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Synthesis of Silver Nanoparticles Using *Bassia eriophora* Plant, Testing their Antibacterial Activity and Cytotoxicity

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Abstract: The present study was designed to synthesize silver nanoparticles using the *Bassia eriophora* (gteena) plant and evaluate their antibacterial efficacy against antibiotic-resistant bacterial species. Additionally, the toxicity of these synthesized nanoparticles was assessed. This research was carried out in the laboratories of the College of Education for Pure Sciences at the University of Basra. AgNPs were synthesized using *B. eriophora* extract as a reducing and capping agent. Hence, it eliminates the need for hazardous chemicals. The characteristics of produced AgNPs were assessed using several methods. These methods include dynamic light scattering (DLS), scanning electron microscopy (SEM), atomic force microscopy (AFM), Zeta potential test, Fourier-transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD). The findings validated the creation of polydispersed and quasi-spherical synthetic nanoparticles with sizes between 39.23 and 60.90 nm. Analytical tests of the active components identified 43 compounds using GC-MS analysis, with arsenous acid tris(trimethylsilyl) ester constituting the greatest proportion at 36.1567%. The AgNPs exhibited significant antimicrobial efficacy against Gram-negative and Gram-positive bacteria, including *Proteus mirabilis* (20-15 mm), *Salmonella* spp. (18 mm), *Pseudomonas aeruginosa* (17-15 mm), *Klebsiella pneumoniae* (13-13 mm), as well as *Shigella* spp, and *Escherichia coli* (15 mm). A human red blood cell lysis assay evaluated the toxicity of nanoparticles. They reveal no hemolysis in the 10 doses tested throughout the experiment. It was determined that AgNPs containing Bassia B. eriophora greatly enhanced their antibacterial properties and cytotoxicity.

Keywords: Antibacterial activity, Phytochemicals, Silver nanoparticles, Green biosynthesis, MDR bacteria.

Introduction

Nanoparticles are entities with several dimensions and measuring 100 nanometers or less in size. The word "Nano" originates from Greek. It denotes a dwarf and signifies a measurement of one billionth (10^{-9}) of a meter. These particles are regarded as the fundamental components of Nanotechnology (Maxwell *et al*., 2021). Nanoparticles are often manufactured from silver, platinum, and gold. They use diverse physical and chemical techniques (Yu,2007; Ying *et al*.,2022). These techniques involve hazardous chemicals and are energy-intensive (Gupta&Xie,2018; Saha *et al*.,2020). Hence, ecologically friendly

procedures without harmful chemicals are needed. Various biological systems, including plant extracts, bacteria, yeast, and fungi, are used in green biosynthesis techniques (Barhulolum *et al*., 2021; Hawar *et al*., 2022). Silver has been historically used in all its forms as an antimicrobial agent alone or in combination with other technologies (Silva *et al*., 2017). Silver nanoparticles have become the focus of interest for researchers and industries. They have been used to improve biomedical applications such as drug delivery and wound healing. They also work in cosmetic, dental, bandage, surgical, protective coating, and garment applications. Due to their antibacterial qualities, silver nanoparticles offer hope as a new weapon in the battle against pathogens. Several harmful bacteria, including drug-resistant ones, are inhibited by them. (Marambio-jones&Hoek, 2010; Ge *et al*., 2014; Saddiqi *et al*., 2018).

Creating green silver nanoparticles using live cells is among the most effective techniques. It generates more mass than other approaches. Plants provide several biochemical stabilizers and reductants for environmentally beneficial nanoparticles. Green synthesis is cost-effective, safe, and environmentally friendly. The concentration of compounds in plant extracts enhances productivity. It stabilizes and diminishes metal ions while generating nanoparticles. It is also more stable than physical, chemical, and biological approaches (Mustapha *et al*., 2022; Venkataraman, 2023).

Plant-derived phytochemicals, including sugars, terpenoids, flavonoids, and phenols, are very effective for the environmentally sustainable creation of nanoparticles (Widatalla *et al*., 2022). This wild plant from the Chenopodiaceae family is called Gteena or *B. eriophora*. This fibrous, downy herbaceous plant reaches a 25–50 cm height and flowers annually.The stems and branches often sprout from the base and have fine hairs. A cotton ball-sized growth stretches from the flower's margin to the blooming zone axis. Small, flat, connected, or short leaves. They are oblong to oval and contain water. Springtime blossoming glomerulus turns into white cotton ball-like balls (Safiallah *et al*., 2017). Traditionally Iranian medicine has used gteena to treat baldness, gingivitis, and Alzheimer's. Saudi Arabians use it for rheumatism and kidney issues. (Norton *et al*, 2009; Yusufoglu, 2015). This study sought to synthesize silver nanoparticles using plant extract in aqueous solution. The objective was to evaluate the efficacy of these nanoparticles against antibiotic-resistant bacteria in pediatric patients with diarrhea in Basra Governorate. The research investigated the impact of nanoparticles on the lysis of human red blood cells to evaluate their cytotoxicity. No studies have been performed on manufacturing *B. eriophora* silver nanoparticles, their antibacterial properties, or their cytotoxic effects.

Materials & Methods

Plant collection

B. eriophora was collected from some desert areas in Basra on 5/5/2023. It was brought to the laboratory and cleaned of impurities and dirt. Then, spread on cardboard in the outdoor air at room temperature and away from direct sunlight for drying. After it was completely dry, the entire plant was ground with an electric grinder inside the laboratory and kept in a clean box in a dry place until use.

Aqueous extraction

The plant was extracted using an aqueous method and a reflux apparatus. 25 g of *B. eriophora* powder was put in a 500 ml glass flask with distilled water for 18-24 hours. Upon completion of the extraction, it was allowed to cool at ambient temperature. Then, it was filtered through medical gauze to eliminate the larger plant fragments. The extract was further filtered using filter paper to exclude tiny phytoplankton. It was kept at a temperature of 4-8°, as indicated in the source with modifications (Shareef *et al*.,2022; AlKhafaji *et al*.,2024).

