Identification Of Biochemical Compounds Of Silylbum Marianum Seeds At Different Locations Salt Stress

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Abstract

The Iraqi environment is characterized by a large abundance of medicinal plants that grow in various agricultural sites due to their different needs and requirements of the environment, including the Milk thistle *Siylbum marianum* plant, which is one of the most widespread medicinal plants in Iraqi country and is important in the pharmaceutical industries, including its use to regenerate liver cells and treat its diseases, this research was carried out to determine the diagnosis of chemical compounds in the *Siylbum marianum* plant and their proportions using the GC-MS device and The percentage of constant oil in its seeds, the seeds of the plant were collected in May 2024 from eight sites in Diyala governorate in different chemical and physical properties , namely (Khanaqin, Mansoriyat Al-Jabal, Muqdadiya, Al-Khalis, Baquba, Bani Saad, Baladrouz, and Mandali). The oil content of the seeds was estimated on the basis of their dry weight, and the percentages differed significantly between them according to the different agricultural sites geographically, where they ranged between 32,68%-25,42. Biochemical in *Siylbum marianum* seeds varied by chemicals soil characteristics like its salt content.

Keyword ; salt stress , milk thistle , Biochemical Compounds .

Introduction

The World Health Organization (WHO) has estimated that 80% of citizens in developing countries only use traditional medicine for primary health care, plants make up a high percentage of the biochemical components of traditional medicines (18), and more than half of the world's population is still completely dependent on plants for their medicines, the Milk thistle plant belongs to the family Asteraceae which is It is a winter annual plant that is widespread in temperate environments of the world (26), and is believed to be native to the Mediterranean basin and grows in wide ranges of climate (10). Due to the importance of the plant in the pharmaceutical industry, it has become cultivated in large areas in several countries of the world, including some countries of Europe,Asia, Africa, and South America (11 , 15), and for its industrial uses as dietary supplements in Greek and Arab medicine for many countries, its sales in 2005 reached million $ 8.3 (27), as many studies have confirmed the importance of plant seed compounds in the regeneration of liver tissue and the treatment of liver diseases (4), the seeds of the Milk thistle plant contain many biochemical compounds, including Flavonolignan for the treatment of viral hepatitis or liver damage as a result of the repeated use of heart medications or the consumption of alcoholic beverages. (30) as well as its seeds are rich in the bioactive Silymarin complex which amounts to 1-3% of seed weight (16), and other vital antioxidant and lipid oxidizing free radical components (21). and protecting the body from some cancers, molluscs heart diseases, kidney disease, and Alzheimer's in adults (7 and 25). Geographical heterogeneity and different environmental regions in general and in Diyala province have an impact on the production capacities of biochemical compounds in medicinal plants (23), despite the recovery and increased production of plants in fertile lands rich in nitrogen and good rainfall rates (8). However, its presence in different environmental sites has a clear effect on the formation of chlorophyll, the efficiency of carbon representation in plants, and the accumulation of secondary metabolic compounds such as (phenols, flavonoids, etc.) which the plant seeks by building and synthesizing them under stress conditions to inhibit the oxidative pathogens (ROS) growing in the plant cell (9 and 28), and is one of the biodeterminants of the activity and completion of biochemical compounds in plants as well (12 and 19). Diyala Governorate is geographically located in the eastern part of central Iraq between two latitudes (33.3 – 35.6) It is characterized by its mild and semi-arid climate, and due to climatic changes, the relative rise in temperatures and the decrease in the amounts of rainfall throughout the country, the indicators of salt stress in the soils located within these ranges have increased and their proportions have increased, and they have become an obstacle to the growth and spread of plants by affecting a number of chemical properties of agricultural soils, including the hydrogen number and the imbalance in the readiness and absorption of nutrients and its capacity to retain water and create a kind of unfavorable conditions for plant life and soil revitalization and thus the formation of active substances in the plant (31 and 22) . (9) showed that the proportions of biochemical components in plant seeds are significantly affected by the environmental variables surrounding them. (20) found that the nature of the soil and its chemical properties have a clear effect on the chemical components of *Solanum nigruml*. (5) concludes that agroecological sites have a significant impact on the Silymarin content in plant seeds. Due to the importance of the plant and its multiple uses in the health aspect, this study was conducted to diagnose the chemical compounds and their ratios in *Siylbum marianum* seeds under different levels of salt stress.

