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Green Synthesis of Silver Nanoparticles Using Aqueous Extract of *Typha domingensis* Pers. Pollen (qurraid) and Evaluate its Antibacterial Activity

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Abstract:

In this study, the aqueous extract of (*Typha domingensis* Pers.) pollen grain (qurraid) to know its ability to manufacture silver nanoparticles. Qurraid is a semi-solid yellow food substance, sold in Basra markets and eaten by the local population. It is made from the pollen of the *T. domingensis* Pers. plant after being pressed and treated with water vapor. The Gas chromatography–mass spectrometry (GC-MS) reaction was done to identify the active compounds of qurraid aqueous extract. The ability of the aqueous extract of qurraid to manufacture silver nanoparticles was tested, and the construction of silver nanoparticles was inferred by the reaction mixture's color, which ranged from yellow to dark brown. The synthesized silver nanoparticles (AgNPs) were described by UV-Vis, FTIR, XRD, SEM, and EDX. Then its anti-bacterial activity was estimated by the agar well diffusion method. The findings of the GC-MS analysis of the qurraid aqueous extract showed the major components with their ratio were: 5-Hydroxymethylfurfural with RT% 13.6196, 3-Deoxy-d-mannoic lactone 6.4285,. alpha.-L-lyxo-Hexopyranoside, methyl 3-amino-2,3,6-trideoxy- 4.264, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- 3.2078, and 1,3-Methylene-d-arabitol 3.1257. The construction of silver nanoparticles was described by spectroscopic methods, where the highest peak was recorded at 400nm by UV-Vis spectrum, which indicates the silver spectrum. The mineral nature of AgNPs was confirmed by XRD analysis, in which the highest peaks were, 111, 300, and 330 were recorded. In addition, the qrdAgNPs nanoparticles were spherical with sizes ranging from 20-70nm. The results of the EDX confirmed that the chemical composition of AgNPs was silver. The ability of the AgNPs was tested against four bacterial species, three of which were Gram-negative *Escherichia coli* A1, *Escherichia coli* A2, *Alcaligenes faecalis* AL1, and the fourth was Gram-positive bacteria *Bacillus zanthoxyli* B1 , which were identified by traditional and molecular methods using 16SrRNA gene sequencing, antibacterial activity results of AgNPs showed that it increases with increasing of AgNPs concentration, and the most sensitive species to silver particles was *Alcaligenes faecalis* AL1bacteria.

Keywords: Antibacterial activity, Biogenic synthesis, AgNPs, aqueous extract, *Typha domingensis*.

Introduction:

Taniguchi Norio was the first to coin the term nanotechnology in 1974, which means nanomaterials with dimensions 1-100 nanometers. Nanoscience includes many branches of knowledge and has many applications in the medical and pharmaceutical fields[1]. Due to the unique characteristics and the good inhibitory effect of silver nanoparticles to inhibit pathogenic bacteria, researchers tend to use nanoparticles, especially silver nanoparticles, as alternatives to the antibiotics currently used in the treatment of diseases resulting from infection with antibiotics resistant bacteria, because of the increase of bacterial resistance to available antibiotics[2]. In the past, silver was used to prevent or inhibit human pathogenic microorganisms, due to its ability to fight these organisms. Silver nanoparticles have many applications in the medical field, where silver in its various forms was used in treating burns or skin infections and as dressings. In industrial application, it was used in many household appliances such as refrigerators and other industrial applications[3]. Because of the increase in bacterial resistance to

many antibiotics, it is necessary to search for a modification in the antibacterial compounds to overcome the bacterial resistance. Here, the role of nanoparticles, especially silver nanoparticles emerges as one of the most promising sources in killing or inhibiting these microorganisms, due to a variety of properties that allow them to do so. By modifying the structure of silver nanoparticles and creating them in nanoscale sizes, which enhances their surface area and improves their ability to bind microbes, it is feasible to increase the silver nanoparticles' antibacterial activity. Numerous researchers have examined the silver nanoparticles' antibacterial activity against multi-drug resistant (MDR) and susceptible strains of bacteria, and it has been demonstrated that these particles are effective weapons against MDR bacteria[4]. There are typically two techniques to create nanoparticles. The first process, which works from top-down, relies on the solid form of the element silver to create nanoparticles, which are then physically prepared using techniques like grinding or laser ablation. By converting metallic components like silver into nanoparticles through chemical or biological processes, the second technique of nanoparticle synthesis consists of bottom-up approaches and incremental procedures[5, 6]. Nanoparticles are created via physical and chemical processes, but these procedures are financially expensive and consume energy in addition to that, toxic chemicals are used in the production, so they are excluded in medical applications and thus the trend to use biological sources such as plants, bacteria, fungi, and algae in the second procedure, which characterized as environmentally friendly, inexpensive and highly efficient in reducing nanoparticles, especially silver nanoparticles[7]. MDR bacteria constitute one of the most problems facing the treatment of bacterial infections, and therefore the physician uses a higher dose of antibiotics to reduce these infections, and this will lead to the emergence of side effects accompanying these treatments such as hypersensitivity or reduced patient's immunity, and here the role of nanoparticles as a possible alternative for the treatment of this antibiotic-resistant bacteria[8]. Therefore, living organisms such as plants have been reported to the biosynthesis of nanoparticles due to the presence the biomolecules that act as reductants and stabilized silver nanoparticles[9]. T. domingensis belongs to the family Typhaceae it is distributed in swamps, shallow ponds, and salt marshes in southern Iraq. In order to create qurraid, a yellow, amorphous food, pollen grains were compressed with water vapor. This substance is known locally as qurraid, is sold in southern markets, and eaten by people[10]. The current study aimed to use the aqueous extract of T. domingensis pollen grains (qurraid) to create silver nanoparticles and test their antibacterial efficacy against some bacteria, because it is cheap, edible, non-toxic, and widely available in the local markets of Basra Governorate.

Materials and Methods:

Preparation of plant extract

Qurraid was purchased and delivered to the laboratory from a local market in the Basra Governorate, and used as a base material in preparing the plant extract by adding 50 grams of the plant material to 250 ml of distilled water, mixed well with an electric mixer to break down the pollen wall, transferred to reflex. The extraction was done for 18 hours; the extract was given time to cool at ambient temperature before passing through NO.1 Whatman filter paper. Stored at 4°C until needed. To know the chemical composition of the biomolecules of the plant extract, a GC/MS analysis was performed.