Synthesis of Silver nanoparticles

0.05 g of silver nitrate was added into an opaque glass flask containing 300 ml of previously filtered plant extract. The mixture should be shielded from light to avert the self-oxidation of silver and incubated at room temperature in a shaking incubator for 5-7 days. Then, the plant extract was examined for color changes indicative of silver nanoparticle formation. Subsequently, the solution was aliquoted into a test and centrifuged for 10 minutes at 3000 rpm. The filtrate was discarded, and 10 ml of distilled water was added to the remaining residue to remove impurities.

Then, it was placed again in the centrifuge, and the process was repeated three times. Then, the concentrated precipitate was placed in glass Petri dishes and dried at 40 °C using an oven. Then, the dry precipitate was collected and kept until use (Ajayi &Afolayan, 2017). In the current study, drying the nano-extract at room temperature, using air

Diagnosis of active compounds in *B. eriophora* using GC-MS technique

This analytical method separates and detects sample mixture chemical components and determines their existence, absence, or amount. The chemical components are generally organic compounds or gases (Technology Networks, 2021).

Fourier Transform Infrared Spectroscopy analysis (FT-IR)

Using infrared spectroscopy, this study identified functional groups in *B. eriophora* plant water-based and nano-extracted extracts. These active groups enclose, stabilize, and decrease silver nanoparticles. This study uses infrared scanning to evaluate chemical bonds and discover which functional groups produced silver nanoparticles. An absorption spectrum showing discrete peaks for highly concentrated chemical bonding shows the analytical findings. Functional groups, including alkanes, ketones, and amines, absorb wavelength infrared photons to identify biomolecules (Palithya *et al*., 2022).

Characterization of silver nanoparticles:

The formation of AgNPs was monitored using the following instruments:

X-ray Diffraction Analysis (XRD)

For quantitative and qualitative nanoparticle research, X-ray diffraction is useful. It helps identify critics, measure crystallinity, and calculate nanoparticle size (Mashwani *et al*., 2015). It also helps determine if a substance is pure or impure. This material testing method employs diffraction patterns since each material has a distinct diffraction beam. Analyze the diffraction beams against the Joint Committee on Powder Diffraction Standards

(JCPDS) reference database to identify the substance (Zhang *et al*., 2016).

Scanning electron microscope (SEM)

Scientists use scanning electron microscopy to study silver nanoparticle size, morphology, and dispersion (Sadeghi&Gholamhoseinpoor, 2015).

Energy Dispersive X-ray Spectroscopy (EDX\EDS)

This device includes the interaction of X-rays with the sample. EDX was used to determine the presence of elements, relative abundance, and impurities in the nanoparticles. The presence of peaks at three kiloelectron volts indicates the presence of silver crystals. The absence of other peaks indicates that the silver nanoparticles are free of other impurities (Hamouda, 2019).

Atomic force microscope (AFM)

This microscope measures material size, shape, structure, diffusion, and aggregation. An imaging approach that detects particle nanoscale architecture in 3D (Song *et al*., 2013; Lin *et al*., 2014).

Zeta potential measurement:

Zeta potential is an indicator of physical stability, and for dispersion to be considered stable, the absolute value of its zeta potential should exceed 25 mV) (Alzubaidi *et al*.,2023).

Antibacterial activity of synthesized silver nanoparticles

This essay used seven human pathogenic bacterial strains. It includes *Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa, Shigella sp.,* and *Salmonella sp*. These strains were obtained from our previous study. 100 stool samples were isolated from children with diarrhea under eight for both sexes. The antibacterial activity of AgNPs was evaluated using the Agar Well Diffusion method (Chavez-Esquivel *et al*., 2021), with bacteria cultured in Mueller-Hinton Agar (MHA) medium at 37C for 24 h.

In this method, the antimicrobials of samples are permitted to diffuse into the surrounding medium. They enable interaction with the microorganisms cultivated on the plate. The bacterial suspension was generated during 24 hours of incubation at 37°C, and its growth was compared to the 0.5 McFarland standard. A cotton swab was used to saturate the MHA plates, and a cork borer was employed to create five wells with a diameter of 6 mm. The wells were loaded with 62.5, 125, 250, 500, and 1000 μg ml^{-1} of synthesized AgNPs concentrations. The inhibition zone diameters are measured millimetres by forming it around the well after 24 hr of incubation at 37C.

Detection of Cytotoxicity of silver nanoparticles using erythrocyte lysis test

The cytotoxicity of silver nanoparticles synthesized from the aqueous extract of the plant *B. eriophora* was tested according to the source (He *et al*.,1994).

Statistical Analysis

In the current study, the Statistical Package for the Social Sciences (SPSS), version 28, was utilized for statistical analysis of the results. A Chi-square test was employed, with a significance threshold set at $p>0.05$ to determine statistical significance.

Results & Discussion

Green biosynthesis of silver nanoparticles from the aqueous extract of *B. eriophora*

The color of the aqueous extract of the *B.eriophora* plant solution changed from yellowish-brown to milky brown after the addition of silver nitrate. The solution was then incubated in a shaking incubator, away from light, for 5-7 days. This change in color serves as an initial sign of the creation of silver nanoparticles (Fig. 1) Below are the stages of the plant's aqueous extraction and the shape of the extract after the formation of silver nanoparticles. The color changes due to the reduction of silver ions to silver nanoparticles. The active biomolecules in the aqueous extract of the *B.eriophora* plant act as natural reducing agents (Nallappen, 2021).

a B c D

Fig. (1): (a) *B. eriophora* after drying (b) after grinding (c) Aqueous extract (d) Aqueous extract after the formation of silver nanoparticles.

Determination of active compounds by (GC-MS)

Gas chromatography-mass spectrometry (GC-MC) was used to evaluate the active components in *Bassia eriophora's* aqueous extract. The results showed the presence of 43 different compounds in Table (1) Fig (2). Arsenic acid and tris (trimethylsilyl) ester had the greatest proportion of 36.1567% in the

aqueous extract with an area of 2652152 and a retention period of 28.022 minutes. Its chemical formula is $C_9H_27AsO_3Si_3$, followed by the compound Cyclotetrasiloxane, octamethyl- with a percentage of 7.4904% and an area of 549432 and a retention time of 9.232 minutes, its molecular formula is $C_8H_{24}O_4Si_4$, followed by the compound Cyclopentasiloxane, decamethyl- with a chemical formula of $C_{10}H_{30}O_5Si_5$ and the compound 1-Oxaspiro[4.5]decan-2-one,6-isopropenyl-9-methyl-chemical formula $C_9H_{14}O_2$, 4.4806%, 4.1118%, area 328663, 301604, retention time 12.091 and 18.894 respectively, and the compound n-Hexadecanoic acid, 3.1419%, area 230464, retention time 21.871, chemical formula $C_{16}H_{32}O_2$, which is a saturated fatty acid with a long straight chain containing sixteen carbon atoms (National Center for Biotechnology Information (NCBI), 2024). Table (2) Molecular structure and chemical formula of active compounds in *B. eriophora* plant.