Methods and materials

A field experiment was carried out to diagnose the biochemical compounds in the seeds of the Milk thistle plant and the oil content in the seeds by taking random samples from the soil of different agricultural sites in Diyala province with a depth of 0-30 cm, then the samples were mixed homogeneously and one sample was taken for each site after it was air-dried, then it was ground and sifted for the purpose of analyzing it and studying its chemical and physical properties in the central laboratories - University of Baghdad Table (1) , (RCBD) was used to estimate oil percentages and the differences between the averages were tested according to the Duncan Multiple Rang Test at a probability level of 0.05 (3) and the statistical program (24) was used in the statistical analysis, and a number of chemical compounds in the seeds were diagnosed and the following were performed:

1. Preparation of plant material: Seed samples were taken for various plants of the Milk thistle plant for the winter season in 2024 to extract the plant oil and identify the chemical compounds present in the seeds.
2. The study was conducted in eight sites that differ in the level of salts in their soil and soil samples were taken at a depth of 5-30 cm.

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Table (1): Physical and Chemical Properties of Soil Sites of *Siylbum marianum*

Soil	Mg Mmo l^{-1}	SO4^{-2} Mmol l^{-1}	Ec.dsm^{-1}	pH	Potassium	Phosphorus	$CaCo_3$	N	Organic Matter g.kg^{-1}	Location Name
Sand Clay Loam	1.15	1.35	3.1	8.0	187	5.1	27.5	1.5	15.0	Khanaqin
Clay Loam	2.75	5.35	3.1	7.9	192	2.6	15.5	1.9	13.4	Mansoriyat Al-Jabal
Clay Loam	4.51	7.45	3.9	7.8	206	7.8	24.5	2.4	13.2	Muqdadiya
Silty Loam	17.52	6.00	10.3	7.2	220	3.9	10	2.0	10.0	Al-Khalis
Silty Loam	7.51	12.90	9.2	7.6	230	10.8	15	1.1	12.1	Baquba
Clay Loam	5.00	8.00	15.4	7.6	280	2.5	20.5	1.2	10.1	Bani Saad
Clay loam	15.00	4.85	14.7	7.5	310	13.6	17.6	1.3	12.2	Baladrouz
Clay	18.23	19.0	13.5	7.4	321	11.5	17.1	0.9	8.3	Mandali

Table (2) Rainfall Rate for the Year 2023-2024 in the Khanaqin and Al-Khalis Regions - Diyala

Total mm	2024					2023			Region
	May	April	March	February	January	December	November	October	
323.8	50.4	54.6	73.6	51.0	10.0	28.4	55.8	0	Khanaqin
219	32.0	34.6	49.2	50.8	3.6	33	15.4	0.4	Al-Khalis

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Table (3) Temperatures for the year 2023-2024 in the regions of Khanaqin and Al-Khalis - Diyala

2024					2023			Temperatures	Region
May	**April**	**March**	**February**	**January**	**December**	**November**	**October**		
35.25	33.14	23.61	18.89	19.13	20.9	24.9	33.4	Max	Khanaqin
21.18	18.04	10.55	8.36	7.87	9.3	14.0	19.7	Min	
35.67	33.66	24.09	19.78	20.09	19.9	25.5	33.8	Max	Al-Khalis
19.51	16.75	10.30	7.11	6.66	7.9	12.7	18.1	Min	

1. Oil extraction
Random samples were taken of the seeds of fully mature plants from each agricultural site after they were dried in an electric oven at a temperature of 75 °C after they were cleaned of dust and impurities and then they were crushed and 15 g were taken from it, 250 ml of hexane concentration (95%) was added to it to extract the oil in the saxolite device for 6 hours at a temperature of 75 °C, then the solvent was evaporated with a rotary evaporator at a temperature of 45 °C and Concentrate the oil extract and estimate its content in a separating flask using a laboratory sensitive scale according to the following equation:

$$\text{The oil extraction precentage(g\textbackslash g)} = \frac{\text{Extraction oil weight(g)}}{\text{Dry seeds weight (g)}}$$

2. Determination of biochemical components of *Siylbum marianum* seeds (%) The chemical composition of the ground seeds and the removal of the oil and after extracting them with methanol alcohol were determined by the amount of 10 g dried seeds as mentioned in paragraph (2) at the Science and Technology Commission – Department of Environment and Water using the Gas-Chromatography-Mass Spectrometry device with FID detector. Nitrogen as a carrier gas, injection of one microliter of methanol extract in GC-ms Shimadzu LAB and column temperature elevation from (80-280), flow control model (pressure): 100 pka at total flow: 11.8 ml/min and column flow: 1.46 ml/min (6)

