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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. Ourraid is a semi-solid vellow 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 gurraid aqueous extract. The ability of the aqueous extract of gurraid 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-Deoxyd-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 fields¹. 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 antibiotics². 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 applications³. 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 bacteria⁴. 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 nanoparticles⁷. 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 bacteria⁸. 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 nanoparticles⁹. 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 people¹⁰. 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 PrestoTM 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 Gramnegative Escherichia coli A1, Escherichia coli A2, Alcaligenes faecalis AL1, and the fourth was Grampositive 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 ⁰C, then 35 cycles of denaturation at 95⁰C for 1 minute, annealing at 55°C for 30 seconds, and extension at 72°C for 1.5 minute. Final 5-minutes extension at 72°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 AgNO₃ 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 Science⁵.

Antibacterial activity of AgNPs

Silver nanoparticles created by aqueous extract of domingensis pollen grains (qurraid) were T. 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 $62.5 \,\mu$ l/ml. The diameter of the inhibitory zone was measured in mm, after dishes were incubated at 37 Celsius for 24 hours⁵.

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-4H-Pyran-4-one, (4.264),2,3-dihydro-3,5dihydroxy-6-methyl-(3.2078), 1.3-Methylene-darabitol (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 gurraid 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 viscosum¹⁴, in addition, to the ethanolic extract of the plant Geodorum densiflorum¹⁵, and extracts of Punica granatum. In addition, it was recorded to have antibacterial, antioxidant and antiproleferative activity¹². The second compound was 3- Deoxy-dmannoic lactone, which was also recorded in the methanolic extract of Clerodendrum viscosum, and it has antimicrobial activity¹⁴, ethanol extract from *Geodorum densiflorum* ¹⁵ and in *Moringa oleifera* leaves extracts¹⁶. It also has antimicrobial activity¹⁵. 5-Hydroxymethylfurfural (aldehyde sugar), alpha.-L-lyxo-Hexopyranoside, methyl 3-amino-2,3,6trideoxy-(glycoside), glycerin, xylitol (alcohol), 1,3-Methylene-d-arabitol (carbohydrate), sorbitol [1,2,3,4]Tetrazolo[1,5-b][1,2,4] (alcohol sugar), triazine. 5,6,7,8-tetrahydro-(amine) and others, responsible in the formation of silver nanoparticles¹⁶. 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 nanoparticles¹⁷.

File :D:\MassHunter\GCMS\1\data\Dr Ali AB00D\T1.D Operator : Hassan alshawi Acquired : 05 Jul 2022 15:59 using AcqMethod HP5 ms UI Column 230 C Pulsed splitless.M Instrument : 5977A MSD Sample Name: T1 Misc Info : Vial Number: 3



Figure 1. GC/MS graph of aqueous extract of *Typha 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

1 8.13 900 No 192 2-2-minuty - polynym sunokce 13 8.57 11300 Ke 900 No 900 No<	11	0 127	00070120	0.7102	2 Chloroothyl 1 propyrnyl gylfoyida
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24 11.365 401779044 3.2078 41-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-c-methyl- 28 12.383 285631073 2.2805 Cyclopentanol, 2.4, 4-trimethyl- 29 12.814 1708854685 13.6196 S-Hydroxymethylimidarol 30 13.079 198038104 1.5811 3.4-Dihydroxyacetophenone 31 13.372 62407470 0.4983 1-Ethyl-2-hydroxymethylimidarole 31 13.925 76647878 0.612 2(31)-Foranone, dihydro-3-(thioacetyl)-silane 33 13.925 76647878 0.612 2(31)-Foranone, dihydro-3-(thioacetyl)-silane 34 14.412 184703288 1.4747 3-Docxyd-mannonic acid 35 14.377 160207778 1.2791 (2.5-Dimethyl-[1.3]dioxan-4-yl)-methanol 36 14.732 5458255 0.4368 4-Chloro-3-n-hexyltetahydropyran 36 14.53309 0.905 Cyclohexane, 1.2,3-trimethyl- 31	23	11.024	17708329	0.1414	Acetylene dicarboxamide
