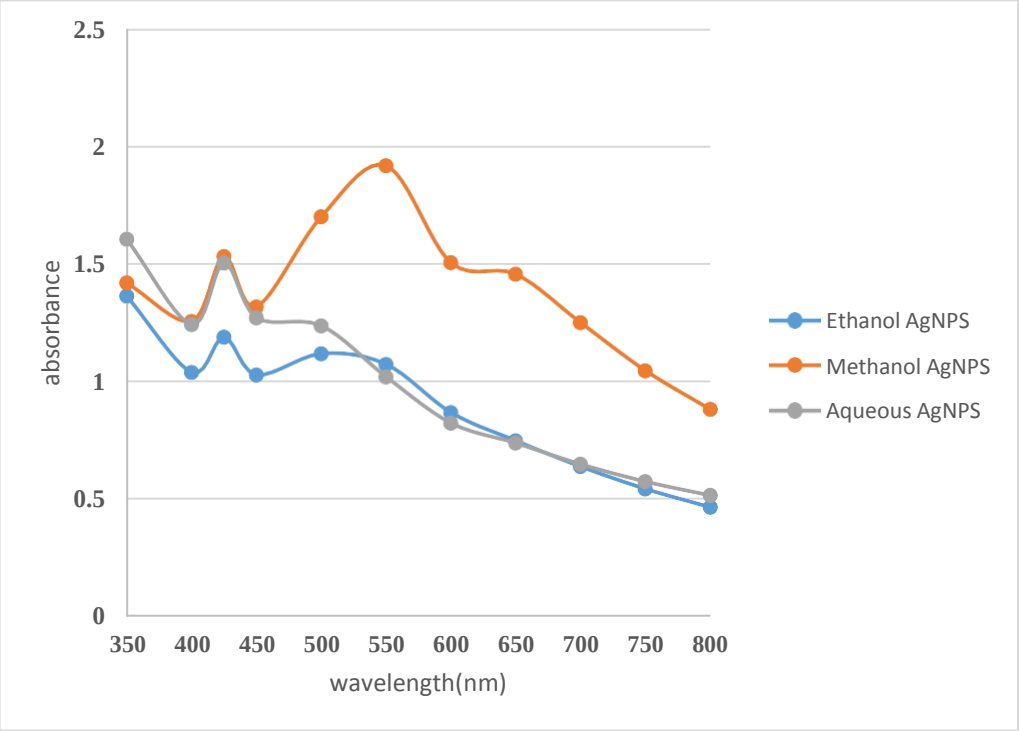

Fig. 2 results of UV-VIS analysis.

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b-Fourier Transform Spectroscopy (FTIR) analysis:

FTIR was done to prove the role of plant extract as reductant, capping, and stabilizing agents in synthesis of AgNPs and the functional groups of all three *F.carica* leaves extractsAgNPS. The results of FTIR analysis recorded broad spectra at: 34441, 2920,2850,1649, and 1539 cm^{-1} for AgNPs mediated with aqueous extract, whereas 3423,2920,1734,and 1651 cm^{-1} for AgNPs mediated with ethanol extract, and 3396,2922,2850,1734,1718,1672, 1651, 1566, and1544cm^{-1} for AgNPs mediated with methanol extract (Fig. 3,4,and 5).

