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The First Scientific Conference of the Faculty of Science Sirte University Environmental Pollution and Challenges of Environmental Sustainability (EPESC), Oct. 14, 2025

Green synthesis of AgNPs from *Viburnum tinus* plant and some biological applications on the extracts

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Keywords: anti-inflammatory; Nanoparticles; plant extract; *Viburnum tinus.*

Abstract: This study investigated the biological activities of *Viburnum tinus* L. extracts and their silver nanoparticles (AgNPs). Leaves and stems were collected from the Al–Jabal Al–Akhdar region, Libya, and extracted using two solvents: water and ethanol. The biosynthesized AgNPs were characterized using Scanning and Transmission Electron Microscopy (SEM and TEM), revealing near-spherical particles with average sizes of 16.3 nm and 13.0 nm for the leaf- and stem-derived nanoparticles, respectively.

The extracts and their corresponding AgNPs were evaluated for antibacterial activity against a panel of bacterial species and for anti-inflammatory potential. The results demonstrated that the silver nanoparticles significantly enhanced the bioactivity of the original plant extracts. The AgNPs exhibited superior antibacterial efficacy and a more potent anti-inflammatory effect compared to the crude extracts alone.

Introduction

Silver nanoparticles (Ag NPs) are metallic silver nano particles in the range of 1–100 nm. The broad-spectrum antimicrobial of silver encourages its use in biomedical applications, water and air purification, food production, clothing, and numerous household products (Chernousova & Epple, 2013). There is a great interest in Ag NPs for medical applications compared with the bacterial resistance to other antibiotics. So far, there are no conclusive reports on the development of bacterial resistance to Ag NPs (Panacek et al., 2018). The most common methods to synthesize Ag NPs are chemical reduction and physical method (Mashwani et al., 2018). For example, using strong reducing agents (borohydride or hydrazine) reduce the silver nitrate solution, laser ablation method or ball milling method to prepare Ag NPs. However, those traditional approaches involve the use of hazardous solvents, chemicals, toxic reagents, power-wasting equipment and poor size control (Ge et al., 2014; Park, 2014 & Bhattarai et al., 2018). Silver nanoparticles were considered bio-compatible, but chemical synthesis methods may still lead to the toxic chemical species absorbed on the surface (Paiva-Santos et al., 2021),Therefore, researchers have been persistently searching for more economical, environmentally friendly and safe Ag NPs synthesis with natural products. Green synthesis seems to be a potential strategy (Gour & Jain, 2019). *Viburnum tinus* plant, the laurustinus, laurustine or laurestine, is a species of flowering plant in the family *Adoxaceae*, native to the Mediterranean area of Europe and North Africa (Hartley, 2020).

Experimental

Experimental site

Production of Nanoparticles and antibacterial applications were conducted in the central laboratory of the Chemistry Department, Omar Al-Mukhtar University. The SEM and TEM analysis and anti-inflammatory activity were performed in scientific way for Viral and Microbiological studies lab- Al waha, 10 th- Nasr city- Egypt

Chemicals

1 M Silver nitrate ,98% ethanol, agar.

Samples preparation

The leaves and Stems of the studied plant were separated and washing several times with distilling water. The samples then dried in dark and dry place. Then the samples were grinded by mortar and stored in polyethylene bottles until analysis.

Preparation of Viburnum tinus extracts

10 g of powder plant dissolved in 100ml of distilled water then boiled for 40 min at 60 °C, for aqueous extracts, same ratio and method used for alcoholic extract. The extracts were cooled to room temperature, and connected to the vacuum pump device, after that filtered by filter paper (Salman et al.,2022).

Nanoparticles synthesis:

A mixture (silver nitrate and aqueous extract of plant) was prepared in a 10:1 ratio and a 50 ml solution was made. The solution is covered with aluminum foil and left at room temperature until the color of the mixture changes from yellow to dark brown. The samples are mixed in a centrifuge for 20 minutes until the solution is suspended. 5 ml of distilled water is added to the precipitate, mixed again, and the process is repeated (Gavhane et al., 2012).

Characterization of silver – studied plant nanoparticles

The nanoparticles synthesized in this study were identified by different methods including (SEM and TEM) analysis (M Ali et al., 2016) The morphology size and shape of the AgNPs synthesized were analyzed using SEM, Figures (1-2) and TEM in Figures (3-4).