25 11.66 16183269 0.1292 4-Methoxypyridine-2-carboxamide 26 11.988 88064243 0.7031 4H-Fyran - Aone, 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 1708854685 13.6196 5-Hydroxymethylimidazole 21 13.6179 198038104 1.5811 3.4-Dihydroxya-etophenone 21 13.641 257992842 2.0598 Allyloxy-dimethyl-silane 23 13.925 76647878 0.612 2(3H)-Furanone, dihydroxy-3-trimethyl- 24 14.42 184702388 1.4747 3-Deoxy-d-mannonic acid 25.14 160207778 1.2791 (2.5-Dimethyl-11.3)dioxan-4:yl-methanol 26 15.45285 1.4504 3-Acetyl-2,5-dimethyl furaine, 5.6.7.8-terahydro- 26 15.518 11353091 0.905 Cyclopentanone, 2-methyl-3-(1-methylethyl)- 21 15.638 55960685 0.4468 4-Chloros-3-n-hexy	24	11.365	401779044	3.2078	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
26 11.988 88064243 0.7031 4H-Pyran-4-one, 3,5-dihydroxymethyl- 27 12.144 75183649 0.6003 D-Arabiniol 28 12.383 285631073 2.2805 Cyclopentanol, 2,4,4-trimethyl- 20 12.814 1708584685 13.6196 S-Hydroxymethylimidarole 31 13.372 62407470 0.4983 1-Ethyl-2-hydroxymethylimidarole 31 13.372 62407470 0.4983 1-Ethyl-2-hydroxymethylimidarole 33 13.925 76647878 0.612 2(3H)-Furanone, dihydro-3-(thioacetyl)- 34 14.142 184702388 1.4747 3-Docxy-d-manonic acid 35 14.377 160207778 1.2791 (2,5-Dimethyl-[1,3]dioxan-4:y]-methanol 36 14.732 54852256 0.4358 Cyclopentanonic acid 7.8784740- 36 15.258 65284497 0.5212 [1,2,3,4]Terazolo,1,5-b][1,2,4]triazine, 5,6,73.8784tpdro- 41 15.518 13353091 0.905 Cyclopentanone, 2-methyl-5,4-1-Gmatonop 45 16.685	25	11.66	16183269	0.1292	4-Methoxypyridine-2-carboxamide
27 12.144 75183649 0.6003 D-Arabinitol 28 12.381 285631073 2.2805 Cyclopentanol, 2.4.4-trimethyl- 29 12.814 1708584685 13.6196 S-Hydroxymethylfurfural 30 13.079 198038104 1.5811 3.4-Dihydroxy-acetophenone 31 13.372 62407470 0.4983 1.Ethyl-2-hydroxymethylimidazole 31 13.325 76647878 0.612 2(3H)-Paranore, dihydro-3-(thioacetyl)- 34 14.142 184702388 1.4747 3-Deoxy-d-manonic acid 35 14.371 160207778 1.251 (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-1,2-dimethyl framino- 30 15.519 66345681 0.5297 Pyrimidine, 2.4.5 triamino- 41 15.81 113353091 0.905 Cyclopentanon, 2-methyl-3-(1-methylethyl)- 42 15.03 534069330 4.264 .alpha, 1-1yxo-Hexopyranoside, methyl 3-anino-2,3,6-trideoxy- 44 16.365	26	11.988	88064243	0.7031	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-
28 12.383 2285631073 2.2805 Cyclopentanol, 2,4,4-trimethyl- 30 13.079 198038104 1.5811 3,4-Dihydroxyacetophenone 31 13.372 62407470 0.4983 1-Ethyl-2-hydroxymethylimidzole 31 13.372 62407470 0.4983 1-Ethyl-2-hydroxymethylimidzole 33 13.925 76647878 0.612 2(3H)-Furanone, dhydro-3-dimethyl-silane 34 14.142 184702338 14.747 3-Deoxy-d-manonic acid 35 14.377 160207778 1.2791 (2,5-Dimethyl-1,3]dioxan-4-yl)-methanol 36 14.732 545822576 0.4358 Cyclohexane, 1,2,3-trimino- 36 14.732 5458253 1.4504 3-Acetyl-2,2-dimethyl-1,3-dioxan-4-yl)-methanol 37 14.82 181658285 1.4504 3-Acetyl-2,2-dimethyl-1,3-dioxan-4-yl)-methanol 38 15.258 65284497 0.5217 Pyrimidine, 2,4-5-triamino- 38 15.258 6528497 0.5217 Pyrimidine, 2,4-5-triamino- 41 15.638 55960685 </td <td>27</td> <td>12.144</td> <td>75183649</td> <td>0.6003</td> <td>D-Arabinitol</td>	27	12.144	75183649	0.6003	D-Arabinitol
29 12,814 1705854685 13,6196 5-Hydroxymethylfurinal 30 13,372 62407470 0.4983 1-Ethyl-2-hydroxyacetophenone 31 13,372 62407470 0.4983 1-Ethyl-2-hydroxymethylimidazole 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)-Piranore, dihydro-3-(thioaca+4y)-methanol 36 14.732 54582256 0.4358 Cyclohexane, 1,2,3-trimethyl-1 37 14.828 181658285 1.4504 3-Acetyl-2,3-dimethyl furan 38 15.528 65224497 0.5212 [1,2,3,4]treiznolo] [1,5-b][1,2,4]treizno, ej,3-trimethyl-3-(1-methyleroyran 40 15.63 55960685 0.4468 4-Chloro-3-n-hexylterahydropyran 41 15.58 55240973 0.567 Ethyl mothyl ehylphosphonate 44 16.365 534069330 4.264 .alpha-L-lyxo-Hexopyranoside, methyl 3-amino-2,3,6-trinetoxy-Preseconcancacid	28	12.383	285631073	2.2805	Cyclopentanol, 2,4,4-trimethyl-