Results and discussion

The results of Table (4) show a significant superiority of the cultivation site in Khanaqin and giving it the highest oil content in Milk thistle seeds amounting to 32.68 % compared to the cultivation site in Baladrouz where the oil content decreased to 25.42%, and the reason for the increase in oil may be attributed to the nature and structure of the soil and its content of organic matter and the decrease in the salt content in the Khanaqin site Table (1) , confirmed that (17) that soil properties and their organic matter content play an important role in improving the oil content of Milk thistle seeds, in addition to the

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negative effect of salts of the Baladrouz site on seed fullness and specific weight, which reduces the chance of accumulation of vital compounds, including oil in seeds, as well as high rainfall rates. In the geographical location of Khanaqin (14), and the importance of this in reducing the proportions of salts for agricultural lands.

Table (4): The Effect of Agricultural Sites on the Oil Percentage in *Siylbum marianum* Seeds

Agricultural Location							
Mandali	Baladrouz	Bani Saad	Baquba	Al-Khalis	Muqdadiya	Mansoriyat Al-Jabal	Khanaqin
Percentage of oil seed (%)							
E 25.49	F 25.42	D25.99	D26.1	C 26.56	C 26.53	B 30.12	A32.68

The results of Table (5) show that the cultivation site in Khanaqin was characterized by giving a number of chemical compounds, and the compound t-Butyl-oct-6-en-1-ol-3 was surpassed by the highest percentage, followed by Cyclododecenol 2, which reached 84.84 and. 12.91% respectively and the lowest 0.11% was for N-(Dimethylthiophosphinyl)ethylamine , while a significant increase in compounds 2,3-Pyridinedicarbonitrile and 4-(1-Hydroxyallyl)-2-methoxyphenol was found in the seeds of the Milk thistle plant at the planting site in Baqubah and amounted to 28.13 and 24.13% respectively, with the lowest percentage of Ketene compound amounted to 2.45%, and it was also found that the content of the compound was high Benzene, 1-methyl-3-[(2-methylpropyl)thio]- followed by 1H-Pyrazole, 4,5-dihydro-3-methyl-1-propyl- with the highest percentages in the plant seed extract at the planting site in Mansouriyat Al-Jabal and 24.21 and 12.08% respectively and the lowest percentage was for Dioxane-2,6-dione-1.4 amounted to 0.64 % in the same site, and due to the different characteristics of the soil and climate, the diversity of compounds and their proportions to the plant in the agricultural sites, the cultivation site in the district of Mandali was distinguished by giving the highest compound, which is Furan, 2-hexyl- followed by Benzene, 1-(butylthio)-4-methyl in the plant extract of the seeds, which reached 22.12 and 21.3% respectively, compared to the lowest 3.32% of the compound Thiophene, 2-propyl. The results of the abundance of trans-2-Dodecen-1-ol, trifluoroacetate and formamidoxime compounds in the plant extracts of the agricultural site at the district of Muqdadiya and giving the highest percentages amounted to 47.08 and 25.03% respectively, while the lowest percentage was 0.17% for the compound Cyclobutylamine in the same site, as indicated by the results of the chemical analysis of the GC mas apparatus. For plant extracts and the same table, a number of phytochemical compounds in the agricultural site of Baldroz district had the highest percentage of 54.51 for Propanedioic acid, followed by H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy 6-methyl-4 with 7.59% with the lowest percentage of 0.29% for 3-methoxyamphetamine. The results of the table also showed that there were variations in the proportions and types of compounds in the seeds in AL-Kalis district as a result of the effect of the environment and soil characteristics, and the highest percentage was found 22.31% for the compound Acetamide, N-(2-acetyl-3-oxo-4-isoxazolidinyl)- followed by Benzene, 1-methyl-3-[(2-methylpropyl) thio]- 19.39% while the lowest percentage was 2.89% for the compound Hydrogen isocyanate. The results of the agricultural site in Bani Saad also found that the highest compound for chemical analysis was 1,4

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Benzene dicarboxylic acid, bis(2-ethylhexyl) ester with a percentage of 39.38% in the extract, followed by 2-Propanone, 1-(4-hydroxy-3-methoxyphenyl) with a percentage of 16.83% compared to the lowest percentage of 0.69% at the compound.1,3-Dimethyl-4-amino-5-thioxo-4,5-dihydro-1,2,4-(1H)-triazole at the same agricultural site.