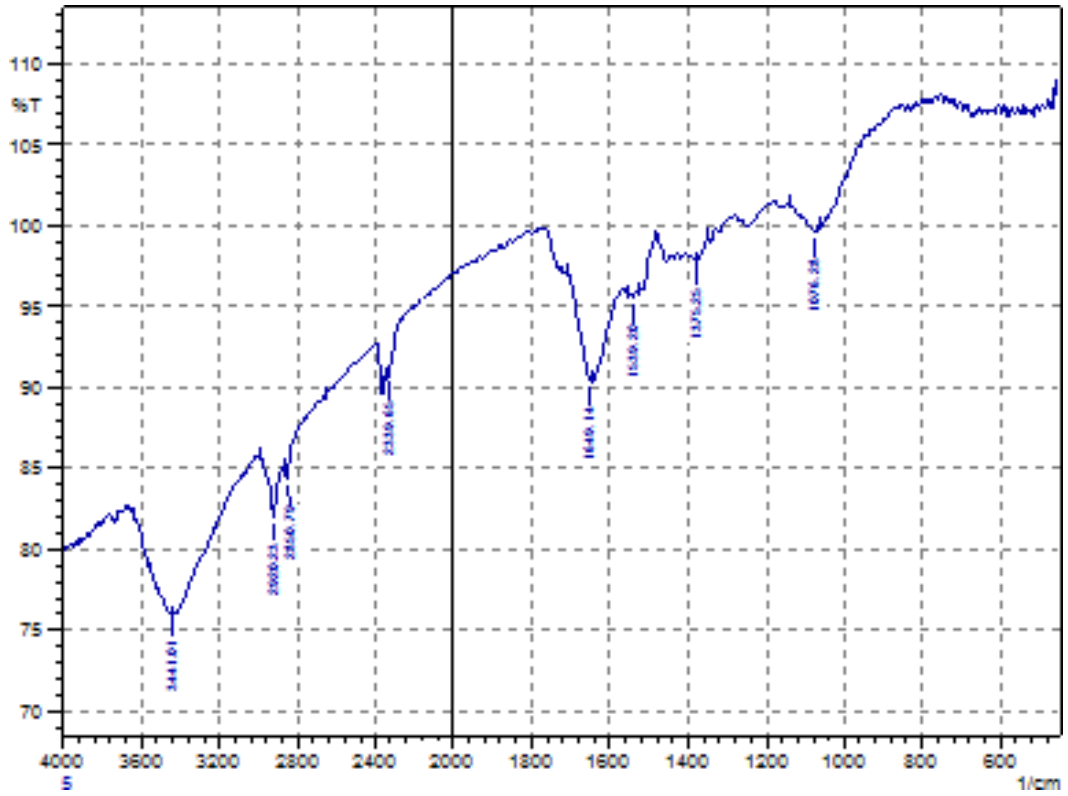

Fig. 3 FTIR of nanoparticles use aqueous extract.

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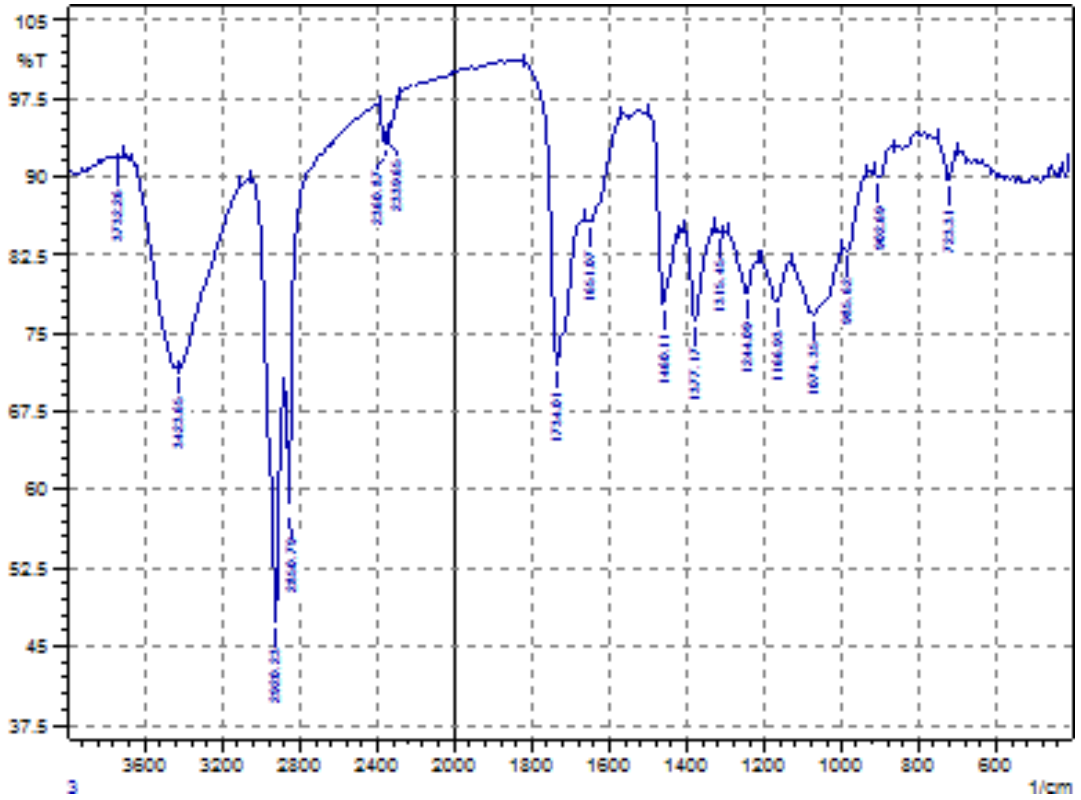