Identification of bacteria

Bacteria used in the current study were diagnosed based on their phenotypic characteristics, and biochemical and molecular methods. The molecular study was carried out by extracting genomic DNA from Presto™ Mini gDNA Bacteria Kit in accordance with the manufacturer's prescriptions. The presence of genomic DNA was confirmed by transferring it onto agarose gel at a concentration of 0.8%. The genomic DNA obtained from studied bacteria three of which were Gram-negative *Escherichia coli* A1, *Escherichia coli* A2, *Alcaligenes faecalis* AL1, and the fourth was Gram-positive bacteria *Bacillus zanthoxyli* B1 was used to amplify the universal 16SrDNA primer 27F (5'-AGA GTT TGA TCC TGG CTC AG - 3') and 1492R (5'- GGT TAC CTT GTT ACG ACT T - 3') was used to amplify about 1500bp *16SrRNA* gene. [11]. PCR reaction was performed using the following mixture: five µl of genomic DNA, 12.5 µl (Promega) master mix, and one µl (10pmol) of each primer, then complete the volume to 25 µl using distilled water free of nuclease. The following program was used for amplification: Denature the DNA for 5 minutes at 95 ^{0}C, then 35 cycles of denaturation at 95^{0}C for 1 minute, annealing at 55^{0}C for 30 seconds, and extension at 72^{0}C for 1.5 minute. Final 5-minutes extension at 72^{0}C. The PCR product was detected by 2% agarose gel electrophoreses supplied with 90 volts for 30minutes, and then sent to the Macrogen Company (Korea) for sequencing. Sequence alignment was aligned according to NCBI Blast.

Synthesis of silver nanoparticles

AgNPs were created by combining an aqueous extract of *T. domingensis* pollen grains qurraid with 100 ml of $AgNO_3$ solution at a concentration of 1

mM in the conical flask at room temperature. Then the mixture was heated to 80 degrees Celsius for two hours and incubated in the dark at room temperature for 24 hours. The color of the reaction mixture shifted from bright yellow to dark brown to show the creation of silver nanoparticles. The resulting solution was used to evaluate the antibacterial activity of AgNPs against Gram negative and Gram positive bacteria, also this solution was used for characteristics of synthesized AgNPs

Characterization of silver nanoparticles

A variety of methodologies were used in the current investigation to confirm the formation of AgNPs, which are UV-visible spectrophotometer (UV-vis) with a wavelength of 300-800nm were done in Biology department, College of Education for pure science, Fourier transform infrared (FTIR) was completed in chemistry department, College of Education for pure science to find out the active groups in the extract, Scanning Electron Microscopy （SEM）to find out the size and shape of nanoparticles along with Elemental analysis, (EDX) analysis to verify the presence of silver, and X-ray diffraction (XRD) to determine whether qrdAgNPs are crystalline done in Physics department, College of Science[5] .

Antibacterial activity of AgNPs

Silver nanoparticles created by aqueous extract of T. domingensis pollen grains (qurraid) were examined for it is antibacterial properties against four species of bacterial species, three of which were Gram-negative *Escherichia coli* A1, *Escherichia coli* A2, *Alcaligenes faecalis* AL1, and the fourth was Gram-positive bacteria *Bacillus zanthoxyli* B1 by agar well diffusion method on Mueller Hinton agar, briefly: The bacterial suspension was prepared at the age of 18-24 hours at a temperature of 37 °C and the growth was compared with 0.5 McFarland tube standard. The MHA dishes were impregnated with a cotton swab and then five wells were made by cork porer. The wells were filled with different concentrations of AgNPs 1000, 500, 250, 125, and 62.5 µl/ml. The diameter of the inhibitory zone was measured in mm, after dishes were incubated at 37 Celsius for 24 hours[5].

Results and Discussion:

GC/MS analysis results

The results of the GC-MS spectrometry analysis of the aqueous extract of the qurraid revealed 88 compounds, as illustrated in Fig. 1 and Table 1.The major compounds were recorded with their ratio: 5-Hydroxymethylfurfural with RT% (13.6196) , 3-Deoxy-d-mannoic lactone (6.4285), alpha.-L-lyxo-Hexopyranoside, methyl 3-amino-2,3,6-trideoxy-(4.264), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-(3.2078), 1,3-Methylene-d-arabitol (3.1257). When examining Fig. 1, it is noted that there are many other peaks with RT% close to the major component peaks, and this indicates that the aqueous extract of qurraid contained many other compounds, which, in addition to the previous compounds, act as a reduction, capping, and stabilization of silver nitrate to silver nanoparticles (AgNPs). Several studies recorded the presence of the 5-Hydroxymethylfurfural compound in the extracts of some plants, as in the study of Yassin *et al.* [12] those who mentioned the existence of this compound in the extract of Punica granatum L. In terms of its role in the manufacturing of silver nanoparticles, a study conducted by Asmat-Campos *et al.* [13] they recorded that the ethanolic extract of blueberry *Vaccinium corymbosum* L. containing this compound has a good role in preparing those nanoparticles. 5-Hydroxymethylfurfural was also recorded in the methanol extract of *Clerodendrum viscosum*[14], in addition, to the ethanolic extract of the plant *Geodorum densiflorum*[15], and extracts of *Punica granatum*. In addition, it was recorded to have antibacterial, antioxidant and antiproleferative activity[12]. The second compound was 3- Deoxy-d-mannoic lactone, which was also recorded in the methanolic extract of *Clerodendrum viscosum*, and it has antimicrobial activity[14], ethanol extract from *Geodorum densiflorum* [15] and in *Moringa oleifera* leaves extracts[16]. It also has antimicrobial activity[15]. 5-Hydroxymethylfurfural (aldehyde sugar), alpha.-L-lyxo-Hexopyranoside, methyl 3-amino-2,3,6-trideoxy-(glycoside), glycerin, xylitol (alcohol), 1,3-Methylene-d-arabitol (carbohydrate), sorbitol (alcohol sugar), [1,2,3,4]Tetrazolo[1,5-b][1,2,4] triazine, 5,6,7,8-tetrahydro-(amine) and others, responsible in the formation of silver nanoparticles[16]. Plants have many biomolecules that reduce silver nitrate to silver nanoparticles, in addition to sticking to these nanoparticles, making them preferred in the manufacture of these nanoparticles[17].