The difference in the proportions of organic matter in the soils of agricultural sites Table (5) and according to the characteristics of their soil and the biological diversity of the plants in it, which constitutes an important factor and source of nutrients in the soil and enhances its ability to retain water, air and the activity of microorganisms in it, in addition to its vital role in regulating soil temperatures and acidity, and this certainly improves the readiness of the elements present in the soil, confirmed by (13). The presence of organic matter in the soil of the fields of the Moringa oleifera crop led to the improvement of the active compounds in the plant, this was confirmed (32) that the fertility of the soil and its readiness with nutrients plays an important role in improving the content of active compounds in the seeds of Milk thistle, and the presence of organic matter at the level indicated in the table improves the penetration of water into the soil and reduces its salt content. Also, the fluctuation of salt ratios between (15.4 - 3.1) dsm^{-1} in the soil of the study sites has a negative effect of physiological drought and increase of osmozyness in the plant cell, a stress factor affecting the absorption of elements and their readiness from the soil, and a deficit in the efficiency of vital processes inside the plant and according to its levels in the soil of agricultural sites. Also, the heterogeneity of the proportions of biochemical compounds in plant seeds may explain the extent to which the plant tolerates salt stress levels and their interaction with other environmental factors, this was confirmed by (29) that the high concentrations of salts in the fields of Salvia mirzayanii plants reduced the content of the active compounds in the plant. The same table indicates that the structure and texture of the soil has changed according to the agricultural sites and a clear effect on its salt content (Table (1), as heavy soils are weak to wash the halic compounds, but they become dense and less ventilated and negatively affect the extent to which they retain water and nutrients, and this has affected the diversity of active compounds and their content in plant seeds (20), Also , the difference in agricultural sites and rainfall rates in them Table (2) as regular rainfall rates improve soil fertility and moisture and support biological activity, which has clearly affected the chemical properties of soils and their role in determining the level of salt stress in the environments in which these plants grow , confirmed by (1 , 2) The active compounds in Milk thistle seeds may vary according to the rainfall rates in agricultural sites.

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Table (5) Percentage of Chemical Compounds in Seeds under the Influence of Agricultural Location for *Siylbum marianum* Plant Growth

Chemical Component Percentage in seeds %	**Molecular Formula**	**Chemical Group**	**Compound Name**	**No.**
Khanaqin				
1.23	C2H7NO	Amines	1-Methanamine, N-hydroxy-N-methy	1
0.12	C9H11NO	Amides	2-Ethylformanilide	2
0.11	C4H12NPS	Dimethyl Thiophosphorus	N-(Dimethylthiophosphinyl)ethylamine	3
0.83	C7H12	Hexadiene	1.5-Hexadiene, 3-methyl-	4
0.32	C4H6O	Alcohols	3 -Butyn-2-ol	5
84.84	C16H30O	Alkyl Acyl	3- t-Butyl-oct-6-en-1-ol	6
12.91	C12H22O	Cycloalkanes	2 - Cyclododecenol	7
Baquba				
2.45	C2H2O	Ketones	Ketene	1
8.57	C4H9NO2	Nitroalkanes	Propane, 1-methyl-1-nitro-	2
13.54	C5H6N6	Triazoles	1-Methyl-bis (1,2,4)-trizole-5,1	3
24.13	C10H12O3	Phenylpropanoids	4-(1 -Hydroxyallyl)-2-methoxyphenol	4
28.13	C7H3N3	Pyridinides	2,3-Pyridinedicarbonitrile	5
4.30	C3H6N4S	Triazoles	3-Methyl-4-amino-1,2,4-triazole-5-thiol	6
3.88	C21H23BrO4	Esters	Phthalic acid, 4-bromophenyl heptyl ester	7
Mansoriyat Al-Jabal				
0.64	C4H4O4	Aldehydes And Esters	1,4-Dioxane-2,6-dione-	1
0.77	CH2N2	Diazocarbon	Diazirine	2
10.81	C18H33ClN2O5S	Lincosamide	Clindamycin	3
9.47	C3H4O2	Beta-Propiolatones	Propiolactone	4