Fig. 4 FTIR of nanoparticles use ethanol extract.

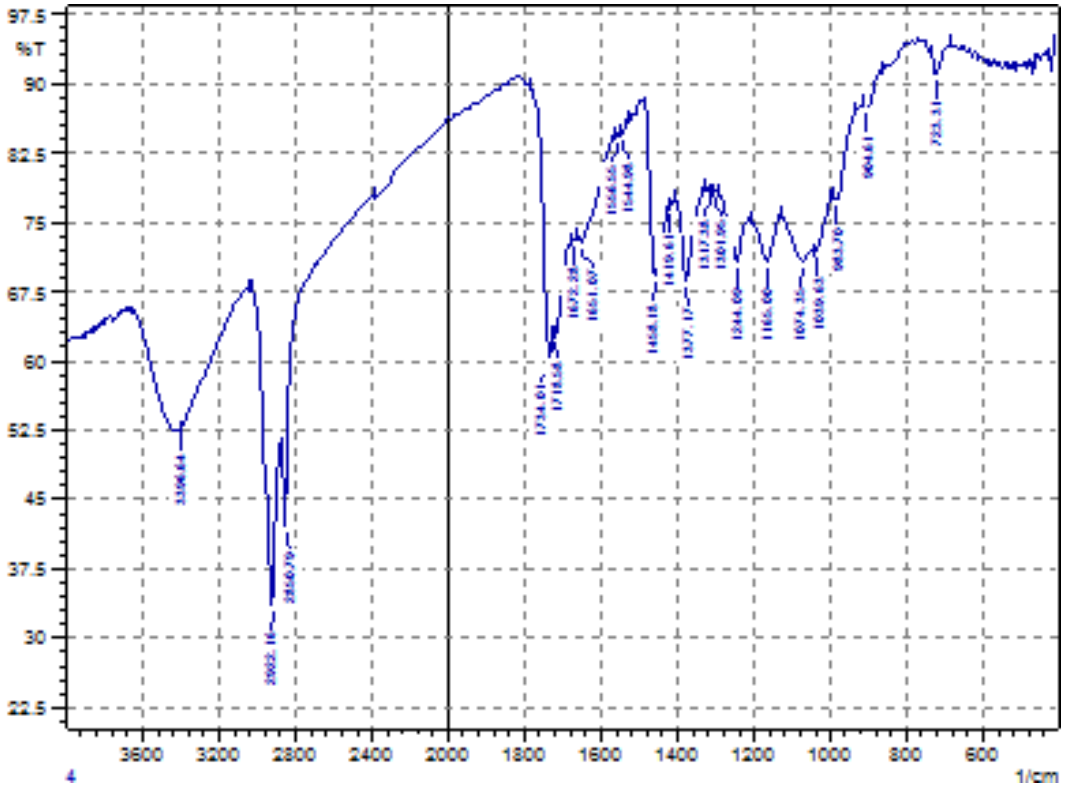

Fig. 5 FTIR of nanoparticles use methanol extract.