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Vial Number: 3
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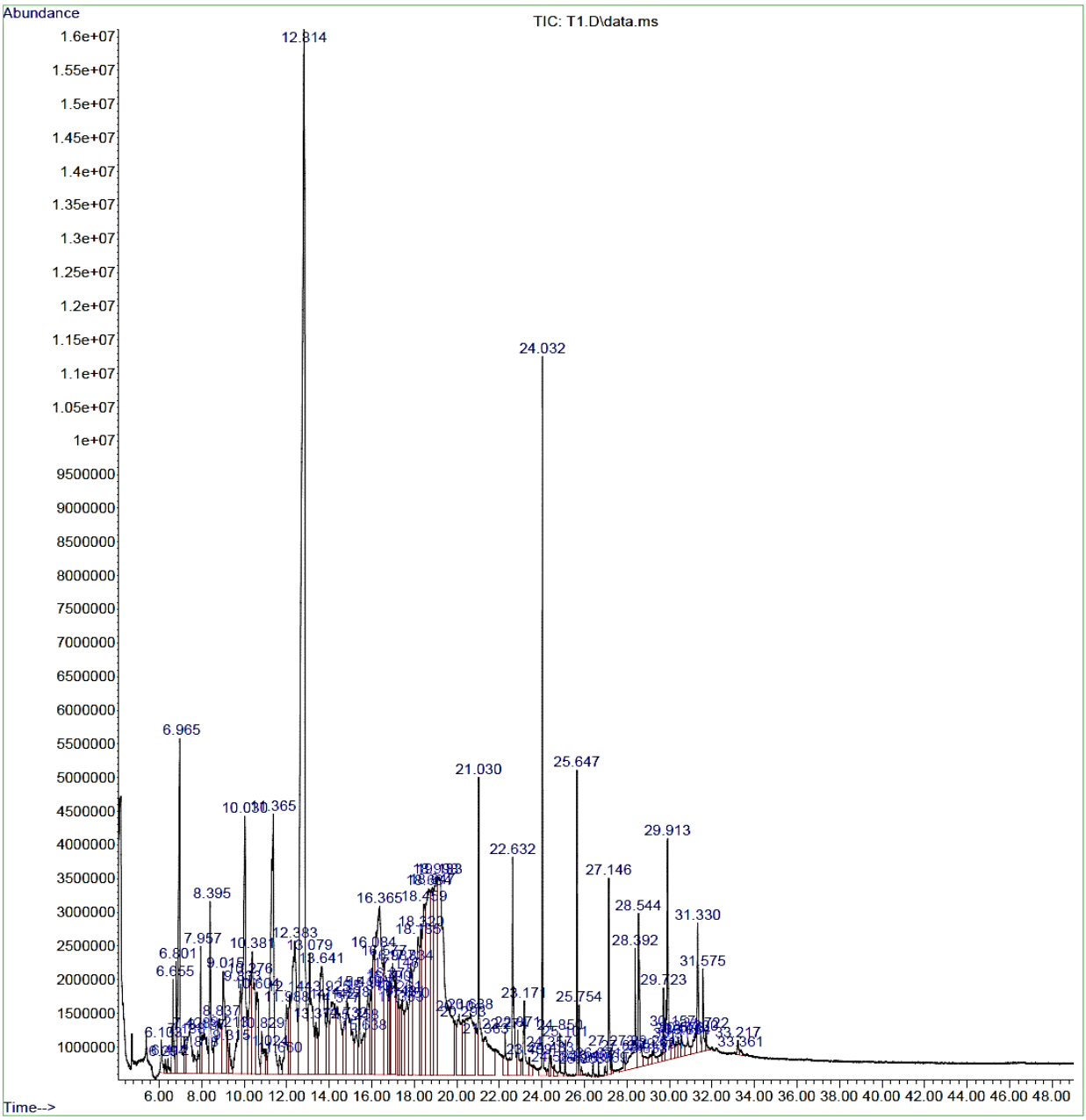

Figure 1. GC/MS graph of aqueous extract of *Typha domingensis* pollen grains (qurraid).

Table 1. Component detected in the aqueous extract of *T domingensis* pollen grains (qurraid).

Peak	R.T.	Area	Area %	Library/ID
1	6.103	25893546	0.2067	2-Imidazolidinethione
2	6.294	6585674	0.0526	Thiophene, tetrahydro-3-methyl-
3	6.412	9448676	0.0754	Cyclopentanethiol
4	6.655	57713924	0.4608	2(5H)-Furanone
5	6.801	53443271	0.4267	Oxime-, methoxy-phenyl-_
6	6.965	263933849	2.1073	1,2-Cyclopentanedione
7	7.188	25199090	0.2012	6-Deoxy-D-mannono-4-lactone
8	7.428	120660386	0.9634	2-Furancarboxaldehyde, 5-methyl-
9	7.818	21481310	0.1715	Pentanoic acid, ethyl ester
10	7.957	55571290	0.4437	2-Pyrrolidinethione