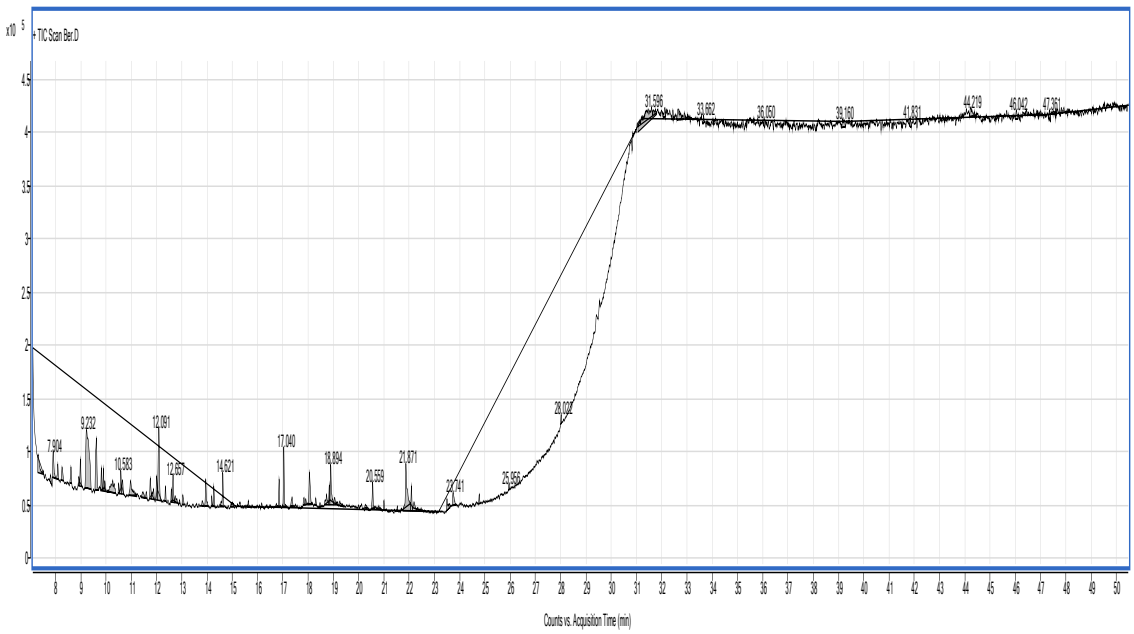


Fig. (2) Mass Spectrum of the aqueous extract of *B. eriophora*.

Characterization of silver nanoparticles:

FT-IR analysis

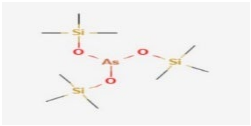
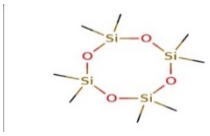
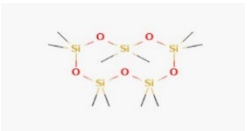
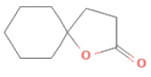
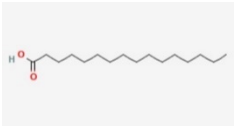
An infrared spectrum (FT-IR) analysis was conducted on the aqueous extract of the *B. eriophora* plant and the nanoparticles produced from the extract. The purpose was to investigate any potential interaction between silver and the biologically active groups in the extract. The findings indicated the existence of several active groups and compounds that participate in the conversion of silver nitrate into silver nanoparticles. The current results were compared with the Infrared Spectroscopy Absorption Table. The peak cm-1 3400 indicates, as shown in (Fig. 4), the expansion of the primary amine group N-H of amino acids. Furthermore, the peaks 2924 cm^{-1} and 2850 indicate the expansion of the alkanes group C=C, and the alkene group C=C with a double bond appeared at the peak 1631 cm^{-1}. The peak 1411 cm-1 indicates the extension of the sulfate group S-O and the alcohol group O-H and Carboxylic acid. The peak 1327 cm^{-1} indicates the extension of the phenol group O-H. The band indicates the presence of 1139 cm^{-1}Aliphatic Ether (C-O) Tertiary alcohol groups. The band 1097 cm^{-1} indicates the secondary alcohol groups C-O Secondary alcohol. Thus, the aqueous extract of the *B. eriophora* plant contains many active groups. These groups include amino acids, alkanes, alkenes, organic sulfur compounds, sulfates, alcohols, carboxylic acids and phenolic groups. These results agreed mainly with (Mohammed & Hawar, 2022).

Table (1) Chemical compounds detected in the aqueous extract in B. eriophora plant