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c-Scanning Electron Microscope (SME) analysis:

The SEM of silver nanoparticles synthesized using aqueous, ethanol and methanol leaf extracts of *F.carica* recorded the size and shape of nanoparticles were spherical in shape and had diameter ranged 46-93 for aqueous extract whereas 48-89 for ethanol extract, and 27-60 nm for methanol extract (fig.6-8).

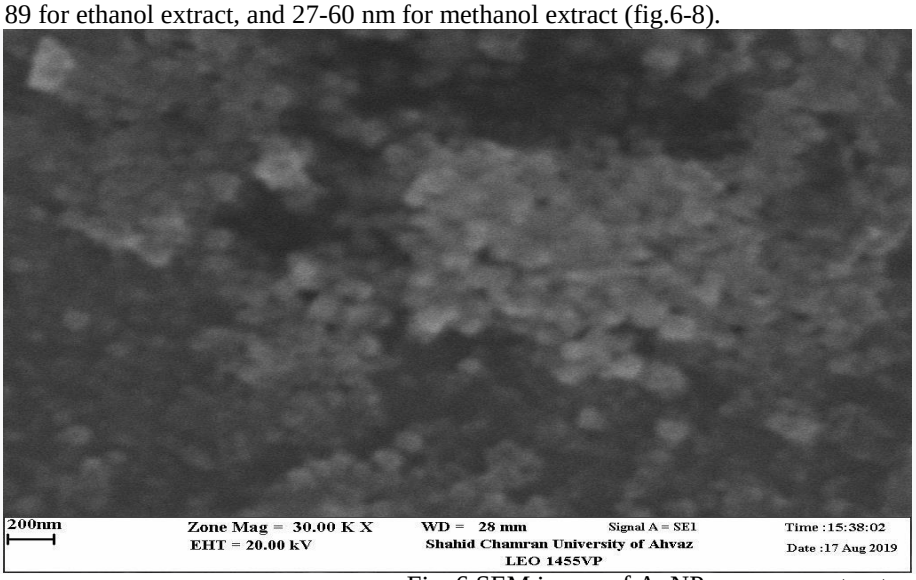

Fig. 6 SEM image of AgNPs aqueous extract.

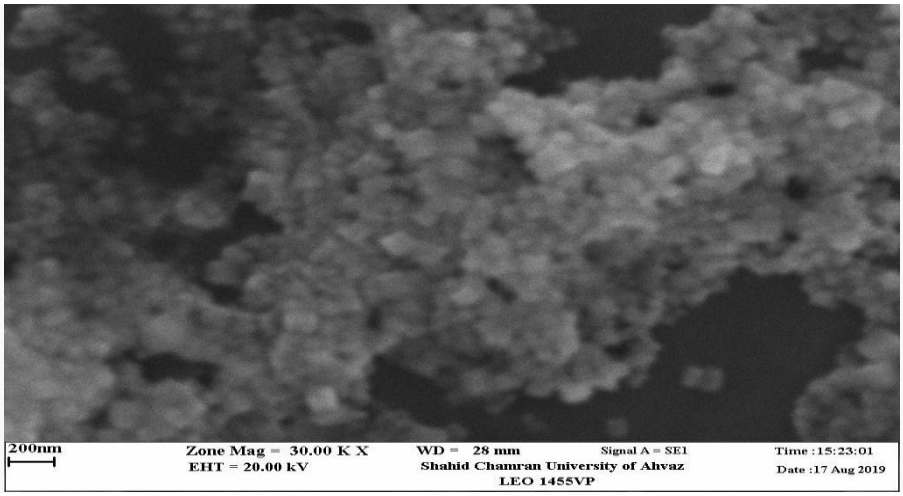

Fig.7 SEM image of AgNPs ethanol extract.