30 13,079 198038104 1.5811 3.4-Dihydroxyacetophenone 31 13,372 62407470 0.4983 1-Ethyl-2-hydroxyacetophenone 31 13,925 76647878 0.612 2(3H)-Furanone, dihydro-3-(tinocetyl)- 34 14,142 184702388 1.4747 3-Deoxy-4-mannonic acid 35 14,377 160207778 1.2791 (2,5-Dimethyl-1,3]dioxan-4-y)-methanol 36 14,377 160207778 1.2791 (2,5-Dimethyl-1,3]dioxan-4-y)-methanol 36 14,377 160207778 1.2791 (2,5-Dimethyl-1,3]dioxan-4-y)-methanol 37 14.828 181658285 1.4504 3-Acetyl-2,5-dimethyl furan 38 15.258 65284497 0.5217 Pyrimidine, 2,4.5-riamino- 40 15.638 55960685 0.4468 4-Chloro-3-n-hexylternahydropyran 41 15.635 58265908 0.4652 1-Bromocicosane 41 16.367 6770349 0.3976 Hethyl mylphophonate 42 16.637 67720349 0.5407 3-M	29	12.814	1705854685	13.6196	5-Hydroxymethylfurfural
31 13.372 62407470 0.4983 1-Ethyl-2-hydroxymethylimidazole 32 13.641 257992842 2.0598 Allyloxy-dimethyl-silane 33 13.325 76647878 0.612 2(3H)-Furanone, dihydro-3-(thioacetyl). 34 14.142 184702388 1.4747 3-Deoxy-d-mannoine acid 35 14.371 160207778 1.2791 (2,5-Dimethyl-1,13)dioxan-4-yl)-methanol 36 14.732 54582255 0.4358 Cyclohexane, 1.2.3-trimethyl- 37 14.828 181658285 1.4504 3-Acctyl-2,5-dimethyl-11ytarine, 5,6,7,8-tetrahydro- 39 15.528 65284497 0.5212 [1,2,3,4]Tetrazolo[1,5-b][1,2,4]triazine, 5,6,7,8-tetrahydro- 39 15.648 5806085 0.4468 4-Choro-3-n-bexyltetrahydropyran 41 15.81 113353091 0.905 Cyclopentanone, 2-methyl-3-(1-methylenyl)- 42 15.938 58265908 0.4652 1-Bromoeicosane 43 16.054 72240875 0.976 Ethyl methyl-3-(1-methylenyl 3-amino-2,3,6-trideoxy- 44	30	13.079	198038104	1.5811	3,4-Dihydroxyacetophenone
32 13.641 25792842 2.0598 Ållylox-dimethyl-islane 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-4y)-methanol 36 14.372 54852256 0.4358 Cyclohexane, 1.2.3-trimethyl-1 37 14.828 181658285 1.4504 3-Accetyl-2,5-dimethyl furaite, 5,6,7,8-tetrahydro- 37 14.828 181658285 0.4468 4-Chloro-3-n-hexylterthydropyran 40 15.638 55960685 0.4468 4-Chloro-3-n-hexylterthydropyran 41 15.81 113353091 0.905 Cyclopentanone, 2-methyl-3-(1-methylethyl)- 42 16.538 53806930 0.4562 1-Boromeicosane 43 16.084 122240875 0.976 Ethyl methyl ethylphosphonate 44 16.577 174190239 1.3907 Pentadecanoic acid 45 16.577 174190239 1.29	31	13.372	62407470	0.4983	1-Ethyl-2-hydroxymethylimidazole
33 13.925 76647878 0.612 2(3H)-Furnone, 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 14.372 54582256 0.4358 Cyclohexane, 1,2,3-trimethyl- 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- 41 15.81 113353091 0.905 Cyclopentanone, 2-methyl-3-timatino-2,3,6-trideoxy- 42 15.935 58365908 0.4652 I-Bromoeicosane 1.4194194194194194194194194194194194194194	32	13.641	257992842	2.0598	Allyloxy-dimethyl-silane
3414.1421847023881.47473-Decxy-d-mannoic add3514.3771602077781.2791(2,5-Dimethyl-[1,3]dixan-4-yl)-methanol3614.732545822560.4558Cyclohexane, 1,2,3-trimethyl-13714.8281816582851.45043-Accetyl-2,5-dimethyl-13815.258652844970.5212[1,2,3,4]Tetrazolo[1,5-b][1,2,4]triazine, 5,6,7,8-tetrahydro-4015.638559606850.44684-Cchloro-3-n-bexylterthydropyran4115.811133530910.905Cyclopenanone,2-methyl-3-(1-methylethyl)-4215.938582659080.46521-Bromoeicosane4316.0841222408750.976Ethyl methyl ethylphosphonate4416.355340693304.264.alpha-1-1yxo-Hexopyranoside, methyl-3-amino-2,3,6-trideoxy-4516.5771741902391.3907Pentadecanoic acid4616.79497963180.3976Methyl 6-O-[1-methylpropyl]-betad-galactopyranoside4716.6871620353021.29375,6-Dimethoxy-1-indanone4816.9871620353021.29375,6-Dimethoxy-1-indanone5017.261579811160.46291,4-Dimethyl-2,3-dimitrobenzene5117.35487446760.3892Octan-2-one, 3,6-dimethyl-5117.451127617160.9003Cyclopropane, 1-ethylptene-3-methyl-2-trimethylsilyl-5317.851127617160.9003Cyclopropane, 1-ethylptene-3-methyl-2-trimethylsilyl-5417.84 <td< td=""><td>33</td><td>13.925</td><td>76647878</td><td>0.612</td><td>2(3H)-Furanone, dihydro-3-(thioacetyl)-</td></td<>	33	13.925	76647878	0.612	2(3H)-Furanone, dihydro-3-(thioacetyl)-