eak	R.T.	Area	Pct Total	Area Pct	Library/ID
1	8،093	41758	0،569	0،5693	Dimethyl Sulfoxide
2	8،258	90300	1،231	1،2311	Dimethyl Sulfoxide
3	8،611	106501	1،452	1،4519	Dimethyl Sulfoxide
4	8،988	111719	1،523	1،5231	Dimethyl Sulfoxide
5	9،232	549432	7،49	7،4904	Cyclotetrasiloxane, octamethyl-
6	9،617	193988	2،645	2،6446	Cyclotrisiloxane, hexamethyl-
7	10،269	158675	2،163	2،1632	Acetic acid butyl-methyl-phosphinoylmethyl ester
8	10،583	176445	2،405	2،4055	Acetic acid butyl-methyl-phosphinoylmethyl ester
9	10،968	187664	2،558	2،5584	Azetidine, 1-chloro-
10	11،596	86499	1،179	1،1792	1H-Indole, 5-methyl-2-phenyl-
11	11،753	118049	1،609	1،6094	6-Methoxybenzofuroxan
12	12،091	328663	4،481	4،4806	Cyclopentasiloxane, decamethyl-
13	12،358	59798	0،815	0،8152	Dimethyl Sulfoxide
14	12،657	189534	2،584	2،5839	Ethane, 1,1-dichloro-
15	13،042	34020	0،464	0،4638	Dimethyl Sulfoxide
16	13،945	85489	1،165	1،1655	1,2-Bis(trimethylsilyl)benzene
17	14،267	71824	0،979	0،9792	6-Methoxybenzofuroxan
18	14،621	117698	1،605	1،6046	Cyclohexasiloxane, dodecamethyl-
19	15،005	45960	0،627	0،6266	Ethyl 3-oxo-3-(pyridine-2-yl) propanoate
20	15،288	41274	0،563	0،5627	Azetidine, 1-chloro-
21	15،634	38249	0،521	0،5214	Propanoic acid, 2-chloro-, ethyl ester
22	16،16	32497	0،443	0،443	1-Ethynylcyclohex-2-en-1-ol
23	16،372	30572	0،417	0،4168	Spiro [7,8-diazatetracyclo [4.3.0.0(2,4).0(3,5)] non-7-ene-9,1'-cyclopropane]
24	16،851	66406	0،905	0،9053	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane
25	17،04	125645	1،713	1،7129	2,4-Di-tert-butylphenol
26	17،37	31924	0،435	0،4352	Brocresine
27	17،503	30262	0،413	0،4126	Spiro[cyclopentane-1,3'-[3H] indole], 2'-methyl-
28	17،841	83219	1،135	1،1345	Brocresine
29	18،053	140221	1،912	1،9116	Diethyl Phthalate

Peak	R.T.	Area	Pct Total	Area Pct	Library/ID
30	18،305	29242	0،399	0،3987	Brocresine
31	18،894	301604	4،112	4،1118	1-Oxaspiro [4.5] decan-2-one, 6-isopropenyl-9-methyl-
32	19،255	35758	0،487	0،4875	3-Azabicyclo [3.1.0] hexane-1,5-dicarbonitrile, 2,4-dioxo-
33	20،559	126299	1،722	1،7218	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane
34	21،007	30829	0،42	0،4203	6H-Thieno[2,3-b] pyrrole-5-carboxylic acid
35	21،871	230464	3،142	3،1419	n-Hexadecanoic acid
36	22،091	98974	1،349	1،3493	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-
37	23،497	72628	0،99	0،9901	Silanamine, N-[2,6-dimethyl-4-[(trimethylsilyl)oxy] phenyl]-1,1,1-trimethyl-
38	23،741	105532	1،439	1،4387	Octadecanoic acid
39	24،063	55206	0،753	0،7526	Benzofuran-2-one, 2,3-dihydro-3,3-dimethyl-4-nitro-
40	24،777	95936	1،308	1،3079	N-Methyl-1-adamantaneacetamide
41	25،775	57631	0،786	0،7857	Cyclotrisiloxane, hexamethyl-
42	26،097	68627	0،936	0،9356	2-Ethylacridine
43	28،022	2652152	36،157	36،1567	Arsenous acid, tris(trimethylsilyl) ester

Table (2): Molecular structure and chemical formula of the highest percentage compounds in *B. eriophora* plant.

n	Chemical compound	Chemical formula	Molecular formula of the compound
1	Arsenous acid, Tris(trimethylsilyl) ester	$C_9H_{27}AsO_3Si_3$	
2	Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_4Si_4$	
3	Cyclopentasiloxane,decamethyl-	$C_{10}H_{30}O_5Si_5$	
4	1-Oxaspiro [4.5] decan-2-one,6-isopropenyl-9-methyl-	$C_9H_{14}O_2$	
5	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	

Compared with the results of the infrared spectrum in the sample of nanoparticles, the band 1411 cm-1 disappears. This indicates the sulfate group (S-O) and O-H extension for alcohol and carboxylic acid and the disappearance of the band 1139 cm^{-1}. This shows the expansion of the Aliphatic Ether (C-O) and Tertiary alcohol groups. Consequently, this led to their contribution to reducing silver nitrate to silver nanoparticles, as shown in (Fig 5). New bands that were not present in the plant's aqueous extract. It includes bands 3466 to 3363 cm-1 for primary amine and aliphatic groups N-H, a strong band at 1641 cm-1 for alkane groups C-H, a band at 1463 cm-1 for alkene groups, and a band at 1328 cm-1 for phenolic groups. Phenolic chemicals and proteins reduce and stabilize. They may prevent agglomeration by binding to silver nanoparticles through free amino groups or cysteine residues (Chougule *et al*., 2020; Shareef *et al*.,2023).

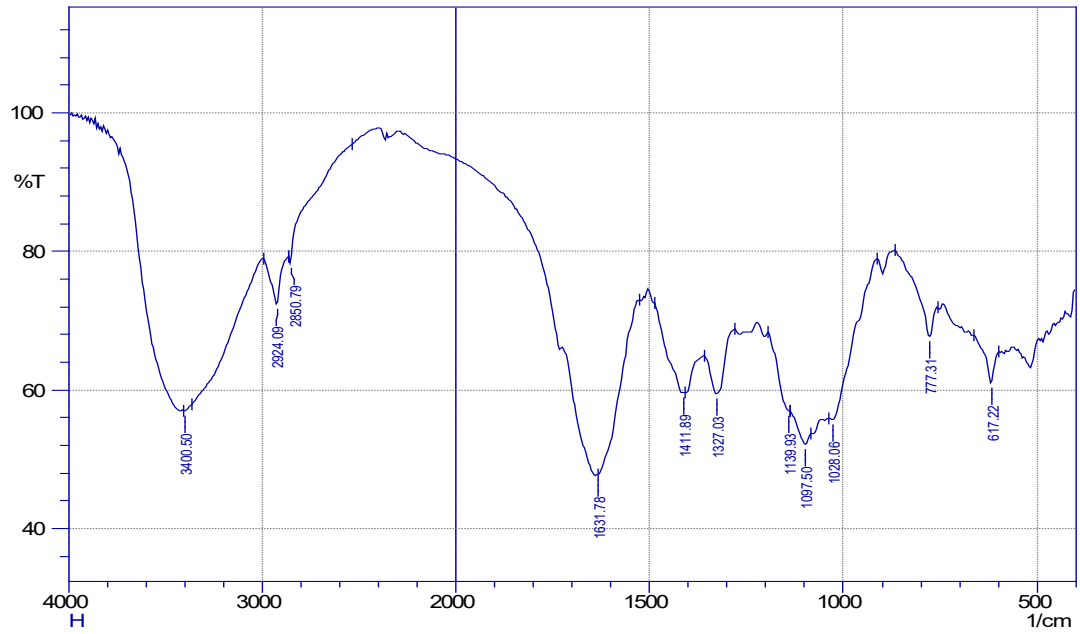


Fig. (4): Infrared spectrum analysis of the dried aqueous extract of *B. eriophora*.