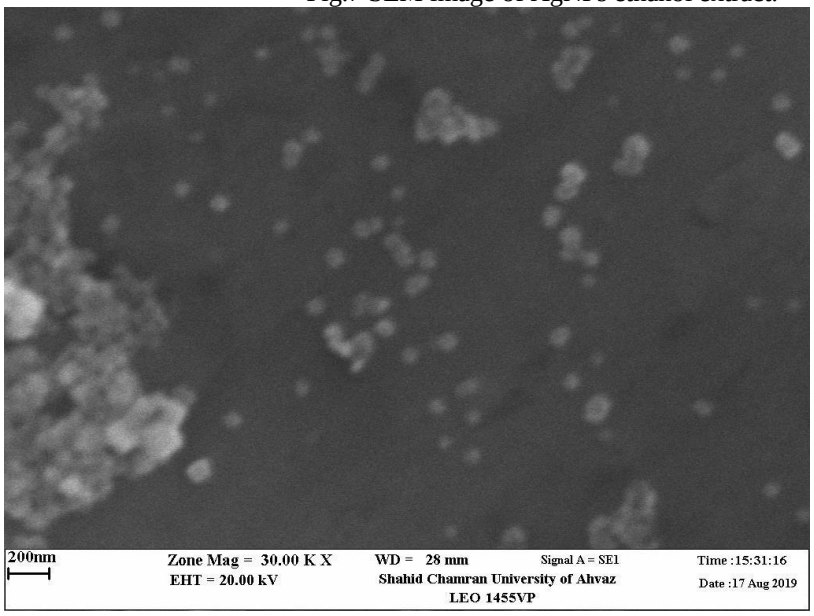

Fig. 8 SEM image of AgNPs methanol extract.

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Antibacterial activity of silver nanoparticles:

The antibacterial activity of silver nanoparticles was founded to inhibit all tested bacteria at most of concentrations for three extracts used for synthesis silver nanoparticles (Table 1) (Fig 9).

Table (1): Antibacterial activity of silver nanoparticles of *Ficus carica* leaf extracts. (Inhibition zone in mm).

Bacteria	AgNPs aqueous extract					AgNPs ethanol extract					AgNPs methanol extract				
AgNPs Concentration µg/ml	1000	500	250	125	63	1000	500	250	125	63	1000	500	250	125	63
E1	22	22	21	19	17	28	24	19	15	14	24	24	23	21	19
E2	19	15	15	14	12	20	15	14	13	11	15	15	15	14	14
E3	13	12	12	11	11	12	12	12	12	11	13	12	11	11	10
E4	16	16	15	14	13	17	16	14	13	12	16	15	15	13	11
E5	19	19	15	14	14	16	12	11	11	10	15	15	14	14	14
E6	13	13	12	12	12	15	15	15	14	13	14	14	14	14	13
E7	14	12	11	11	-	18	18	17	17	15	19	19	18	18	17
E8	14	12	11	11	-	18	18	17	17	15	19	19	19	18	17
E9	11	11	10	10	-	18	18	17	15	11	18	18	18	11	11
E10	12	11	11	11	11	19	19	17	14	15	17	17	16	16	16

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Table (2): Antibiotics susceptibility test (AST) of MDR E.coli strains.

Antibiotics	**strains MDR *E.coli***									
	E1	**E2**	**E3**	**E4**	**E5**	**E6**	**E7**	**E8**	**E9**	**E10**
Ampicillin		R			R	R		R	R	R
Amoxilin-clavulanic acid									R	R
Piperacilin	R		R				R			
Piperacillin/Tazobactam			R							
Cefuroxime								R		R
Cefuroxime Axetil		R						R	R	R
Cefoxitin					R				R	R
Cefixime		R							R	R
Ceftazidime	R	R	R		R	R			R	R
Ceftriaxone		R			R			R	R	R
Cefepime	R		R							
Aztreonam /monobactam	R		R							
Gentamicin									R	
Ciprofloxacin			R			R		R	R	R
Levofloxacin			R							
Tetracycline			R				R			
Nitrofurantoin		R								
Trimethoprime		R	R				R	R	R	R