3514.3771602077781.2791 $(2,5-Dimethyl-[1,3]dioxan-4-yl)-methanol3614.732545822560.4358Cyclohexane, 1,2,3-trimethyl-3714.8281816582851.45043-Acetyl-2,5-dimethyl Iruran3815.258652844970.5212[1,2,3,4]Tetrazolo[1,5-b][1,2,4]triazine, 5,6,7,8-tetrahydro-3915.419663456810.5297Pyrimidine, 2,4,5-triamino-4115.811133530910.905Cyclopentanone, 2-methyl-3(-1-methylethyl)-4215.938582659080.46521-Bromocicosane4316.0841222408750.976Ethyl methyl ethylphosphonate4416.3655340693304.264.alphaL-lyxo-Hexopyranoside, methyl 3-amino-2,3,6-trideoxy-4516.5771741902391.3907Pentadecanoic acid4616.79497963180.3976Methyl 6-O-[1-methylproyl]-beta-dgalactopyranoside4716.873677203490.54073-Methylmanonicide4816.8731620353021.293756-Dimethoxy-1-indanone4917.146952141300.7602Diethyl Phthalate5017.261579811160.46291,4-Dimethyl-2,3-dimitroberzene5117.35348746760.3892Octano-2-methyl-25217.431734284710.5863Cyclopropane, 1-(E)-hexylidene-2-trimethylsilyl-5318.853578470642.8571Cyclopropane, 1-methylene-45417.851127617160.9003Cyclopropane, 1-me$	34	14.142	184702388	1.4747	3-Deoxy-d-mannonic acid
36 14.732 54582256 0.4358 Cyclohexane, 1.2,3-trimethyl- 37 14.828 181658285 1.4504 3-Acetyl-2,5-dimethyl furan 38 15.528 65284497 0.5212 [1,2,3,4]Tetrazlo[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 55960865 0.4468 4-Chloro-3-n-hexyltetrahydropyran 41 15.81 113353091 0.905 Cyclopentaone, 2-methyl-3-(1-methylphosphonate 43 16.084 122240875 0.976 Ethyl methyl ethylphosphonate 44 16.365 534069330 4.264 .alphaL-lyxo-Hexopyranoside, methyl 3-amino-2,3,6-trideoxy- 45 16.577 174190239 1.3907 Pentadecanoic acid 46 16.79 49796318 0.3976 Methyl 6-0-[1-methylpropyl]-betad-galactopyranoside 47 16.673 67720349 0.5407 3-Methylbranoside 3 48 16.987 162035302 1.2937 5,6-Dimethoxy-1-indanone 5	35	14.377	160207778	1.2791	(2.5-Dimethyl-[1.3]dioxan-4-yl)-methanol
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- 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 58265008 0.4652 1-Bromocicosane 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 16.679 49796318 0.3976 Methyl 6-O-[1-methylpropyl]-beta-d-galactopyranoside 47 16.873 67720349 0.5407 3-Methylmanoside 48 16.987 162035302 1.2937 5,6-Dimethoxy-1-indanone 51 17.353 48744676 0.3892 Octan-2,0e, 3,6-dimethyl- 51 17.351 1374647 0.5863 Cyclopropane, 1-(E)-hexyldene-2-trimethylsilyl- 55 <td< td=""><td>36</td><td>14.732</td><td>54582256</td><td>0.4358</td><td>Cyclohexane, 1.2.3-trimethyl-</td></td<>	36	14.732	54582256	0.4358	Cyclohexane, 1.2.3-trimethyl-
3815.258652844970.5212 $[1,2,3,4]$ Tetrazolo $[1,5^{+}b][1,2,4]$ triazine, 5,6,7,8-tetrahydro-3915.419663456810.5297Pyrimidine, 2,4,5-triamino-4015.638559606850.44684-Chloro-3-n-hexyltetrahydropyran4115.811133530910.905Cyclopentanone, 2-methyl-3-(1-methylethyl)-4215.938582659080.46521-Bromocicosane4316.0841222408750.976Ethyl methyl ethylphosphonate4416.3655340693304.264.alpha-L-lyxo-Hexopyranoside, methyl 3-amino-2,3,6-trideoxy-4516.5771741902391.3907Pentadecanoic acid4616.79497963180.3976Methyl 6-O-[1-methylpropyl]-betad-galactopyranoside4716.873677203490.54073-Methylmannoside4816.9871620353021.29375,6-Dimethoxy-1-indanone5017.261579811160.46291,4-Dimethyl-2,3-dinitrobenzene5117.353487446760.3892Octan-2-one, 3,6-dimethyl-5317.651127617160.9003Cyclopropane, 1-(E)-hexylidene-2-trimethylsilyl-5417.8341548993801.2367(E)-Hexadec-9-enoic acid5518.1853578470642.8571Cyclopropane, 1-methylene-3-methyl-2-trimethylsilyl-5618.321229608740.98173-Deoxy-d-mannoic lactone5718.4592221680901.7738Silane, dimethyl(methylene-3-methyl-2-trimethylsilyl-581	37	14.828	181658285	1.4504	3-Acetyl-2.5-dimethyl furan
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-(1-methylpropyl]-betad-galactopyranoside 47 16.873 67720349 0.5407 3-Methylimanoside 48 16.087 162035302 1.2937 5(6-Dimethyl-2.3-dimitrobenzene 49 17.146 95214130 0.7602 Diethyl Phthalate 50 17.251 57981116 0.4629 (1,4-Dimethyl-2.3-dimitrobenzene 51 17.353 4874676 0.3892 Octan-2-one, 3,6-dimethyl-3 51 17.854 112761716 0.9003 Cyclododecanone, 2-methylene-3-methyl-2-trimethylsilyl- 53 18.85 35	38	15 258	65284497	0.5212	[1 2 3 4]Tetrazolo[1 5-b][1 2 4]triazine 5 6 7 8-tetrahydro-