Scanning electron microscope (SEM)

A scanning electron microscope (SEM) examined the size, shape, and homogeneity of water-based B. eriophora plant extract-derived silver nanoparticles. The result of the scanning electron microscope, pictured at a 500 nm scale, is shown in (Fig 6). The silver nanoparticles were either completely spherical or somewhat semi-spherical. The sizes ranged from 39.23 to 60.90 nm. Several studies show that silver nanoparticles may exhibit a wide range of non-uniform shapes when seen by scanning electron microscopy (Vijayaraghavan *et al*., 2012; Jagat & Bapat, 2013).

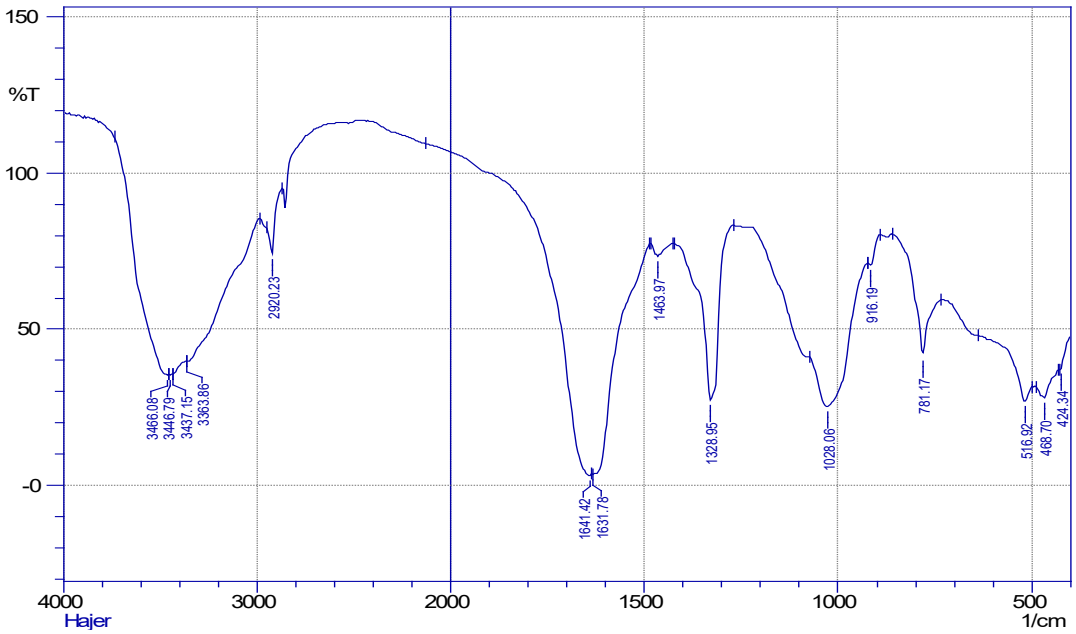


Fig. (5): Infrared spectrum analysis of silver nanoparticles manufactured from the aqueous extract of the *B. eriophora*.

Energy Dispersive X-ray Spectroscopy (EDX\EDS) Analysis

The energy-dispersive X-ray study showed that the *B.eriophora* nano-extract included silver. In surface plasmon resonance (SPR) examination, the nanoparticle-containing material displayed peaks in the 3 KEV range, typical for silver. Silver comprised 17.3% of weight. The sample contains chlorine due to analytical processing. Surface metabolite interaction with silver nanoparticles or oxidation may explain oxygen and other pollutants in the sample (Radhakrishnan *et al*., 2018; Hamouda, 2019) (Fig.7).

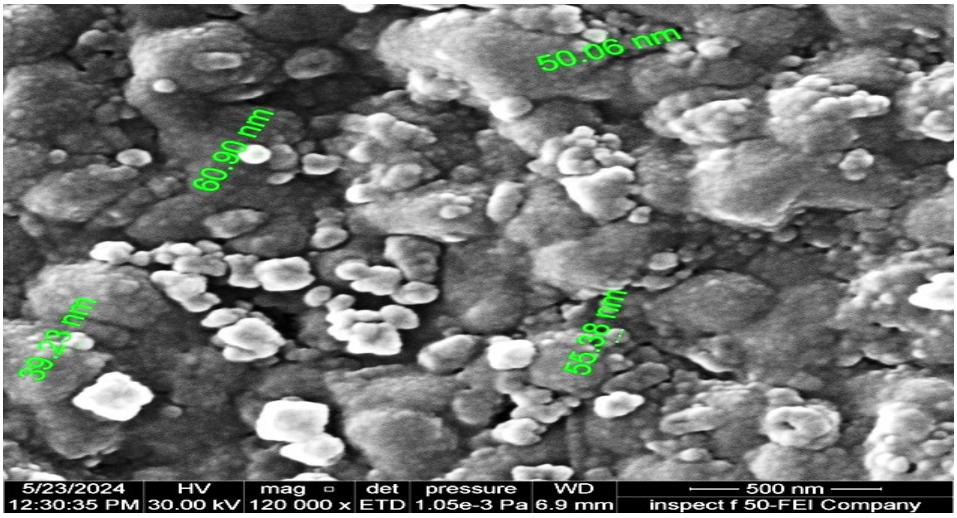
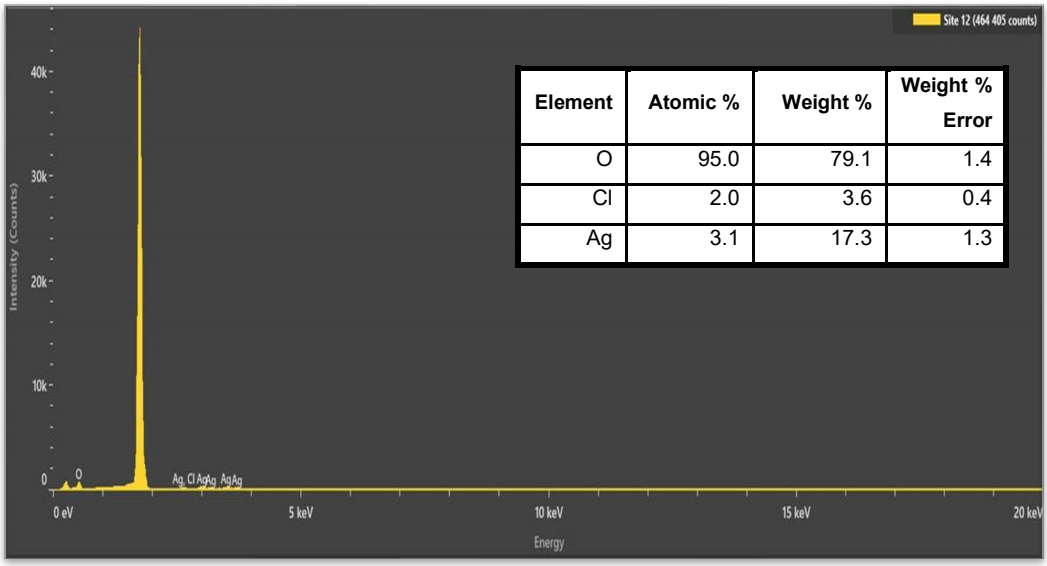