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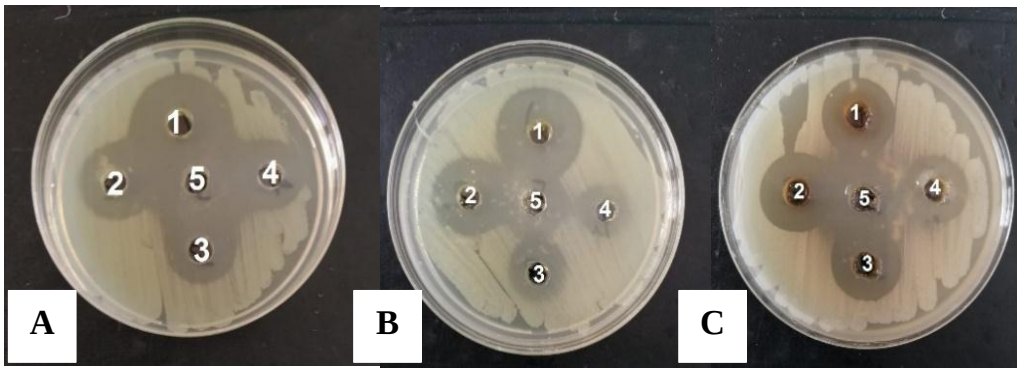

Fig. 9 Antibacterial activity of synthesized silver nanoparticles using A: aqueous extract; B: ethanol extract; C: methanol extract; 1:(1000 µg/ml); 2:(500 µg/ml) ; 3:(250 µg/ml); 4:(125 µg/ml) ; 5:(63 µg/ml).

Discussion

The biosynthesis of nanoparticles have been the focus of recent times. Plants possess secondary metaboliets, which have a major role in the manufacture of these nanoparticles, especially silver nanoparticles. These secondary metabolites were used to synthesis nanoparticles because these compounds, such as proteins, polysaccharides, and flavonoids, have the ability to reduce silver nitrate to AgNPs, capping and stabilizing these particles (Ahmed *et al*.,2016).In the present study, three extracts were used to synthesized silver nanoparticles(aqueous, ethanol, and methanol) of *Ficus carica* leaves by adding them to(1mM) silver nitrate solution. The formation of AgNPs was indicated by the color changed of the solution from pale yellow to dark brown. The formation of these nanoparticles can be tested by spectroscopic and microscopic analysis (Ayad, *et al.,* 2019). The use of biological methods in synthesizing silver nanoparticles using plant extracts is eco-friendly, low-cost and non-toxic compared with the chemical methods for synthesis of these nanoparticles. When these chemical compounds were used to reduced and stabilized silver nitrate, these compounds were known to be toxic, expensive and harmful to the environment. (Abdel-Aziz *et al*.,2014).

The ultraviolet spectrum of silver nanoparticles made using three *F.carica* leaf extracts (aqueous, ethanol, and methanol) were shown in the figure (2). Change the solution color from pale yellow to dark brown because of surface plasmon resonance absorption in AgNPs at 425 nm fig(3,4).Aldebasi *et al.* (2018) recorded maximum peak at 423nm when they used aqueous leaf extract of *F.carica* for synthesized AgNPs . The variations in UV-Vis were recorded by many authors, such as Saware *et al*.(2014) the UV-Vis peak of AgNPS of *Ficus bengalensis* was at 280 nm, and 310 nm recorded by (Siddiqi *et al*.,2018).Plasmon resonance of AgNPs indicate formation of these particles, it exhibit a strong absorption band in visible region, and it responsible for color changed of the solutions (Khandelwal *et al.,* 2010).