15.638 5596085 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 .alphaL-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-methylpropyll-betad-galactopyranoside 47 16.873 67720349 0.5407 3-Methylmannoside 48 16.987 162035302 1.2937 5,6-Dimethyl-phthalate 50 17.261 57881116 0.4629 1(4-Dimethyl-2,3-dinitrobenzene 51 17.353 48744676 0.3892 Octan2-one, 3,6-dimethyl- 52 17.431 73428471 0.5863 Cyclopropane, 1-(E)-hexylidene-2-trimethylsilyl- 53 18.15 357847064 2.8571 Cyclopropane, 1-methylene-3-methyl-2-trimethylsilyl- 54 <	39	15 419	66345681	0.5297	Pyrimidine 2.4.5-triamino-
1 15.81 11333091 0.905 Cyclopentators, 2-methyl-3-(1-methylethyl)- 2 15.938 58265908 0.4652 1-Bronocicosane 43 16.084 122240875 0.976 Ethyl methyl ethylphosphonate 44 16.365 534069330 4.264 .alphaL-lyxo-Hexopyranoside, methyl 3-amino-2.3,6-trideoxy- 45 16.577 174190239 1.3907 Pentadecanoic acid 46 16.79 49796518 0.3976 Methyl 6-O-[1-methylproyl]betad-galactopyranoside 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-dethylene-2-trimethylsilyl- 52 17.431 73428471 0.5863 Cyclopropane, 1-E)-hexyldee-2-trimethylsilyl- 54 17.834 154899380 1.2367 (E)-Hexadec-9-enoic acid 55 18.185 357847064 2.8571 Cyclopropane, 1-methylene-4-manbicl lactone <t< td=""><td>40</td><td>15.638</td><td>55960685</td><td>0.3257</td><td>4-Chloro-3-n-bexyltetrahydropyran</td></t<>	40	15.638	55960685	0.3257	4-Chloro-3-n-bexyltetrahydropyran
11 15.938 582:55908 0.4652 1-Bromocicosane 43 16.084 122240875 0.976 Ethyl methyl ethylphosphonate 44 16.365 534069330 4.264 .alphaL-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]-betad-galactopyranoside 47 16.873 67720349 0.5407 3-Methylmannoside 48 16.987 162035302 1.2937 5,6-Dimethoxy-1-indanone 91 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 Octan2-one, 3,6-dimethyl- 52 17.431 73428471 0.5863 Cyclopropane, 1-(E)-hexyldene-2-trimethylsilyl- 53 18.185 357847064 2.8571 Cyclopropane, 1-(E)-hexyldene-2-trimethylsilyl- 54 17.834 154899380 1.2367 (E)-Hexyade-9-enoic acid 57 18.459	41	15.81	113353091	0.905	Cyclopentanone 2-methyl-3-(1-methylethyl)-
16.084 122240875 0.976 Ethyl methyl ethyl phosphonate 44 16.365 53406930 4.264 .alphaL-lyxo-Hexopyranoside, methyl 3-amino-2,3,6-trideoxy- 45 16.577 174190239 1.3907 Pentadecanoic acid 46 16.77 47976318 0.3976 Methyl 6-0-[1-methylpropyl]-betad-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- 51 17.351 137428471 0.5863 Cyclopropane, 1-(E)-hexylidene-2-trimethylsilyl- 53 17.65 112761716 0.9003 Cycloddecanone, 2-methylene- 54 17.834 154899380 1.2367 (E)-Hexadec-9-enoic acid 57 18.459 22168090 1.7738 Silane, dimethylendylendylendylendylendylendylendylend	42	15 938	58265908	0.4652	1_Bromoeicosane
44 16.365 534069330 4.264 .alpha.1-1-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].betad-galactopyranoside 47 16.873 67720349 0.5407 3-Methylmanoside 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 Cycloptogane, 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 <td< td=""><td>43</td><td>16 084</td><td>122240875</td><td>0.4052</td><td>Ethyl methyl ethylphosphonate</td></td<>	43	16 084	122240875	0.4052	Ethyl methyl ethylphosphonate
44 10.503 5.9400730 4.204 apha.5-ryx041cx0y1ardistic, itekny15-animos2,5041de0xy1 45 16.577 174190239 1.3907 Pentadecanoic acid 46 16.79 49796318 0.3976 Methyl 6-O-[1-methylpropyl]betad-galactopyranoside 47 16.873 67720349 0.5407 3-Methylmannoside 48 16.987 162035302 1.2937 5,6-Dimethoxy-1-indanone 91 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- 51 17.65 112761716 0.9003 Cyclopropane, 1-(E)-hexylidene-2-trimethylsilyl- 53 17.65 112761716 0.9003 Cyclopropane, 1-methylene-3-methyl-2-trimethylsilyl- 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	43	16 365	53/060330	4 264	alpha I lyvo Hevopyranoside methyl 3 amino 2.3.6 trideovy