11	8.137	90078138	0.7192	2-Chloroethyl 1-propynyl sulfoxide
12	8.395	113091657	0.9029	2H-Pyran-2,6(3H)-dione
13	8.837	145999453	1.1657	Ethanamine, N,N-dimethyl-2-[2-[(trimethylsilyl)oxy]ethoxy]-
14	9.015	128489194	1.0259	1,2-Cyclopentanedione, 3-methyl-
15	9.213	23267616	0.1858	5-Dodecanone
16	9.315	22294109	0.178	Isothiazole, 3-methyl-
17	9.833	151235232	1.2075	[(2-Amino-3-hydroxypropanoyl)amino]acetic acid
18	10.03	303401323	2.4224	Furaneol
19	10.276	119680383	0.9555	Ethane, 1,1,1-triethoxy-
20	10.381	178926217	1.4286	Xylitol
21	10.604	96601090	0.7713	Glycerin
22	10.829	47813664	0.3817	Dimethylvinyl(n-pentyl)silane
23	11.024	17708329	0.1414	Acetylene dicarboxamide
24	11.365	401779044	3.2078	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
25	11.66	16183269	0.1292	4-Methoxypyridine-2-carboxamide
26	11.988	88064243	0.7031	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-
27	12.144	75183649	0.6003	D-Arabinitol
28	12.383	285631073	2.2805	Cyclopentanol, 2,4,4-trimethyl-
29	12.814	1705854685	13.6196	5-Hydroxymethylfurfural
30	13.079	198038104	1.5811	3,4-Dihydroxyacetophenone
31	13.372	62407470	0.4983	1-Ethyl-2-hydroxymethylimidazole
32	13.641	257992842	2.0598	Allyloxy-dimethyl-silane
33	13.925	76647878	0.612	2(3H)-Furanone, dihydro-3-(thioacetyl)-
34	14.142	184702388	1.4747	3-Deoxy-d-mannonic acid
35	14.377	160207778	1.2791	(2,5-Dimethyl-[1,3]dioxan-4-yl)-methanol
36	14.732	54582256	0.4358	Cyclohexane, 1,2,3-trimethyl-
37	14.828	181658285	1.4504	3-Acetyl-2,5-dimethyl furan
38	15.258	65284497	0.5212	[1,2,3,4]Tetrazolo[1,5-b][1,2,4]triazine, 5,6,7,8-tetrahydro-
39	15.419	66345681	0.5297	Pyrimidine, 2,4,5-triamino-
40	15.638	55960685	0.4468	4-Chloro-3-n-hexyltetrahydropyran
41	15.81	113353091	0.905	Cyclopentanone, 2-methyl-3-(1-methylethyl)-
42	15.938	58265908	0.4652	1-Bromoeicosane
43	16.084	122240875	0.976	Ethyl methyl ethylphosphonate
44	16.365	534069330	4.264	.alpha.-L-lyxo-Hexopyranoside, methyl 3-amino-2,3,6-trideoxy-
45	16.577	174190239	1.3907	Pentadecanoic acid
46	16.79	49796318	0.3976	Methyl 6-O-[1-methylpropyl]-.beta.-d-galactopyranoside
47	16.873	67720349	0.5407	3-Methylmannoside
48	16.987	162035302	1.2937	5,6-Dimethoxy-1-indanone
49	17.146	95214130	0.7602	Diethyl Phthalate
50	17.261	57981116	0.4629	1,4-Dimethyl-2,3-dinitrobenzene
51	17.353	48744676	0.3892	Octan-2-one, 3,6-dimethyl-
52	17.431	73428471	0.5863	Cyclopropane, 1-(E)-hexylidene-2-trimethylsilyl-
53	17.65	112761716	0.9003	Cyclododecanone, 2-methylene-
54	17.834	154899380	1.2367	(E)-Hexadec-9-enoic acid
55	18.185	357847064	2.8571	Cyclopropane, 1-methylene-3-methyl-2-trimethylsilyl-
56	18.32	122960874	0.9817	3-Deoxy-d-mannoic lactone
57	18.459	222168090	1.7738	Silane, dimethyl[(methylsilyl)methyl]-
58	18.684	391490862	3.1257	1,3-Methylene-d-arabitol
59	18.847	219695693	1.7541	Ethyl 2,3-epoxybutyrate
60	18.998	290087274	2.3161	3-Deoxy-d-mannoic lactone
61	19.183	805171089	6.4285	3-Deoxy-d-mannoic lactone
62	20.104	118277807	0.9443	D-glycero-D-manno-Heptitol
63	20.293	65324147	0.5216	D-glycero-D-manno-Heptitol
64	20.638	245509465	1.9602	Sorbitol
65	21.03	174011890	1.3893	n-Hexadecanoic acid
66	21.363	146934281	1.1731	1-Deoxy-d-mannitol
67	22.274	39762130	0.3175	Heneicosane
68	22.632	152775255	1.2198	9,12-Octadecadienoic acid (Z,Z)-
69	22.871	36183248	0.2889	Octadecanoic acid
70	23.171	62199157	0.4966	Butyric acid, 2-(3-oxo-3H-benzo[d]isothiazol-2-yl)-
71	23.409	21887522	0.1748	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
72	24.032	190089903	1.5177	Hexadecane, 2,6,10,14-tetramethyl-
73	24.357	10954038	0.0875	Z,Z-3,13-Octadecedien-1-ol
74	24.433	14755485	0.1178	2-(3-Hydroxybutyl)cyclooctanone

75	24.584	9488524	0.0758	Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-
76	24.85	16735451	0.1336	Tetracosane
77	25.101	11071279	0.0884	Docosanal
78	25.647	93131890	0.7436	Pentacosane
79	25.754	22348200	0.1784	1-Nonadecene
80	25.868	4510881	0.036	Adamantane, 1-isothiocyanato-3-methyl-
81	26.407	5252756	0.0419	Eicosane
82	26.671	4389261	0.035	Tetradecanal
83	26.969	5251686	0.0419	Nonadecyl trifluoroacetate
84	27.146	78971676	0.6305	Heptacosane
85	27.273	9780927	0.0781	2-Pentacosanone
86	27.854	13456407	0.1074	Octacosane
87	28.392	96311490	0.769	Nonacos-1-ene
88	28.544	103512941	0.8265	Tetracosane

Identification of bacteria

Morphological, biochemical, and molecular methods (polyphasic taxonomy) were used to identify four bacterial isolates included in this study. Molecular identification was done by amplification of the 16SrRNA gene by PCR technique. All bacterial isolates gave positive results and this was confirmed using gel electrophoresis. The resulting gene size was about 1500bp (Fig. 2). Four species were identified in the current study three of which were Gram-negative, and the fourth was Gram-positive bacteria as shown in Table 2, with their accession numbers.

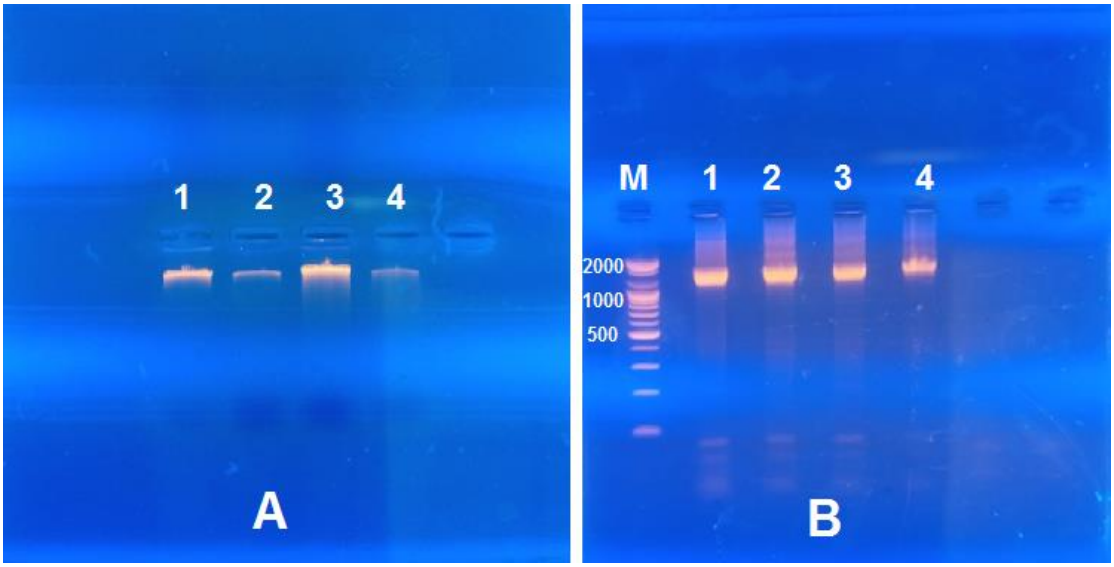

Figure 2. Electrophoresis of agarose gel for *16SrRNA* gene: (A) genomic DNA; (B) PCR product, 1:(*E.coli* A1), 2:(*E.coli* A2), 3:(*Bacillus zanthoxyli* B1), 4:(*Alcaligenes faecalis* AL1), M:(2000bp) ladder.