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12.08	C7H14N2	Dihydropyrazole	1H-Pyrazole, 4,5-dihydro-3-methyl-1-propyl-	5
11.02	C4H9NO	Nitroalkane	Propane, 2-methyl-1-nitro -	6
2.49	C5H6N6	Triazoles	1- Methyl-bis(1,2,4)-triazole-5,1	7
24.21	C11H16S	Isobutyl Thioethe	Benzene, 1-methyl-3-[(2-methylpropyl)thio]-	8
9.03	C22H42O4	Fatty Alcohol Esters	Hexanedioic acid, dioctyl ester	9
8.47	C16H22O4	Phthalate Esters	Phthalic acid, disobutyl este	10
10.99	C5H4N2O3	Imidazole	(1 H-Imidazol-4-yl) oxoacetic acid	11
Mandali				
8.11	C2H7NO	Hydroxyl Amines	Methanamine, N-hydroxy-N-methyl-	1
11.00	C10H11NO4	Alpha-Amineacide Esters	D-Alanine, N-propargyloxycarbonyl-, propargyl ester	2
16.75	C2H5N	Aziridine	Ethylenimine	3
3.32	C7H10S	Thiophenes	Thiophene, 2-propyl -	4
21.3	C11H16S	Benzene	Benzene, 1-(butylthio)-4-methyl	5
8.82	C22H42O4	Fatty Alcohol Esters	Hexanedioic acid, dioctyl ester	6
8.54	C26H42O4	Phthalate Esters	Di-2-nonyl phthalate	7
22.12	C10H16O	Furans	Furan, 2-hexyl-	8
Muqdadiya				
25.03	CH4N2O	Amidoximes	Formamidoxime	1
0.25	C2H5NO	Amides	Acetamide	2
0.17	C4H9N	Aliphatic Amines	Cyclobutylamine	3
1.90	C6H8O4	Pyranonic Amines	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6 methyl-	4
0.42	C6H12O2S	Thioester	Methyl 3-(methylthio)butanoate	5
0.37	C20H30O4	Phthalate Ester	1,2 -Benzenedicarboxylic acid, dihexyl ester	6
2.56	C11H16O2	Benzenediol	1,3 -Benzenediol, 5-pentyl-	7
5.04	C10H20O2	Carboxylic Acid	Decanoic acid	8
1.49	C4H5N3	Pyridine	4- Pyrimidinamine	9
0.44	C9H13F3O2	Ester	Cyclohexylmethanol, trifluoroacetate (ester)	10

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47.08	C14H23F3O2	Carboxylic Acids	trans-2-Dodecen-1-ol, trifluoroacetate	11
0.77	C7H11N3OS	Amide	N-(5-Ethyl-1,3,4-thiadiazol-2-yl)propanamide	12
1.46	C5H3F3O2	Alcohol Propargyl	Propargyl alcohol, trifluoroacetate	13
0.51	C13H15NOS	Alcoholic And Indolic	3-Mercaptoindole, S-trimethylacetyl	14
12.51	C19H34O2	Fatty Alcohols	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	15
Baladrouz				
54.51	C3H4O4	Dicarboxylic Acid	Propanedioic acid	1
0.79	C4H4O4	Carboxylic Acids Anhy	Acetic acid, oxydi-, cyclic anhydride	2
0.29	C10H15NO	Amphetamine	Methoxyamphetamine-3	3
0.08	C4H8O	Hydroxyl	2-Buten-1-ol	4
7.59	C6H8O4	Pyranone	H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy 6-4 -methyl	5
2.81	C7H14N2	Pyrazole	1 H-Pyrazole, 4,5-dihydro-3-methyl-1-propyl	6
1.36	C11H16S	Aromatic Hydrocarbon	Benzene, 1-(butylthio)-4-methyl-	7
5.55	C6H6N4O	Pteridines	7,8-Dihydro-4(1H)-pteridinone	8
1.06	C7H14O2	Carboxylic Acid	2-Hexanecarboxylic acid	9
1.30	C3H9NO2	Amino Alcohol	2-Amino-1,3-propanedio	10
0.98	C7H7ClO2	Organochlorides	2,3-Norbornanedione, 1-chloro	11
3.14	C4H8N2	Aldehyde With Hydrazine	Acetaldehyde, ethylidenehydrazone	12
2.87	C22H42O4	Fatty Alcohol Ester	Hexanedioic acid, dioctyl ester	13
1.83	C19H37NO4	Ester	Alanine, N-methyl-N-ethoxycarbonyl-, dodecyl ester	14
1.26	C6H12O	Aldehydes	Pentanal, 2-methyl-	15
1.39	C13H15NOS	Thiol With Pivaloyl	3-Mercaptoindole, S-trimethylacetyl	16
13.17	C12H22O	Secondary Alcohol	2-Cyclododecenol	17
Al-Khalis				
2.89	CHNO	Hydrogen Isocyanate	Hydrogen isocyanate	1
22.31	C7H10N2O4	Acetamide	Acetamide, N-(2-acetyl-3-oxo-4-	2