4616.57174702.591.570Methyl $6-O-[1-methylpropyl]-betad-galactopyranoside4716.873677203490.54073-Methylmannoside4816.9871620353021.29375,6-Dimethoxy-1-indanone4917.146952141300.7602Diethyl Phthalate5017.261579811160.46291,4-Dimethyl-2,3-dinitrobenzene5117.353487446760.3892Octan-2-one, 3,6-dimethyl-5217.431734284710.5863Cyclopropane, 1-(E)-hexylidene-2-trimethylsilyl-5317.651127617160.9003Cyclododecanone, 2-methylene-5417.8541548993801.2367(E)-Hexadec-9-enoic acid5518.1853578470642.8571Cyclopropane, 1-methylene-3-methyl-2-trimethylsilyl-5618.321229608740.98173-Deoxy-d-mannoic lactone5718.4592221680901.7738Silane, dimethyl[methylsilyl]5818.6843914908623.12571,3-Methylene-d-arabitol5918.8472196956931.7541Ethyl 2,3-epoxybutyrate6018.9982900872742.31613-Deoxy-d-mannoic lactone6119.1838051710896.42853-Deoxy-d-mannoic lactone6220.1041182778070.9443D-glycero-D-manno-Heptitol6320.293653241470.5216D-glycero-D-manno-Heptitol6420.6382455094651.9602Sorbitol6521.03174011890$	44	16 577	17/100230	4.204	Pentadecanoic acid
40 10.79 477 16.873 67720349 0.5407 3-Methyl 0-0-[1-31e,Methyl]mannoside 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 Cyclopropane, 1-eb-Hexadec-9-enoic acid 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 60 18.998 290087274 2.3161 3-Deoxy-d-mannoic lactone <t< td=""><td>45</td><td>16.70</td><td>174190239</td><td>0.3076</td><td>Mathyl 6 Ω [1 mathylpropyl] beta d galactopyranogida</td></t<>	45	16.70	174190239	0.3076	Mathyl 6 Ω [1 mathylpropyl] beta d galactopyranogida
4710.81307.403490.34073-Methylinalioside4816.9871620353021.29375,6-Dimethyl-x.3-dinitrobance4917.146952141300.7602Diethyl Phthalate5017.261579811160.46291,4-Dimethyl-2,3-dinitrobenzene5117.353487446760.3892Octan-2-one, 3,6-dimethyl-5217.431734284710.5863Cyclopropane, 1-(E)-hexylidene-2-trimethylsilyl-5317.651127617160.9003Cyclododecanone, 2-methylene-5417.8341548993801.2367(E)-Hexadec-9-enoic acid5518.1853578470642.8571Cyclopropane, 1-methylene-3-methyl-2-trimethylsilyl-5618.321229608740.98173-Deoxy-d-mannoic lactone5718.4592221680901.7738Silane, dimethyl(methylsilyl)methyl]-5818.6843914908623.12571,3-Methylene-d-arabitol5918.8472196956931.7541Ethyl 2,3-epoxybutyrate6018.9982900872742.31613-Deoxy-d-mannoic lactone6119.1838051710896.42853-Deoxy-d-mannoic lactone6220.1041182778070.9443D-glycero-D-manno-Heptitol6320.293653241470.5216D-glycero-D-manno-Heptitol6420.6382455094651.9602Sorbitol6521.031740118901.3893n-Hexadecanoic acid6621.3631469342811.17311-De	40	16.79	67720240	0.3970	2 Mathylmannosida
4610.987102033021.29373.30-Dimetholy-1-intainone4917.146952141300.7602Diethyl Pthalate5017.261579811160.46291,4-Dimethyl-2,3-dinitrobenzene5117.353487446760.3892Octan-2-one, 3,6-dimethyl-5217.431734284710.5863Cyclopropane, 1-(E)-hexylidene-2-trimethylsilyl-5317.651127617160.9003Cyclododecanone, 2-methylene-5417.8341548993801.2367(E)-Hexadec-9-enoic acid5518.1853578470642.8571Cyclopropane, 1-methylene-3-methyl-2-trimethylsilyl-5618.321229608740.98173-Deoxy-d-mannoic lactone5718.4592221680901.7738Silane, dimethyl((methylsily))methyl]-5818.6843914908623.12571,3-Methylene-d-arabitol5918.8472196956931.7541Ethyl 2,3-epoxybutyrate6018.9982900872742.31613-Deoxy-d-mannoic lactone6119.1838051710896.42853-Deoxy-d-mannoic lactone6220.1041182778070.9443D-glycero-D-manno-Heptitol6320.293653241470.5216D-glycero-D-manno-Heptitol6420.6382455094651.9602Sorbitol6521.031740118901.3893n-Hexadecanoic acid6621.3631469342811.17311-Deoxy-d-mannitol6722.274397621300.3175Heneicos	47	16.027	162025202	0.3407	5 6 Dimethovy 1 independent
4917.140952141300.7002Dreiny Findatae5017.261579811160.46291,4-Dimethyl-2,3-dinitrobenzene5117.353487446760.3892Octan-2-one, 3,6-dimethyl-5217.431734284710.5863Cyclopropane, 1-(E)-hexylidene-2-trimethylsilyl-5317.651127617160.9003Cyclododecanone, 2-methylene-5417.8341548993801.2367(E)-Hexadec-9-enoic acid5518.1853578470642.8571Cyclopropane, 1-methylene-3-methyl-2-trimethylsilyl-5618.321229608740.98173-Deoxy-d-mannoic lactone5718.4592221680901.7738Silane, dimethyl[(methylsilyl)methyl]-5818.6843914908623.12571,3-Methylene-d-arabitol5918.8472196956931.7541Ethyl 2,3-epoxybutyrate6018.9982900872742.31613-Deoxy-d-mannoic lactone6119.1838051710896.42853-Deoxy-d-mannoic lactone6220.1041182778070.9443D-glycero-D-manno-Heptitol6320.293653241470.5216D-glycero-D-manno-Heptitol6420.6382455094651.9602Sorbitol6521.031740118901.3893n-Hexadecanoic acid6621.3631469342811.1731I-Deoxy-d-mannitol6722.274397621300.3175Heneicosane6822.6321527752551.21989.12-Octadecadienoic ac	40	10.967	05214120	1.2937	Distryl Detholoto