Anti-inflammatory Studies:

Preparation of erythrocyte suspension:

Blood from healthy volunteers (3 ml) was collected in tubes containing heparin and spun at 3,000 rpm for 10 minutes to separate the red blood cells. The cells were then washed with saline and adjusted to a 40% concentration using a special salt solution (phosphate buffer at pH 7.4) that mimics the body's fluids. This solution was made by dissolving specific amounts of sodium phosphate salts and sodium chloride in water. The prepared red blood cell suspension was then ready for use in experiments (Aidoo et al., 2021).

Hypotonicity induced hemolysis:

To assess the extract's effects, researchers prepared solutions in varying concentrations (100-1000 µg/ml) using both distilled water (hypotonic) and a balanced salt solution (isotonic). These solutions were then placed in centrifuge tubes, two per concentration level. For comparison, additional tubes contained either just the solvent (distilled water) or a standard reference solution (200 µg/ml indomethacin). To assess the effect of an extract on red blood cell lysis, red blood cell suspensions (0.1 mL) were incubated with the extract for 1 hour at room temperature (37°C). Hemolysis was then measured by quantifying hemoglobin release at 540 nm using a spectrophotometer. Complete hemolysis by distilled water served as a positive control (100% hemolysis). The percentage inhibition of hemolysis by the extract was subsequently calculated.

% Inhibition of hemolysis = 1-((OD2-OD1)/ (OD3-OD1))*100

Where:

OD1 = absorbance of test sample in isotonic solution

OD2 = absorbance of test sample in hypotonic solution

OD3 = absorbance of control sample in hypotonic solution (Sharma et al 2013)

Antibacterial activity:

This study investigates the antibacterial activity of plant extracts from (plant leaves and stems). The extracts were tested both before and after treatment with silver nitrate. The bacterial strains used for testing were ***Escherichia coli*** and ***Salmonella*** (a Gram-negative bacterium) and ***Staphylococcus aureus*** (a Gram-positive bacterium). The method involved spreading the extracts at different concentrations and measuring the inhibition zones to determine antibacterial efficacy (Oussalah et al., 2007).

Results and discussion

SEM and TEM analysis

The detailed imaging and size analysis of Ag nanoparticles synthesized using both leaves and stems reveal that the nanoparticles are nearly spherical. For *Viburnum tinus*

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leaves, the particle size 16.3 nm, the size of the nanoparticles was 13.0 nm for *Viburnum tinus* stems. The nanoparticles were successfully detected and characterized, showcasing their spherical shape and consistent size