Table 2. The identification results of the studied bacteria by traditional methods and 16S rRNA sequences

	Bacteria	Accession numbers (this study)	Gram's stain	3% KOH test	Cell shape
1	*Escherichia coli* A1	OP040007.1	-ve	+	rod
2	*Escherichia coli* A2	OP038673.1	-ve	+	rod
3	*Alcaligenes faecalis* AL1	OP040006.1	-ve	+	rod
4	*Bacillus zanthoxyli* B1	OP040008.1	+ve	-	rod

If the identification of bacteria is based on phenotypic or biochemical tests only without molecular methods, it will give an incomplete picture of the classification of these organisms[18, 19].

Characterization of AgNPs

1-UV-Visble spectrophotometer

The entity of the surface plasmon resonance of AgNPs caused the solution's color to change from yellow to dark brown, to prove the creation of silver

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nanoparticles (Fig. 3). In this research, the synthesized AgNPs were proved by UV-Vis results and the highest peak was recorded at 400nm (Fig. 4). UV-visible spectroscopy was used to measure plasmon absorbance, which causes a shift in the solution's color during the manufacturing process of silver nanoparticles. The plasmon resonance, which recorded the highest peaks near 400 nm, indicates the formation of silver nanoparticles[20].

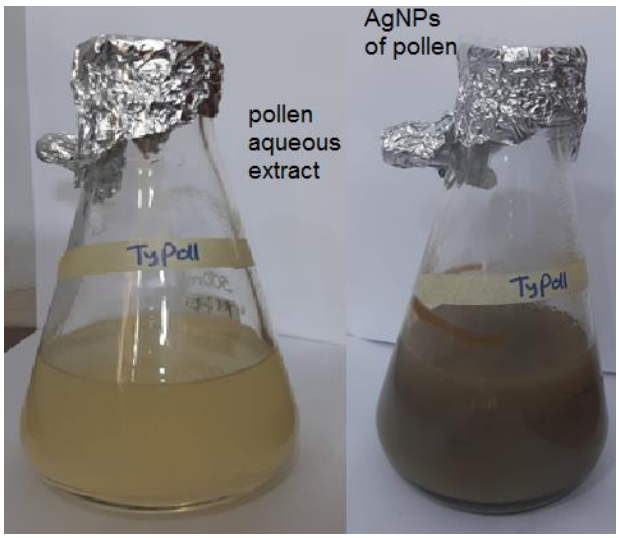

Figure 3. qrdAgNPs composed by aqueous extract of *T. domingensis* pollen grains (qurraid).

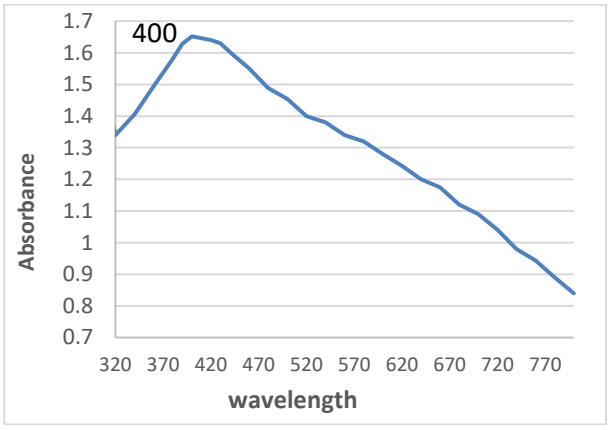

Figure 4. UV spectrum of the synthesized silver nanoparticles by aqueous extract of *T. domingensis* pollen grains (qurraid).

2-Fourier infrared spectroscopy analysis

Fourier infrared spectroscopy analysis (FTIR) spectroscopy was carried out to know the active biomolecules in the aqueous extract of T. domingensis pollen grains (qurraid), which are in charge of silver nitrate reduction and stabilization of AgNPs. Figs. 5 and 6 showed the FTIR spectra of the plant extract and nanoparticle mixture, recorded in this study respectively. The results of the FTIR analysis of the aqueous extract of T. domingensis pollen grains recorded the presence of the following peaks 3390, 3369, 2927, 1618, 1450, 1421, 1261, 1209 cm-1. The peaks at 3390 and 3369 cm^{-1} were referred to the OH group as the primary alcohols, 2927 cm-1 referred to CH of long aliphatic compounds, 1618 cm-1 attributed to NH of amide I of protein, 1450and 1421refer to C-C alkene group. While the FTIR analysis results for qrdAgNPs synthesized in the current study recorded the following peaks at 3414 cm-1 referring to OH group, 2926 and 2854 cm-1 was CH of the aliphatic compound, 1637 and 1620 cm-1 attributed to NH of amide I of protein, and 1454, 1415 cm-1 refer to C-C of alkene group. 3390 shifted to 3414 cm-1, 2927 to 2926 cm-1, 11618 to 1637 cm-1, 1450 to 1454 cm-1, and 1421 to 1415 cm-1 in FTIR peaks of qrdAgNPs. This shifting in peaks was also recorded by[21]. The results of the current study recorded the common peaks that indicate the presence of active biomolecules that helped to reduce and stabilize silver nitrate to AgNPs such as those that are more than 3000 cm-1, indicating the presence of the OH group, which may be found in alcohol or phenol. Also, the peaks at 1618, 1735, and 1637 cm-1 confirm the existence of the amide I compound within the protein structure. These compounds were to be crucial in the stability and reduction of silver nitrate into silver nanoparticles [22].

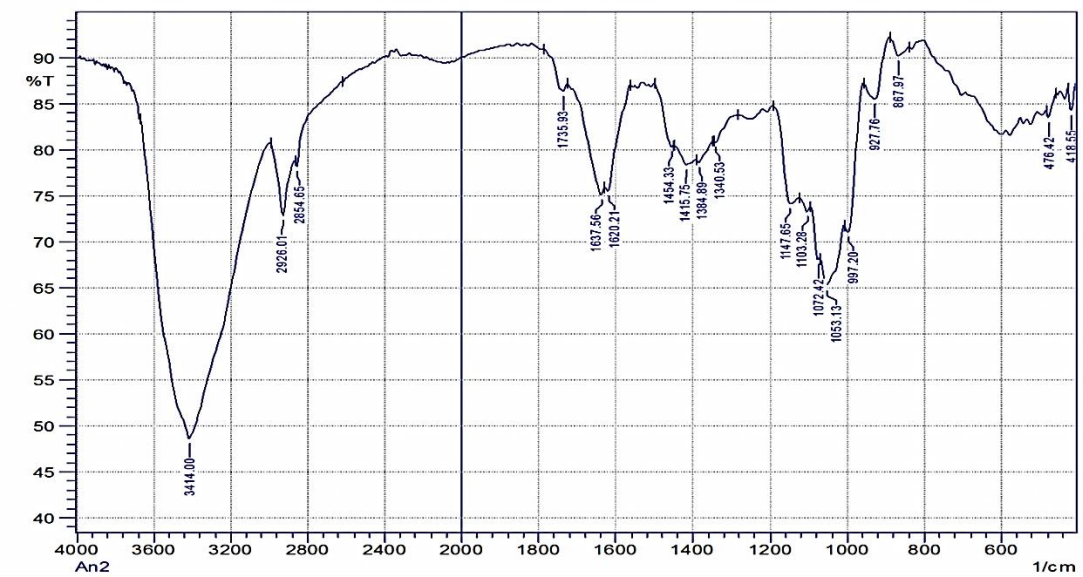

Figure 5. FTIR spectrum of the qrdAgNPs manufactured by aqueous extract of *T. domingensis* pollen grains (qurraid).