Fig. (6): Detection of silver nanoparticles manufactured from the aqueous extract of *B. eriophora* using scanning electron microscopy (SEM).



Element	Atomic %	Weight %	Weight % Error
O	95.0	79.1	1.4
Cl	2.0	3.6	0.4
Ag	3.1	17.3	1.3

Fig. (7): EDX energy dispersion diagram of silver nanoparticles manufactured from the aqueous extract of *B. eriophora*.

X-ray diffraction analysis (XRD)

Fig.8 shows XRD data showing *B.eriophora* plant water extract silver nanoparticles in crystalline form. Peaks at 2θ = 33 degrees indicate a reflection of 111. The current research shows that biologically generated silver nanoparticles are crystalline and face-centered cubic (fcc).

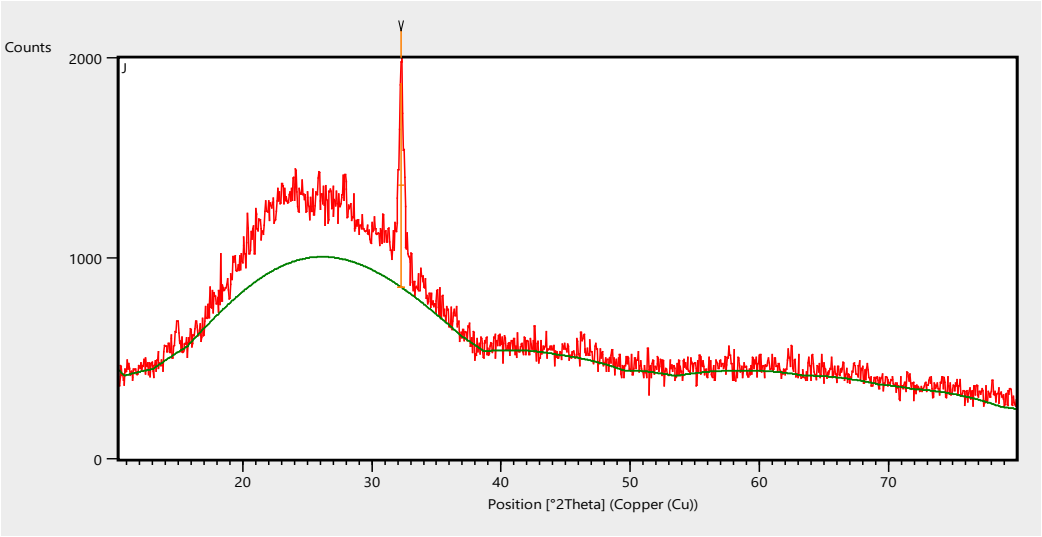


Fig. (8): XRD analysis of nanoparticles manufactured from the aqueous extract of *B. eriophora.*

Atomic force microscope (AFM)

Employing atomic force microscopy, Youssef *et al*. (2019) validated the nanomaterials' size, morphology, dispersion, and aggregation. He also reveals details on their surface topography and roughness. Figure 9, a three-dimensional

graphic, illustrates the findings of the present experiment. Indicating that the nanoparticles in the plant's aqueous extract may be spherical, singular, or aggregated.

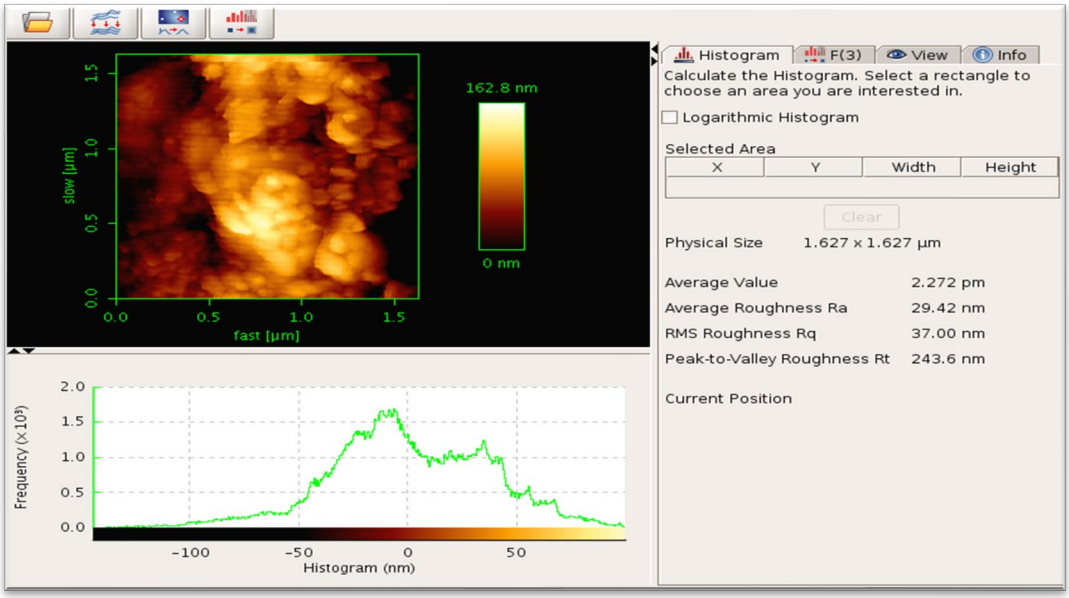


Fig. (9): Examination of nanoparticles synthesized from the aqueous extract of the *B. eriophora* plant using an atomic force microscope (AFM).