Fourier transform infrared spectroscopy(FTIR) analysis was used to determine the functional biological groups of *F.carica* leaf extracts (aqueous, ethanol, and methanol) used to reduced silver nitrate to AgNPs ,and the capping of these nanoparticles fig (3-5).FTIR measurements of AgNPs synthesized by the previous leaf extracts recorded different absorption peaks at values 3441, 2920, 2850, 1649, 1539 cm^{-1} for AgNPs synthesized using aqueous extract, 3423, 2920, 2850, 1734, 1651 cm^{-1} for AgNPs synthesized using ethanol extract, and 3396, 2922, 2850, 1734,1718, 1672, 1651,1556, 1544 cm^{-1} AgNPs synthesized using methanol extract. Bands at 3441, 3423, 3396 cm^{-1} for the previous extracts correspond to O-H stretching which indicate the presence of flavonoid, alcohol and phenols (Zafar *et al*., 2018), and that may indicates the binding of silver ion with hydroxyl or amine (Masum *et al.*, 2019). The values 2920, 2992, 2850 cm^{-1} represent C-H indicate the primary amine group. Similarly, peaks at 1651, 1649,1556,1544,1539 cm^{-1} responsible of N-H bonds indicates the presence of secondary amines (Zafar *et al*., 2018).Primary and secondary amine characteristic of protein and enzymes proteins or enzymes responsible for the reduction and capture of silver ions(Aldebasi *et al*.,2018).

Scanning electron microscope was used to know the shapes and sizes of nanoparticles made using *F.carica* leaf extracts (aqueous, ethanol, and methanol) in the formation of silver nanoparticles. In this study, the size of AgNPs mediated with aqueous extract was ranging from 46-93 whereas 48-89 for AgNPs synthesized using ethanol extract and 27-60nm for AgNPs synthesized with methanol extract. In the present study, there were records of minutes larger than 100 nm and this was also found in previous studies such as Sriraum and Pandidurai (2014), they record size of AgNPs between 100-500nm, Naik et al.(2018)? recorded AgNPs size was 580nm, and Kumar and Sinha (2003) founded size of AgNPs in range of 207-293nm. These formed sizes can be explained as due to the capping of nanoparticles by proteins (Warisnoicharoen *et al*,2011; Aldebasi *et al.*2018), or highly agglomerate nanoparticles resulting in aggregation of nanoparticles during sample preparation(Ayad *et al*.,2019).

Silver nanoparticles synthesized in this study were had antibacterial activity against all MDR *E.coli* bacterial strains in different ratios and variable according to type plant extract used in production of AgNPs and to bacterial strain.(Tabe l1), for example AgNPs synthesized with ethanol showed the highest inhibitory action against E1strain, followed by AgNPs synthesized using methanol, and then AgNPs prepared with aqueous extract. Whereas antibacterial

activity of AgNPs mediated with methanol extract had highest inhibition zone against E3, E7, E8 and E9. Also, antibacterial activity correlated somewhat with a -resistance of bacteria to antibiotics table (2), this is clear with strain E1 that recorded more sensitive to AgNPs mediated with extracts, and E9 more resistant strain to antibiotics showed lowest inhibition zone. This may be a result of these bacteria have developed mechanisms to resist anti-bacterial substances, including AgNPs. Several hypotheses have been proposed to explain the highest antibacterial activity of silver nanoparticles. AgNPs act against Gram-negative bacteria in three mechanisms, first these particles attach to bacterial cell membrane causing disturb of its functions resulting in loss of permeability and respiration, second silver nanoparticles can enter to inside of bacterial cell, and attack DNA. The last is AgNPs release silver ions which known to be had bactericidal effect (Singh *et al*.,2008). Sondi and Sondi (2004) recorded that silver nanoparticles had an effective bactericidal causing damaged of *E.coli* bacteria by formation pits in the cell wall of that bacteria, and AgNPs were accumulated in the bacterial membrane causing increase in permeability, therefore death of bacterial cell.

Conclusions

Leaf extracts of *Ficus carica* L. were a good source of plant metabolites, which had an important role in reducing silver nitrate to silver nanoparticles, and these AgNPS had a good antibacterial against MDR bacteria.

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