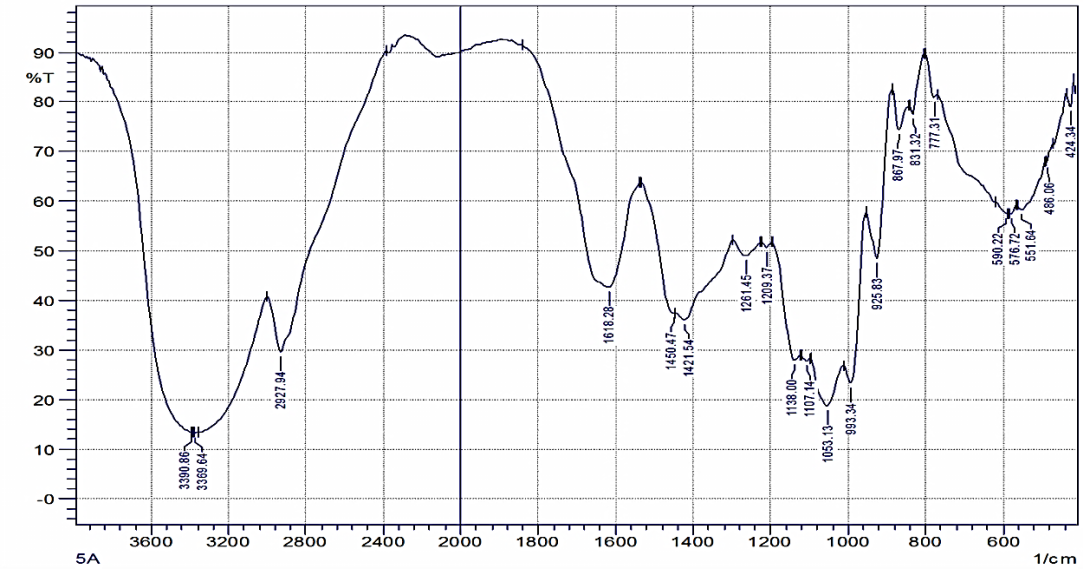

Figure 6. FTIR spectrum of aqueous extract of *T. domingensis* pollen grains (qurraid).

XRD analysis

An XRD analysis was performed to prove the crystalline nature of qrdAgNPs synthesized by the aqueous extract of *T. domingensis* pollen grains (qurraid). XRD diffraction peaks at 2Ø values 27^0, $31^{0,}$ and 450 were assigned to (211), (300), and, (330) face-centered cubic planes (FCC) respectively. Sharp peaks recorded in Fig.7 demonstrate that biomolecules constituent of the qurraid aqueous extract act as capping and stabilizing of AgNPs[23]. Similar results were reported in a study by Rautela *et al.*[24] they were recorded XRD patterns of silver nanoparticles and confirmed the crystalline nature of AgNPs. The values of 2θ were 38.05, 44.23, 64.41, and 76.66 , which are attributed to (1 1 1), (2 0 0), (2 2 0) and (3 1 1) levels of reflection of the cubic face of silver. When referring to Fig. 7, it is noted that there is an additional peak at 2Ø marked with a star, the presence of this peak was resulting from the plant extract that contains organic compounds and it is responsible for reducing and stabilizing silver ions into silver nanoparticles[25].

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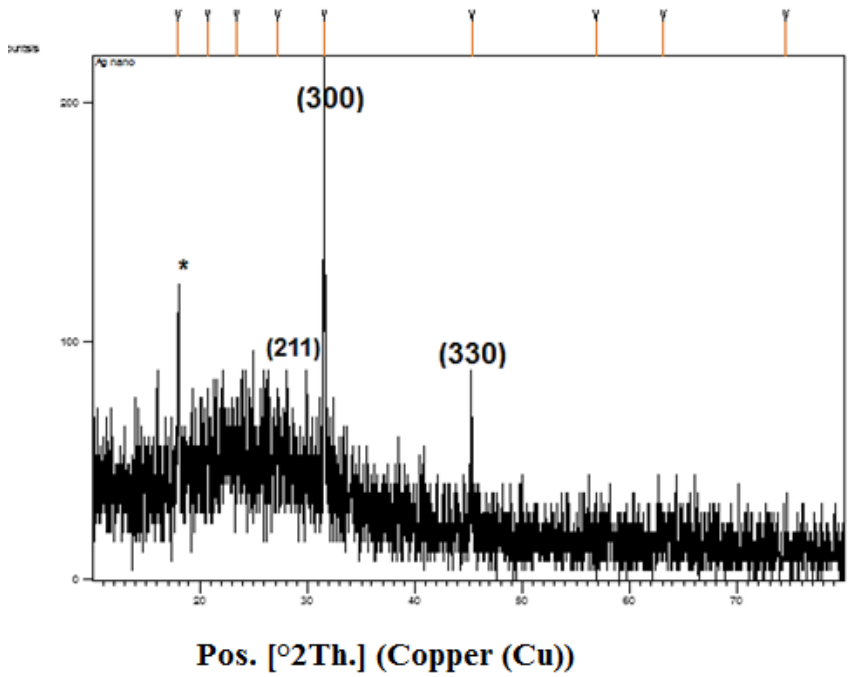

Figure 7. XRD micrograph of the qrdAgNPs synthesized by aqueous extract of *T. domingensis* pollen grains (qurraid).

Scanning Electron Microscopy (SEM)

The formation of qrdAgNPs was confirmed after turning the color of the solution to dark brown and examined by UV-vis spectrophotometer, then those particles were examined with an SEM to know the shape and size of the biogenic synthesized qrdAgNPs manufactured in the current study using the aqueous extract of T. domingensis pollen grains (qurraid), and their shapes were spherical, with dimensions ranging between 20-70 nm as shown in Fig. 8. The same results were recorded by other researchers such as Nasser *et al*. 2020 [26] they reported that the shape of AgNPs synthesized in their study was spherical with dimensions ranging between 32-46 nm. Additionally, Tufail *et al*. 2022 [26] noted that the AgNPs were spherical in shape and ranged in size from 30 to 100nm.