Zeta potential measurement

B. eriophora plant aqueous extract nanoparticles had -38.9 mV surface charges in the zeta potential test. Electrostatic attraction made silver nanoparticles stable. (Fig.10) demonstrates their negative value. Fatima *et al*. (2020) recommend a zeta potential of at least -+30 mV to define nanosuspensions' water stability.

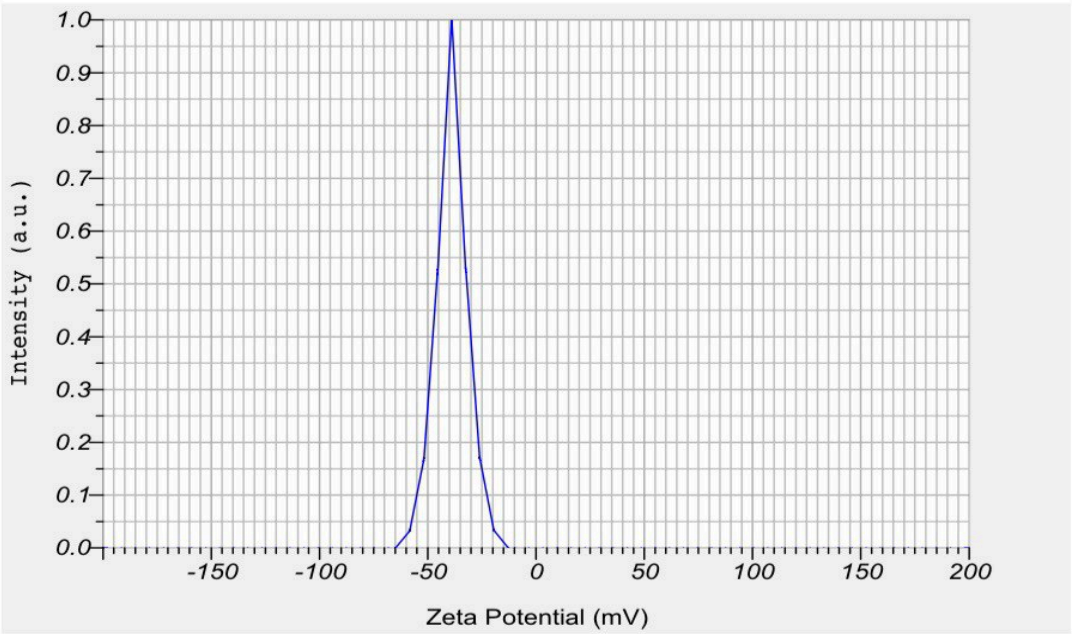


Fig. (10): Zeta potential measurement of nanoparticles manufactured from the aqueous extract of the *B. eriophora*

Antibacterial activity of silver nanoparticles

Due to the problems of multidrug resistance, it has become necessary to produce safer alternatives to antimicrobial agents and antibiotics. The synthesis of nanoparticles from biological sources with antibacterial has opened new avenues against multidrug-resistant bacteria (Geethalakshmi 0 Sarada, 2012). The present study focuses on pathogenic bacteria linked to diarrhea in children. It specifically selected those with the highest antibiotic resistance from the isolates. These were used to test the effectiveness of nanoparticles created from the aqueous extract of the plant *B. eriophora*, as detailed in Table 3 and Figure 11. The statistical analysis showed no significant difference between the concentrations and bacteria at the probability level of $P<0.05$. The manufactured nanoparticles exhibited varying effects on Gram-negative and Gram-positive bacteria. At a concentration of 1000 µg/ml, they achieved the highest inhibition rate against *Salmonella* spp., with an inhibition diameter of 18 mm. In contrast, they showed the lowest inhibition rate against *S. epidermidis*, with a diameter of 12 mm. The 500 µg/ml concentration achieved the highest inhibition rate among the five concentrations used against *P. mirabilis* bacteria with an inhibition diameter of 20 mm. The concentration of 250 µg/ml achieved the highest inhibition rate against *P. aeroginosa* bacteria with an inhibition diameter of 17 mm. The 62.5 µg/ml concentration achieved the highest inhibition rate against *Salmonella* spp and *P. mirabilis* bacteria with an inhibition diameter of 17 mm. The findings matched (Aljazy *et al*., 2019; Almudhafar & Al-Hamdani, 2022). In the current study, better effectiveness was observed when drying the nano-extract at room temperature, using air to preserve the active ingredients of the plant. This may be due to the fact that، the heat of the electric oven may destroy some of the plant's active ingredients. Many ideas explain silver nanoparticles' antimicrobial properties, including:

- Adhesion of silver nanoparticles to the cell wall or membrane surface through the positive surface charge of silver nanoparticles. The hydrostatic attraction between silver nanoparticles and microorganisms' negatively charged cell membrane (Abbaszadegan *et al*., 2015). The interaction of silver ions with sulfur-containing proteins in the bacterial cell wall leads to the destruction of the bacterial cell wall (Keat *et al*., 2015). Silver nanoparticles lead to the destruction of cell membranes, increase their permeability, and disrupt respiratory function (Duran *et al*., 2016; Prabhu & Poulose, 2012).
- Silver nanoparticle penetration and damage to mitochondria, ribosomes, vacuoles, and biomolecules.
- The formation of reactive oxygen species (ROS) and free radicals leads to induced cytotoxicity and oxidative stress. Silver ions can interact with thiol groups of many vital enzymes, which leads to their inactivation and the generation of reactive oxygen species (ROS).(Ahmed *et al*.,2016; Dakal *et al*.,2016; Huang *et al*.,2010).