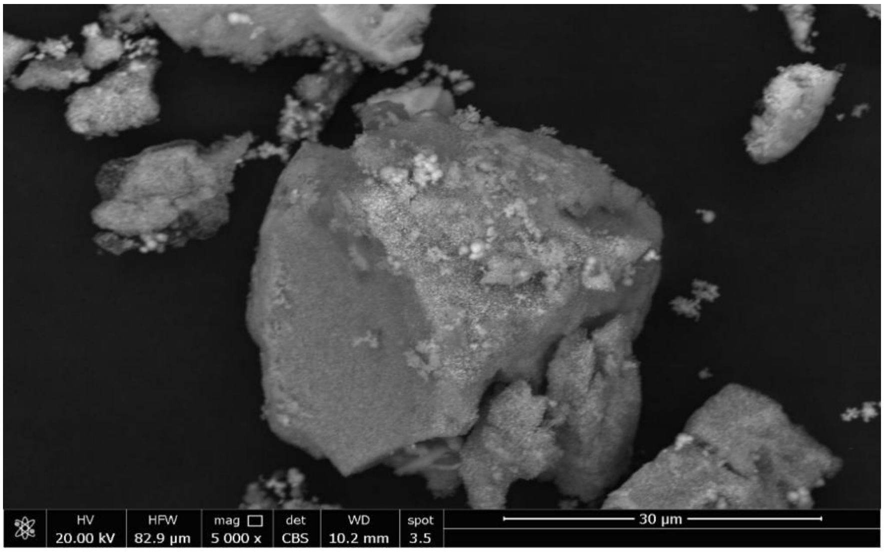

Figure (1): SEM analysis of nanoparticles of Viburnum tinus leafs

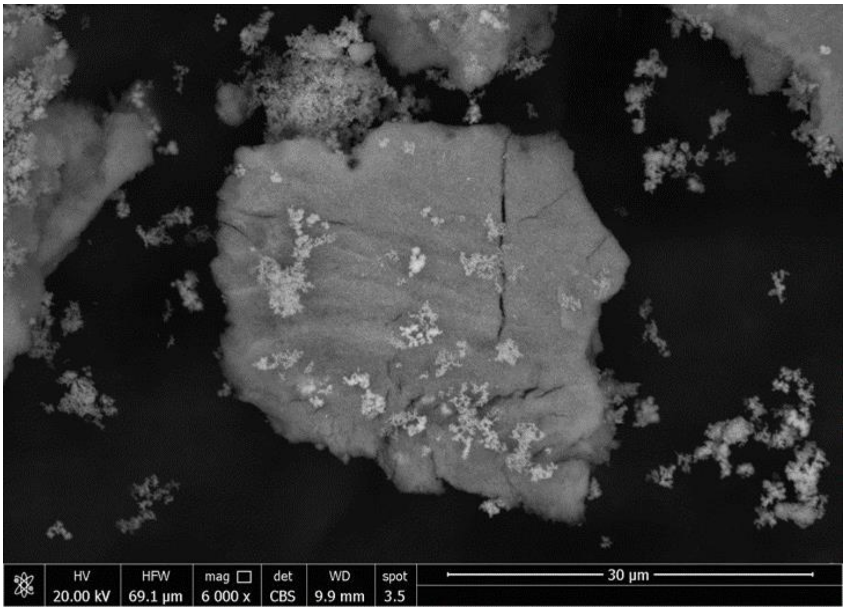

Figure (2): SEM analysis of nanoparticles of Viburnum tinus stems

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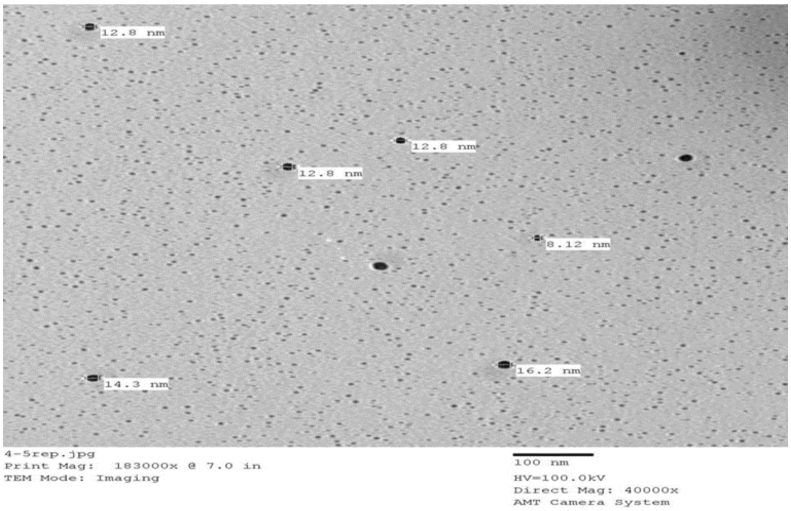