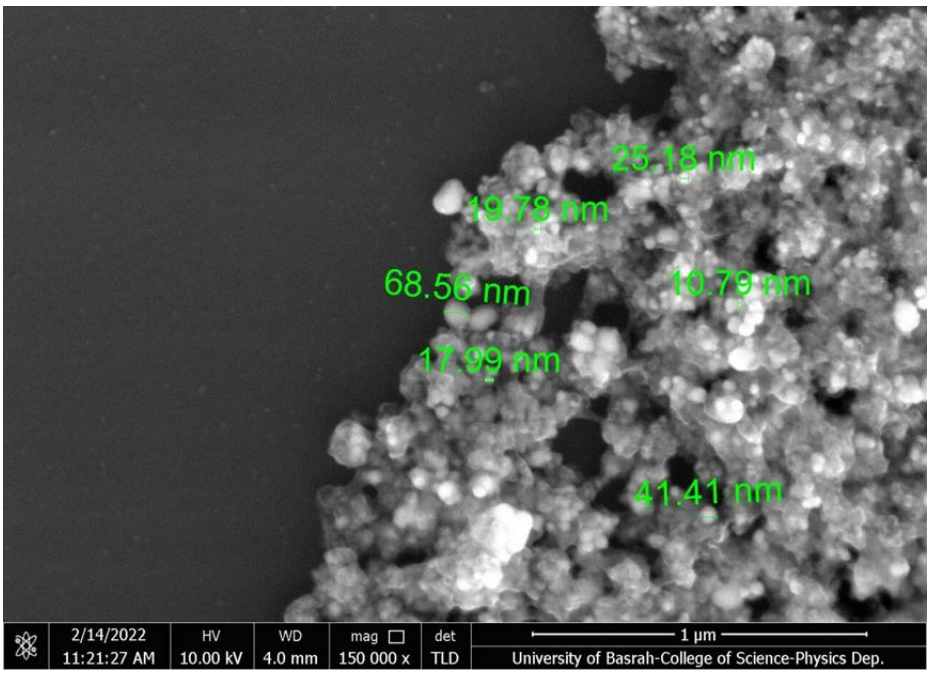

Figure 8. SEM micrograph of the synthesized qrdAgNPs by aqueous extract of *T. domingensis* pollen grains (qurrait).

Energy Dispersive X-ray Spectroscopy (EDX)

To be certain, the chemical composition of qrdAgNPs, EDX analysis was performed, and it appeared that they were composed of the element silver, as shown in Fig. 9. These findings are consistent with the study of Femi-Adepoju *et al.* [27] they found that the nanoparticles were mostly composed of silver.

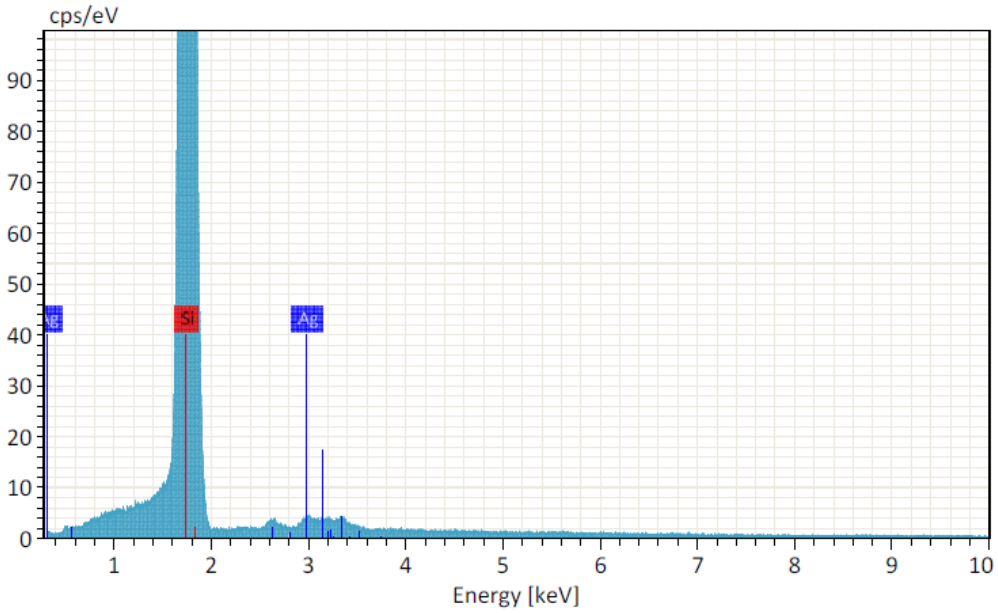

Figure 9. EDX micrograph of the synthesized qrdAgNPs by aqueous extract of *T. domingensis* pollen grains (qurraid).

Antibacterial activity of qrdAgNPs

The activity of the qrdAgNPs produced by the aqueous extract of *T. domingensis* pollen grains (qurraid) to inhibit tested bacteria was assessed using the agar well diffusion method. The results of the current study showed that the concentration of 1000 µg/ml was the most effective against all bacterial species, meaning that the anti-bacterial activity increases with increasing concentration of this qrdAgNPs. Same results were recorded by Hasson *et al.* [28] and Shareef *et.al.* [29] and in their study. In addition, *Alcaligenes faecalis* AL1 bacteria was the most sensitive to these nanoparticles as shown in (Table 3 & Fig.10).

Table 3. Antibacterial properties of qrdAgNPs synthesized by aqueous extract of *T. domingensis* pollen grains (qurraid).

Bacteria	AgNPs concentration (µg/ml)				
	1000	500	250	125	62.5
Escherichia coli A1	13*	13	12	0	0
Escherichia coli A2	15	15	12	0	0
Alcaligenes faecalis AL1	15	15	12	0	0
Bacillus zanthoxyli B1	15	14	12	11	0

*: inhibition zone measured in mm.