Table (3): Antibacterial activity of silver nanoparticles synthesized from *B. eriophora* against bacteria isolated from children with diarrhea.

n	Strain	Concentration µg/ml				
		1000	500	250	125	62.5
1	*Escherichia coli*	15	11	12	14	15
2	*Proteus mirabilis*	15	20	15	14	14
3	*Salmonella* spp.	18	17	16	15	17
4	*Shigella* spp	15	14	14	14	15
5	*Klebsiella pneumonia*	13	13	13	13	13
6	*Pseudomonas aeruginosa*	15	15	16	17	17
7	*Staphylococcus epidermidis*	12	10	12	10	10

Chi sequare: 21; P ≤0.05 Effectiveness measured in mm*

Gram-negative bacteria, including E. coli, Salmonella, and Pseudomonas have cell walls made of lipolysaccharide and a thin peptidoglycan layer (3–4 nm). Gram-positive bacteria, including *Staphylococcus*, *Bacillus*, and *Streptococcus,* have 30-nanometer-thick peptidoglycan layers in their cell walls. Thus, silver nanoparticles kill Gram-negative bacteria regardless of resistance, unlike Gram-positive bacteria. Silver nanoparticles' antibacterial activity depends on the dose, size, shape, zeta potential, and colloidal state (Rai *et al*.,2012; Abbaszadegan *et al*.,2015; Duran *et al*.,2016; Selvaraj *et al*.,2017).

The results of the toxicity test of the nanoparticles manufactured from the aqueous extract of *B. eriophora* in the current study, showed no lysis of red blood cells at all ten concentrations used in the experiment (2, 4, 8, 16, 31.25, 62.5, 125, 250, 500, 1000) as a result can be observed in (Fig. 12). The figure shows complete lysis of red blood cells in the positive control group (tap water). In comparison, the concentrations used in the experiment. No degradation was observed in the negative control element represented by normal saline. This indicates the possibility of using synthetic

Fig.12: Results of the cytotoxicity test of silver nanoparticles manufactured from the *B. eriophora* plant using the human red blood cell lysis test.

Cytotoxicity test of silver nanoparticles through the lysis of human red blood cells

silver nanoparticles as an antibiotic to treat multi-drug-resistant bacteria. The pathogens for children are due to the widespread resistance formed by these bacteria (He *et al*.,1994).

Conclusion

The results demonstrated that silver nanoparticles produced through green biosynthesis from the *B. eriophora* plant demonstrated effective antibacterial activity. The manufactured nanoparticles exhibited varying effects on Gram-negative and Gram-positive bacteria. At a concentration of 1000 µg/ml, they achieved the highest inhibition rate against *Salmonella* spp., with an inhibition diameter of 18 mm. In contrast, they showed the lowest inhibition rate against *S. epidermidis*. At any of the 10 doses tested, the nanoparticle toxicity assay did not detect hemolysis of human RBCs.

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Conflicts of interest

The authors declare no conflict of interest.

Ethical approval

Stool samples were collected from children under the age of eight after obtaining verbal consent from their parents or legal guardians. All samples were anonymized to maintain participant confidentiality. No personal or identifying information was recorded or used in the study. The research was conducted in accordance with the ethical standards of the College of Education for Pure Sciences, University of Basrah.

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تخليق دقائق الفضة النانوية باستخدام نبات *Bassia eriophora* واختبار فعاليتها ضد البكتريا المتعددة المقاومة للمضادات الحيوية واختبار سميتها

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المستخلص: هدفت الدراسة الحالية إلى تصنيع دقائق الفضة النانوية باستخدام نبات *B. eriophora*. واختبار فعالية هذه الدقائق ضد بعض الأنواع الجرثومية المقاومة للمضادات الحيوية واختبار سميتها. اجراء العمل في مختبرات كلية التربية للعلوم الصرفة، جامعة البصرة، تم تصنيع دقائق الفضة النانوية باستخدام المستخلص المائي لنبات B.eriophora كعامل اختزال وتغطية وبالتالي يلغي الحاجة الى المواد الكيميائية الخطرة. تم تشخيص خصائص دقائق الفضة النانوية المصنعة باستخدام عدة طرق. تشمل هذه الطرق تشتت الضوء الديناميكي (DLS)، المجهر الالكتروني الماسح (SEM)، مجهر القوة الذرية (AFM)، اختبار جهد زيتا Zeta potential، مطيافية الاشعة تحت الحمراء (FTIR)، حيود الاشعة السينية (XRD). اثبتت النتائج تكوّن دقائق الفضة النانوية ذات اشكال شبه كروية باحجام تتراوح بين 60.90–39.23 نانومتر. حددت الاختبارات التحليلية للمكونات النشطة 43 مركبا باستخدام تحليل GC–MS. حيث شكل المركب Arsenous acid, tris (trimethylsilyl) ester النسبة الأعلى وبنسبة 36.1567%. أظهرت دقائق الفضة النانوية فعالية مضادة للجراثيم ضد البكتريا السالبة والموجبة لصبغة كرام، بما في ذلك *Proteus mirabilis* (15–20) ملم، spp *Salmonella* (18) ملم، *Pseudomonas aeruginosa* (15–17) ملم، *Klebsiella pneumoniae* (13–13) ملم، بالإضافة الى spp *Shigella* و*Escherichia coli* (15)ملم. تم اختبار سمية الجسيمات النانوية باستخدام اختبار تحلل كريات الدم الحمر في الانسان ولم يلاحظ أي تحلل لكريات الدم الحمر في التراكيز العشر المستخدمة في التجربة. بينت النتائج ان دقائق الفضة النانوية المصنعة من المستخلص المائي لنبات*B. eriophora* قد عززت بشكل كبير خصائصها المضادة للجراثيم.

الكلمات المفتاحية: النشاط المضاد للبكتيريا ، المركبات النباتية الفعالة ، جسيمات الفضة النانوية ، لتخليق الحيوي الأخضر ، بكتريا متعددة المقاومة للمضادات الحيوية.