Figure (3): TEM analysis of nanoparticles of Viburnum tinus leafs

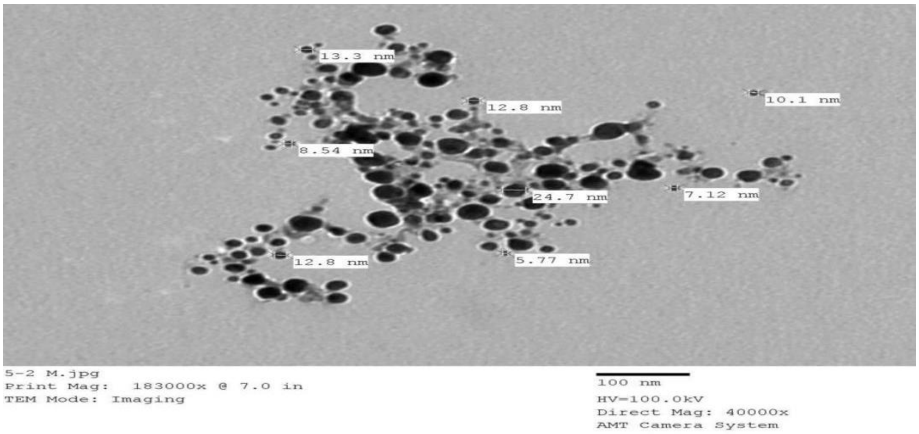


Figure (4): TEM analysis of nanoparticles of Viburnum tinus stems

Anti-inflammatory activity

The anti-inflammatory properties of the studied plant extracts were compared with the standard compound indomethacin, aceclofenac, etoricoxib, and aspirin. The values of Hemolysis % in the Tables of (1-4) are indicating that the compound with less Hemolysis Inhibition % results in good inhibitory activity. Among the standard compounds, responds better to all human red blood cells (HRBC). In this study the Hemolysis values of the plant extracts were ranged as following (68.9 – 97.7 %), (65.6 – 97.2 %), for *Viburnum tinus* leaves, *Viburnum tinus* stems, respectively. On the other side the effect of nanoparticles of the plants showed increase of anti-inflammatory activates, where the values were fluctuated in the ranges of (87.4 – 99.2 %), (83.5 – 97.5%), for nanoparticles of *Viburnum tinus* leaves, *Viburnum tinus* stems, respectively.

Table (1): Effect of aqueous extract of Viburnum tinus leaves on HRBC hemolytic and membrane Stabilization.

Sample (1) Conc .ug/ml	OD R1	OD R2	OD R3	Hypotonic Ab. Mean	Sample with Isotonic solution Ab.	Hemolysis Inhibition%	SD	SE
Control	1.296	1.292	1.291	1.293	0.001	0	0.003	0.001
1000	0.224	0.229	0.225	0.226	0.201	97.7	0.003	0.001
800	0.261	0.263	0.268	0.264	0.148	89.9	0.004	0.001
600	0.291	0.288	0.293	0.291	0.112	84.9	0.003	0.001
400	0.328	0.329	0.334	0.330	0.087	79.8	0.003	0.001
200	0.379	0.386	0.384	0.383	0.063	74.0	0.004	0.001
100	0.441	0.436	0.422	0.433	0.044	68.9	0.010	0.003

Table (2): Effect of aqueous extract of Viburnum tinus stems on HRBC hemolytic and membrane stabilization.

Sample (2) Conc .ug/ml	OD R1	OD R2	OD R3	Hypotonic Ab. Mean	Sample with Isotonic solution Ab.	Hemolysis Inhibition%	SD	SE
Control	1.296	1.292	1.291	1.293	0.001	0	0.003	0.001
1000	0.128	0.120	0.126	0.125	0.091	97.2	0.004	0.001
800	0.204	0.206	0.208	0.206	0.075	89.2	0.002	0.001
600	0.251	0.259	0.253	0.254	0.063	84.4	0.004	0.001
400	0.292	0.297	0.296	0.295	0.055	80.6	0.003	0.001
200	0.356	0.358	0.355	0.356	0.037	74.6	0.002	0.000
100	0.456	0.458	0.460	0.458	0.021	65.6	0.002	0.001

Table (3): Effect of Viburnum tinus leaf nanoparticles on HRBC hemolytic and membrane stabilization.

Sample (3) Conc .ug/ml	OD R1	OD R2	OD R3	Hypotonic Ab. Mean	Sample with Isotonic solution Ab.	Hemolysis Inhibition%	SD	SE
Control	1.296	1.292	1.291	1.293	0.001	0	0.003	0.001
1000	0.028	0.031	0.021	0.027	0.017	99.2	0.005	0.002
800	0.048	0.047	0.053	0.049	0.012	97.1	0.003	0.001
600	0.095	0.091	0.093	0.093	0.009	93.5	0.002	0.001
400	0.111	0.12	0.115	0.115	0.004	91.4	0.005	0.001
200	0.148	0.143	0.137	0.143	0.003	89.2	0.006	0.002
100	0.163	0.161	0.167	0.164	0.001	87.4	0.003	0.001

Table (4): Effect of Viburnum tinus stems nanoparticles on HRBC hemolytic and membrane stabilization.

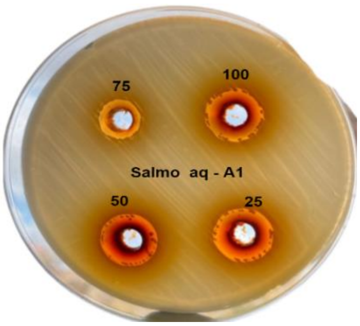
Sample (4) Conc . ug/ml	OD R1	OD R2	OD R3	Hypotonic Ab. Mean	Sample with Isotonic solution Ab.	Hemolysis Inhibition %	SD	SE
Control	1.296	1.292	1.291	1.293	0.001	0	0.003	0.001
1000	0.047	0.043	0.049	0.046	0.015	97.5	0.003	0.001
800	0.076	0.08	0.083	0.080	0.011	94.6	0.004	0.001
600	0.109	0.114	0.113	0.112	0.006	91.8	0.003	0.001
400	0.129	0.129	0.133	0.130	0.003	90.1	0.002	0.001
200	0.178	0.173	0.171	0.174	0.002	86.7	0.004	0.001
100	0.211	0.216	0.217	0.215	0.001	83.5	0.003	0.001