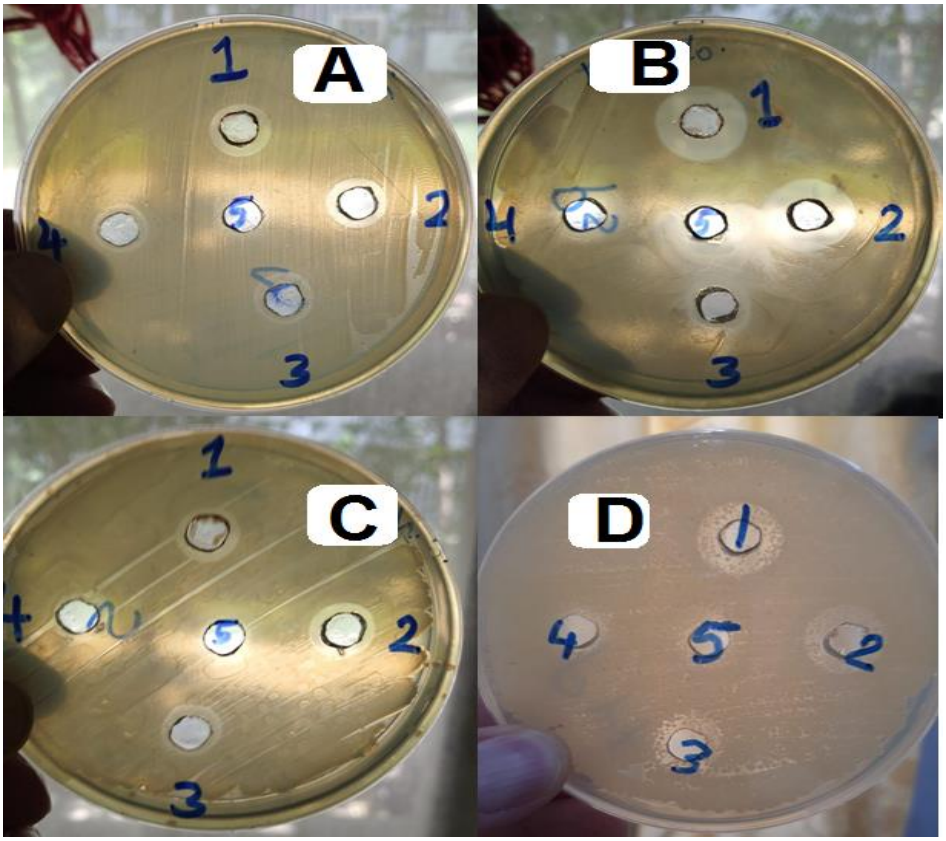

Figure 10. Antibacterial activity of qrdAgNPs synthesized by aqueous extract of *T. domingensis* pollen grains (qurraid), A: *Escherichia coli* A1, B: *Escherichia coli* A2, C: *Alcaligenes faecalis* AL1, D: *Bacillus zanthoxyli* B1, qrdAgNPs concentrations (1:1000, 2:500, 3:250, 4:125, and 5:62.5 µ/ml).

The antibacterial activity of AgNPs was mostly proportional to their size. Although, the mechanism by which nanoparticles, especially silver nanoparticles, can inhibit bacterial growth is still not well known. However, it is believed that silver nanoparticles may emission silver ions frequently, which is the most accepted opinion until now. These positively charged nanoparticles are drawn to the negatively charged cell wall and envelope, changing the permeability of the cell membranes and disrupting the cell envelope, allowing silver nanoparticles to enter the cell and inhibit respiratory enzymes, as a result, reactive oxygen species (ROS) are formed, which inhibit ATP production. In addition, it had the ability to bind to the ribosomes and prevent it from binding to mRNA, and thus inhibiting protein synthesis. All of these changes lead to cell lysis[30].

Conclusion:

AgNPs synthetized in this study had good antibacterial activity against four bacterial species, three of which were Gram-negative and the fourth was Gram-positive bacteria, antibacterial activity results of AgNPs showed that it increases with increasing of AgNPs concentration, and the most sensitive species to these particles was *Alcaligenes faecalis* AL1 bacteria.

Authors' Declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Basrah.

Authors' Contributions Statement:

A. A., F.J., and F.A. contributed to the planning and execution of the study, the findings analysis, and the paper writing.

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التصنيع الأخضر لدقائق الفضة النانوية باستخدام المستخلص المائي لحبوب لقاح نبات البردي (الخريط) وتقييم فعاليته الضد بكتيرية *Typha domingensis* Pers.

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الخلاصة:

تم الدراسة الحالية استخدام المستخلص المائي لحبوب اللقاح (الخريط) لنبات البردي *Typha domingensis* Pers. لمعرفة قابليته في تصنيع دقائق الفضة النانوية. والخريط هو عبارة عن مادة غذائية صفراء اللون شبه صلبة, تباع في اسواق البصرة وتؤكل من قبل السكان المحليين اساسها هو حبوب لقاح نبات البردي *Typha domingensis* بعد ان تكبس وتعامل ببخار الماء. اجري تفاعل الـ Gas chromatography–mass spectrometry (GC-MS) لمعرفة المركبات الفعالة في المستخلص المائي للخريط. اختبرت قابلية المستخلص المائي للخريط في تصنيع دقائق الفضة النانوية وتم الاستدلال على تكون دقائق الفضة النانوية بتحول لون خليط التفاعل من اللون الاصفر الى اللون البني.تم توصيف الدقائق المصنعة بواسطة UV-Visو FTIRو XRDو SEMو EDX . ثم اختبرت فعالياتها المضادة للبكتريا بواسطة الانتشار بالحفر على الآكار Agar Well Diffusion Method. اظهرت نتائج الـGC/MS للمستخلص المائي للخريط هو وجود المركبات الأتية:5-Hydroxymethylfurfural اذ بلغت الـRT% له (13.6196) يليه المركب 3-Deoxy-d-mannoic lactone وبلغت الـRT% له (6.4285) والمركب alpha.-L-lyxo-Hexopyranoside, methyl 3-amino-2,3,6-trideoxy ذو RT% (4.264)والمركب 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl وله RT%هي(3.2078) والمركب-1,3 Methylene-d-arabitol له RT% (3.1257).تم التاكد من تكون دقائق الفضة النانوية وذلك بالطرق الطيفية حيث سجلت القمة 400nm بطيف الـ UV-Vis والطبيعة المعدنية لتلك الدقائق تم بواسطة تحليل الـ XRD. اضافة الى ذلك فقد كانت الدقائق النانوية كروية الشكل وباحجام تراوحت بين20 -70 نانوميتر. وبينت نتائج الـEDX ان التركيب الكيميائي للدقائق النانوية المصنع في الدراسة الحالية هو الفضة.اختبرت قابلية دقائق الفضة النانوية المحضرة بواسطة المستخلص المائي للخريط ضد اربع انواع بكتيرية والتي شخصت بالطرق التقليدية والجزيئية باستخدام تتابعات الجين *16SrRNA* ,ثلاث منها سالبة لصبغة كرام وهي *Escherichia coli* A1 و*Escherichia coli* A2 و *Alcaligenes faecalis* AL1 والرابعة موجبة لصبغة كرام وهي*Bacillus zanthoxyli* B1 .

الكلمات المفتاحية: الفعالية ضد بكتيرية,التصنيع الحيوي ,دقائق الفضة النانوية, *المستخلص المائي*,*Typha domingensis*