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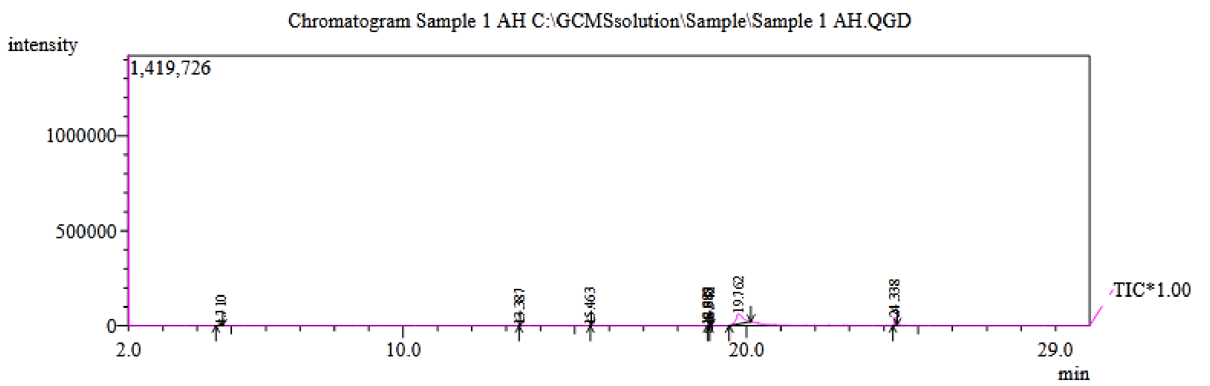
			isoxazolidinyl)-	
19.39	C11H16S	Aromatic Hydrocarbon	Benzene, 1-methyl-3-[(2-methylpropyl)thio]-	3
3.87	C15H15N	Imine Chemical	4-Methylbenzylidene-4-methylaniline	4
13.19	C22H42O4	Dicarboxylic Ester	Hexanedioic acid, dioctyl ester	5
3.18	C9H9N	Nitrile	Bicyclo[4.2.0]octa-2,4-diene-7-carbonitrile	6
6.60	C9H13F3O2	Ester	Cyclohexylmethanol, trifluoroacetate (ester)	7
Bani Saad				
0.69	C4H8N4S	Triazole	1,3-Dimethyl-4-amino-5-thioxo-4,5-dihydro-1,2,4-(1H)-triazole	1
16.65	C8H14O	Alkene	4-Hexen-3-one, 4,5-dimethyl	2
12.34	C9H14N2	Ptrazine	-Pyrazine, 3,5-diethyl-2-methyl	3
16.83	C10H12O3	Aromatic Keton	2-(Propanone, 1-(4-hydroxy-3-methoxyphenyl-	4
3.86	C7H5BrO	Aromatic Aldehydes And Organobromine	-Benzaldehyde, 4-bromo	5
2.78	C7H3N3	Pyridine	2,3-Pyridinedicarbonitrile	6
6.10	C8H6N2	Azanaphthalenes	1,6-Naphthyridine	7
1.36	C4H11N	Amines	2-Propanamine, 2-methyl	8
39.38	C24H38O4	Phthalate	1,4 Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	9