Anti-bacterial activity:

The anti-bacterial activity studies were carried out on three different species (*Salmonella*, *Staph* and E. *coli*). In this study three different extracts of selected plant were applied (aqueous extracts, Alcoholic extract and nanoparticles). The results showed different effects of plant extracts and their nanoparticles. For aqueous and alcoholic extracts, there are small variations of inhibition zones of studied plants, high effect was observed for high concentration of (100 %) of extracts. Where the values of inhibition zone of (6-10 mm) were recorded for the plant. The obtained results indicated that, there is relative decrease in inhibition zone values as the concentration of plant extract decrease, for the bacteria species the low effect of aqueous and alcoholic extract was recorded for concentration of 25 %. Tables (5 and 6) and Figures (5 - 9)

Table (5): The anti-bacterial activity of aqueous extracts on the selective bacteria species.

Extract / Specie	*Viburnum tinus* leaves				*Viburnum* tinus stems			
	100%	75%	50%	25%	100%	75%	50%	25%
Salmonella	9	9	7	6	8	8	8	7
Staphylococcus	9	8	7	7	7	6	6	5
E-coli	8	8	6	6	7	7	7	5



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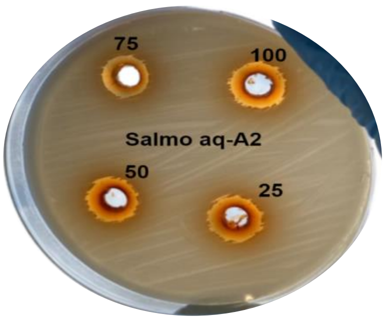

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Figure (5): Inhibition zone of Viburnum tinus (1) leaves (2) stems extract on Salmonella

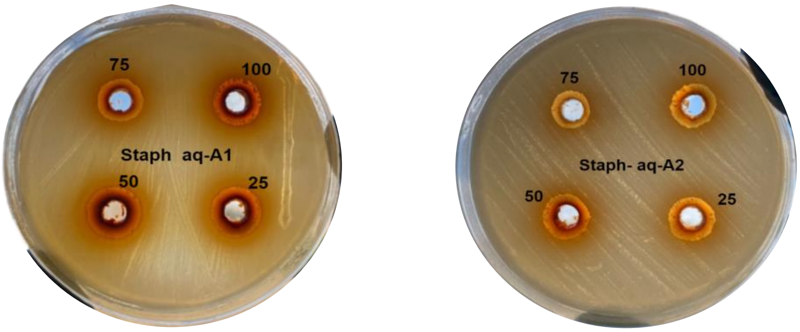

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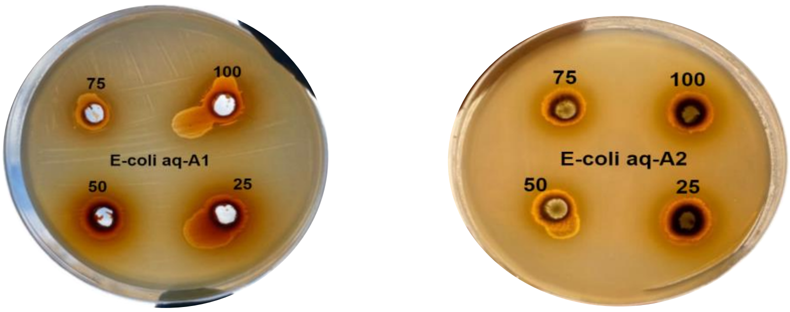
Figure (6): Inhibition zone of Viburnum tinus (1) leaves (2) stems extract on Staph



(1) (2)

Figure (7): Inhibition zone of Viburnum tinus (1) leaves (2) stems extract on E. Coli

Table (6): The anti-bacterial activity of Alcoholic extracts on the selective bacteria species.