50 17.251 57.951116 0.4029 17.4-Differing 2,3-diffiction of the product of the p	49 50	17.140	93214130 57081116	0.7602	1 4 Dimethyl 2 2 dinitrohanzona
51 17.53 46/44076 0.5892 Octain 2-one, 3,0-diffediyl- 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 21.363 14	50	17.201	J7901110	0.4029	1,4-Dimetryi-2,5-dimetryi
52 17.451 73428471 0.3865 Cyclopropane, 1-(E)-flexyfidene-2-trimethylsifyi- 53 17.65 112761716 0.9003 Cyclopropane, 1-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 <t< td=""><td>51</td><td>17.333</td><td>48/440/0</td><td>0.3892</td><td>Octan-2-one, 5,6-dimethyl-</td></t<>	51	17.333	48/440/0	0.3892	Octan-2-one, 5,6-dimethyl-
53 17.65 112/61716 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[(methylsily])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-	52	17.431	/34284/1	0.5863	Cyclopropane, I-(E)-nexylidene-2-trimetnylsilyi-
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 </td <td>53</td> <td>17.65</td> <td>112/61/16</td> <td>0.9003</td> <td>Cyclododecanone, 2-methylene-</td>	53	17.65	112/61/16	0.9003	Cyclododecanone, 2-methylene-
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<	54	17.834	154899380	1.2367	(E)-Hexadec-9-enoic acid
56 18.32 122960874 0.9817 3-Deoxy-d-mannotc 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	22	18.185	35/84/064	2.8571	Cyclopropane, 1-methylene-3-methyl-2-trimethylsilyl-
5718.4592221680901.7738Silane, dimethyl[(methylsilyl)methyl]-5818.6843914908623.12571,3-Methylene-d-arabitol5918.8472196956931.7541Ethyl 2,3-epoxybutyrate6018.9982900872742.31613-Deoxy-d-mannoic lactone6119.1838051710896.42853-Deoxy-d-mannoic lactone6220.1041182778070.9443D-glycero-D-manno-Heptitol6320.293653241470.5216D-glycero-D-manno-Heptitol6420.6382455094651.9602Sorbitol6521.031740118901.3893n-Hexadecanoic acid6621.3631469342811.17311-Deoxy-d-mannitol6722.274397621300.3175Heneicosane6822.6321527752551.21989,12-Octadecadienoic acid7023.171621991570.4966Butyric acid, 2-(3-oxo-3H-benzo[d]isothiazol-2-yl)-7123.409218875220.1748E,E,Z-1,3,12-Nonadecatriene-5,14-diol7224.0321900899031.5177Hexadecane, 2,6,10,14-tetramethyl-7324.357109540380.0875Z,Z-3,13-Octadecedien-1-ol7424.433147554850.11782-(3-Hydroxybutyl)cyclooctanone	56	18.32	122960874	0.9817	3-Deoxy-d-mannoic lactone
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	57	18.459	222168090	1.7738	Silane, dimethyl[(methylsilyl)methyl]-
5918.8472196956931.7541Ethyl 2,3-epoxybutyrate6018.9982900872742.31613-Deoxy-d-mannoic lactone6119.1838051710896.42853-Deoxy-d-mannoic lactone6220.1041182778070.9443D-glycero-D-manno-Heptitol6320.293653241470.5216D-glycero-D-manno-Heptitol6420.6382455094651.9602Sorbitol6521.031740118901.3893n-Hexadecanoic acid6621.3631469342811.17311-Deoxy-d-mannitol6722.274397621300.3175Heneicosane6822.6321527752551.21989,12-Octadecadienoic acid (Z,Z)-6922.871361832480.2889Octadecanoic acid7023.171621991570.4966Butyric acid, 2-(3-oxo-3H-benzo[d]isothiazol-2-yl)-7123.409218875220.1748E,E,Z-1,3,12-Nonadecatriene-5,14-diol7224.0321900899031.5177Hexadecane, 2,6,10,14-tetramethyl-7324.357109540380.0875Z,Z-3,13-Octadecedien-1-ol7424.433147554850.11782-(3-Hydroxybutyl)cyclooctanone	58	18.684	391490862	3.1257	1,3-Methylene-d-arabitol
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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	62	20.104	118277807	0.9443	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	63	20.293	65324147	0.5216	D-glycero-D-manno-Heptitol
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6621.3631469342811.17311-Deoxy-d-mannitol6722.274397621300.3175Heneicosane6822.6321527752551.21989,12-Octadecadienoic acid (Z,Z)-6922.871361832480.2889Octadecanoic acid7023.171621991570.4966Butyric acid, 2-(3-oxo-3H-benzo[d]isothiazol-2-yl)-7123.409218875220.1748E,E,Z-1,3,12-Nonadecatriene-5,14-diol7224.0321900899031.5177Hexadecane, 2,6,10,14-tetramethyl-7324.357109540380.0875Z,Z-3,13-Octadecedien-1-ol7424.433147554850.11782-(3-Hydroxybutyl)cyclooctanone	65	21.03	174011890	1.3893	n-Hexadecanoic acid