Diagrams of Chemical Compounds Using GC-MS in *Siylbum marianum* Seeds



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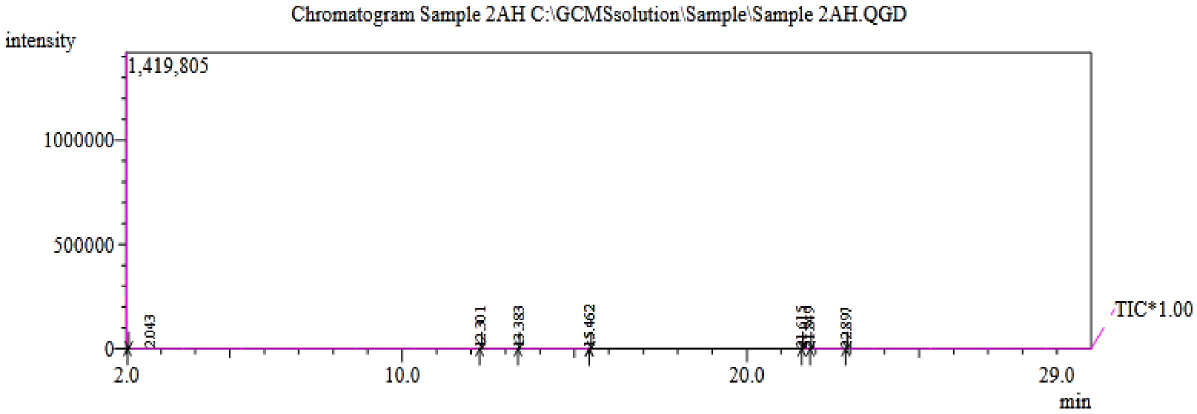
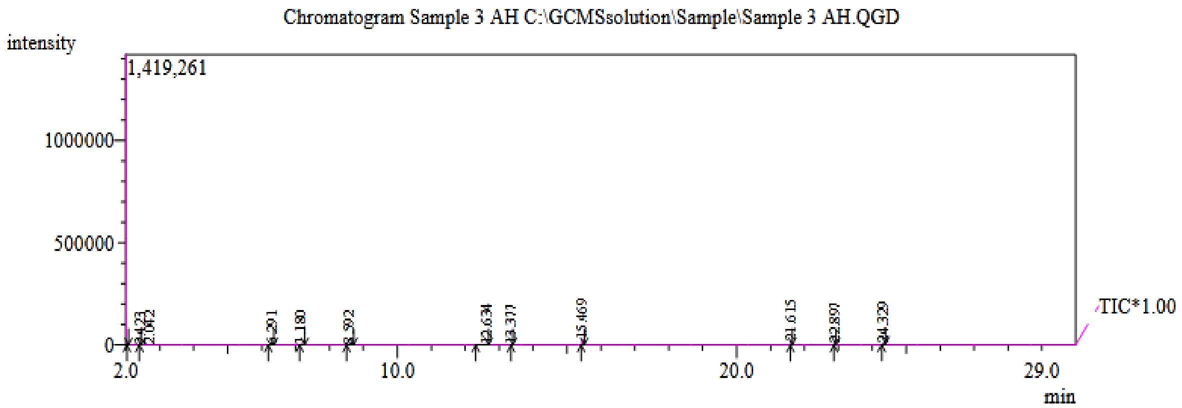
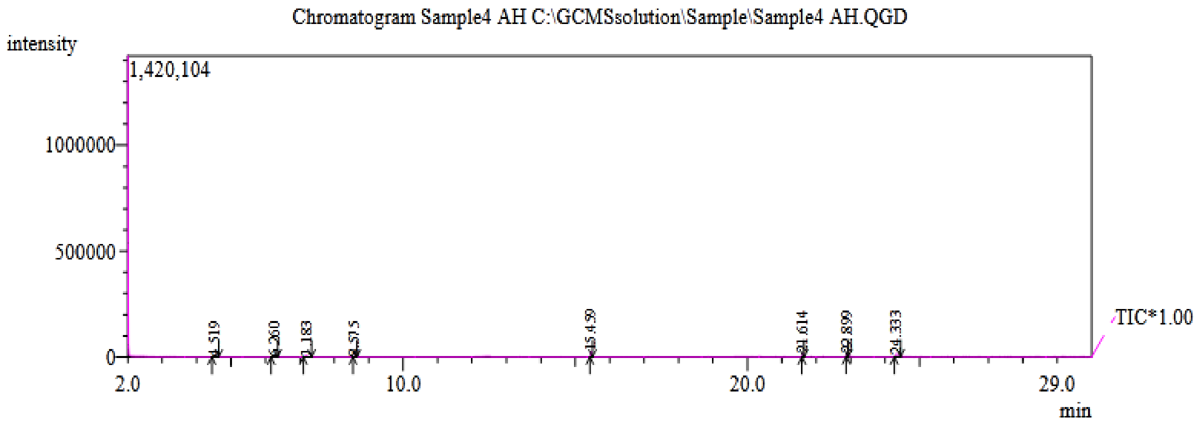
Khanaqin