Extract / Specie	*Viburnum tinus* leafs				*Viburnum tinus* stems			
	100%	75%	50%	25%	100%	75%	50%	25%
Salmonella	9	7	6	6	7	7	6	6
Staphylococcus	7	7	7	6	9	9	7	7
E-coli	10	10	6	6	10	9	6	6

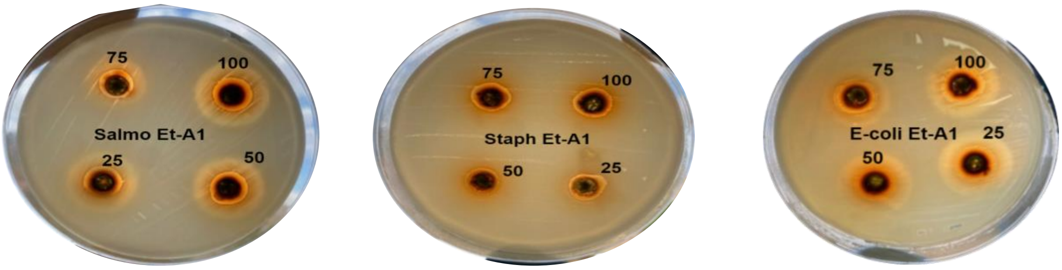


Figure (8): Inhibition zone of Alcoholic extract of *Viburnum tinus* leaves on salmonella, staph and *E-coli*

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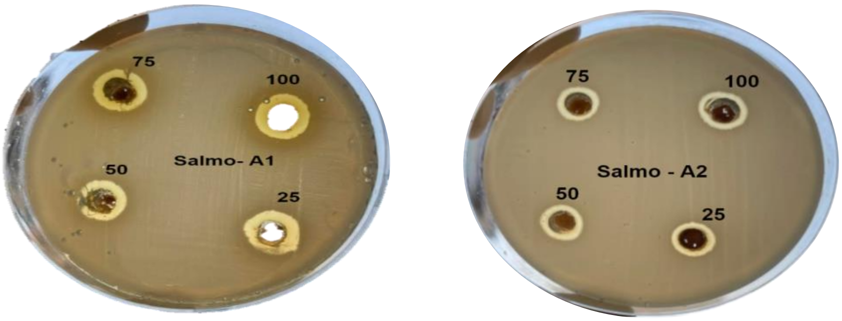
Anti- bacterial activity of nanoparticles:

The nanoparticles synthesized in this showed higher antibacterial activities compared with the aqueous and alcoholic extracts, where high inhibition zones were recorded for plant at concentration of (100 %) of values (12 - 20 mm) on the bacteria species. Also, the results indicated that the leaves of plant gave relative high effect compared with stems used in this study, Table (7) and Figures (9-10).

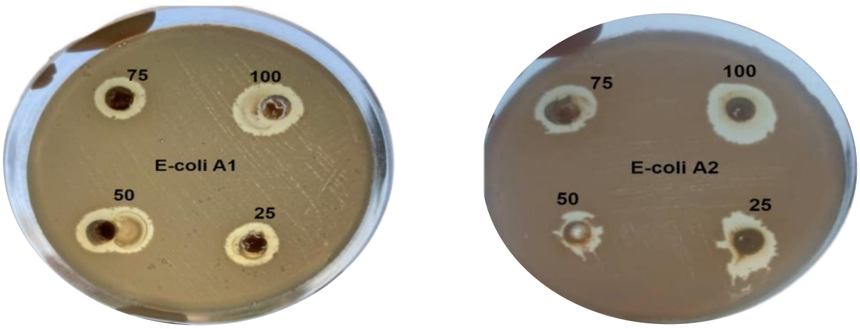
Table (29): The anti-bacterial activity of nanoparticles on the selective bacteria species.

Extract / Specie	Viburnum *tinus* leaves				*Viburnum tinus* stems			
	100%	75%	50%	25%	100%	75%	50%	25%
Salmonella	18	12	12	12	14	14	12	12
Staphylococcus	13	13	12	12	20	20	13	13
E-coli	20	20	13	12	20	18	13	13



(1) (2)

Figure (9): Inhibition zone of nanoparticles of Viburnum tinus (1) leaves (2) stems extracts on Salmonella



(1) (2)

Figure (10): Inhibition zone of nanoparticles of Viburnum tinus (1) leaves (2) stems extracts on E. coli

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Conclusions

According to the results recorded in this study the studied plant extract showed important values as antibacterial, anti-inflammatory activities, besides that, the nanoparticles produced were effective as drug carriers, which gave the studied plant and their AgNPs high medical impact.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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