6722.274397621300.3175Heneicosane6822.6321527752551.21989,12-Octadecadienoic acid (Z,Z)-6922.871361832480.2889Octadecanoic acid7023.171621991570.4966Butyric acid, 2-(3-oxo-3H-benzo[d]isothiazol-2-yl)-7123.409218875220.1748E,E,Z-1,3,12-Nonadecatriene-5,14-diol7224.0321900899031.5177Hexadecane, 2,6,10,14-tetramethyl-7324.357109540380.0875Z,Z-3,13-Octadecedien-1-ol7424.433147554850.11782-(3-Hydroxybutyl)cyclooctanone	66	21.363	146934281	1.1731	1-Deoxy-d-mannitol
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7023.171621991570.4966Butyric acid, 2-(3-oxo-3H-benzo[d]isothiazol-2-yl)-7123.409218875220.1748E,E,Z-1,3,12-Nonadecatriene-5,14-diol7224.0321900899031.5177Hexadecane, 2,6,10,14-tetramethyl-7324.357109540380.0875Z,Z-3,13-Octadecedien-1-ol7424.433147554850.11782-(3-Hydroxybutyl)cyclooctanone	69	22.871	36183248	0.2889	Octadecanoic acid
7123.409218875220.1748E,E,Z-1,3,12-Nonadecatriene-5,14-diol7224.0321900899031.5177Hexadecane, 2,6,10,14-tetramethyl-7324.357109540380.0875Z,Z-3,13-Octadecedien-1-ol7424.433147554850.11782-(3-Hydroxybutyl)cyclooctanone	70	23.171	62199157	0.4966	Butyric acid, 2-(3-oxo-3H-benzo[d]isothiazol-2-yl)-
7224.0321900899031.5177Hexadecane, 2,6,10,14-tetramethyl-7324.357109540380.0875Z,Z-3,13-Octadecedien-1-ol7424.433147554850.11782-(3-Hydroxybutyl)cyclooctanone	71	23.409	21887522	0.1748	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
73 24.357 10954038 0.0875 Z,Z-3,13-Octadecedien-1-ol 74 24.433 14755485 0.1178 2-(3-Hydroxybutyl)cyclooctanone	72	24.032	190089903	1.5177	Hexadecane, 2,6,10,14-tetramethyl-
74 24.433 14755485 0.1178 2-(3-Hydroxybutyl)cyclooctanone	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 Grampositive 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	2.	The ide	ntification	results o	f the s	tudied	bacteria	ı by	traditiona	l method	ls and	16S	rRNA
								•					

	sequences									
	Bacteria	Accession numbers	Gram's stain	3% KOH test	Cell shape					
		(this study)								
1	Escherichia coli A1	OP040007.1	-ve	+	rod					
2	Escherichia coli A2	OP038673.1	-ve	+	rod					
3	Alcaligenes faecalis	OP040006.1	-ve	+	rod					
	ALI									
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 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 nanoparticles²⁰.



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 of analysis 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 27⁰, 31⁰, 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 AgNPs²³. 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 20 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 nanoparticles²⁵.



Pos. [°2Th.] (Copper (Cu))

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 μ g/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 lysis³⁰.

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 البرديTypha 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 الفضّة النانوية بتحول لوّن خليط التفاعل من اللون الاصفر الى اللون البني تم توصيف الدقائق المصنعة بواسطة UV-Vis وTTR وXRD وKEM و EDX . ثم اختبرت فعالياتها المضادة للبكتريا بوأسطة الانتشار بالحفر على الأكار Agar Well Diffusion Method .اظهرت نتائج الـGC/MS للمستخلص المائي للخريط هو وجود المركبات الأتية:Hydroxymethylfurfural اذ بلغت الـRT% له (13.6196) يليه المركب 3-Deoxy-d-mannoic lactone وبلغت الـ RT% له (6.4285) والمركب alpha-L-lyxo-Hexopyranoside, methyl 3-amino-2,3,6-trideoxy ذو RT% (بالمركب RT% والمركب 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl وله RT% والمركب 4H-261) والمركب Methylene-d-arabitol له Mr (3.125). تم التاكد من تكون دقائق الفضة النانوية وذلك بالطرق الطّيفية 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 تر اوحت بين20 -70 نانوميتر. وبينت نتائج الـEDX ان التركيب الكيميائي للدقائق النانوية المصنع في الدر اسة الحالية هو الفضة اختبرت قابليةً دقائق الفضة النانوية المحضرة بوأسطة 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 وEscherichia coli A2 و Alcaligenes و faecalis AL1 والرابعة موجبة لصبغة كرام وهي faecalis AL1

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