Baquba



Mansoriyat Al-Jabal



Mandali

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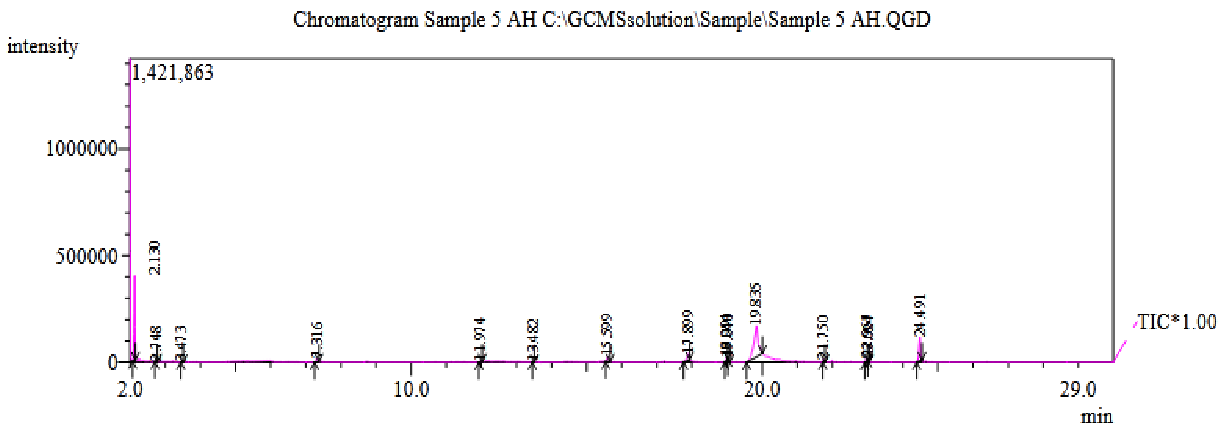

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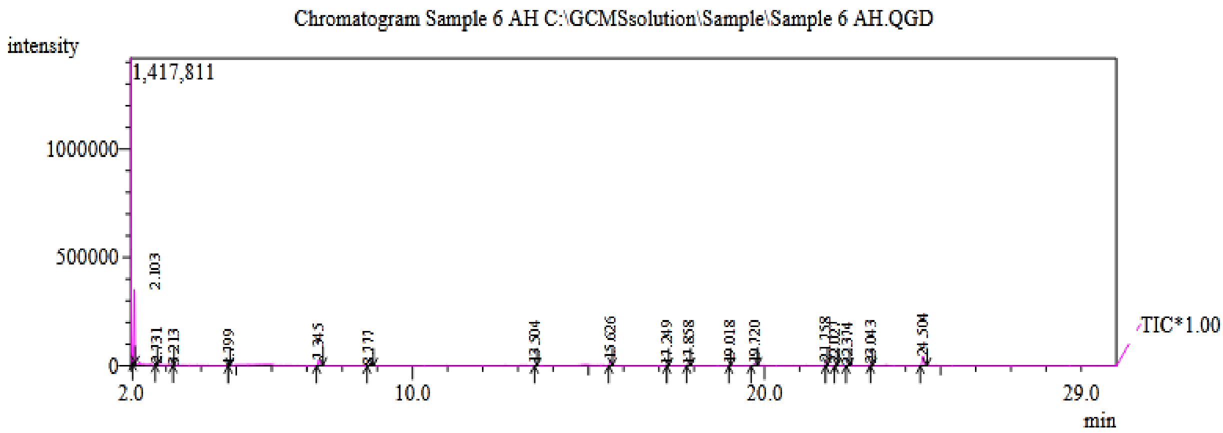
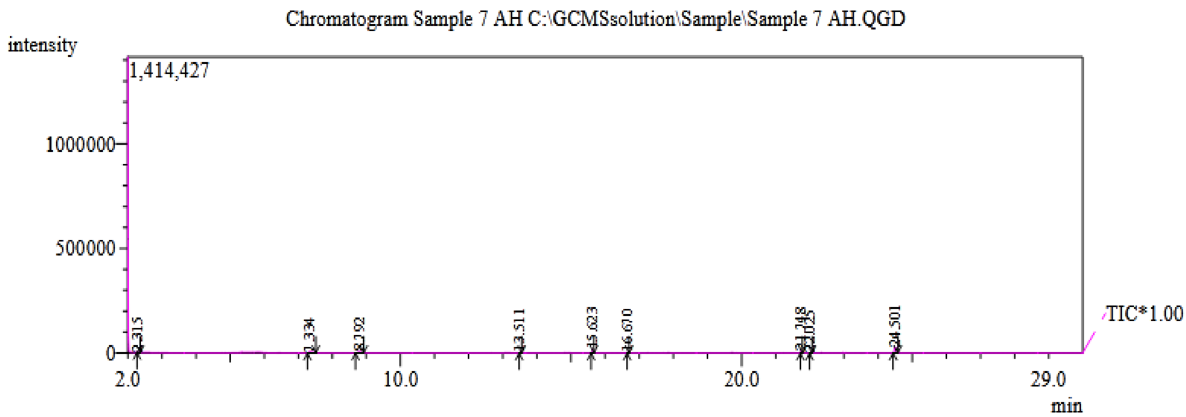


Baladrouz



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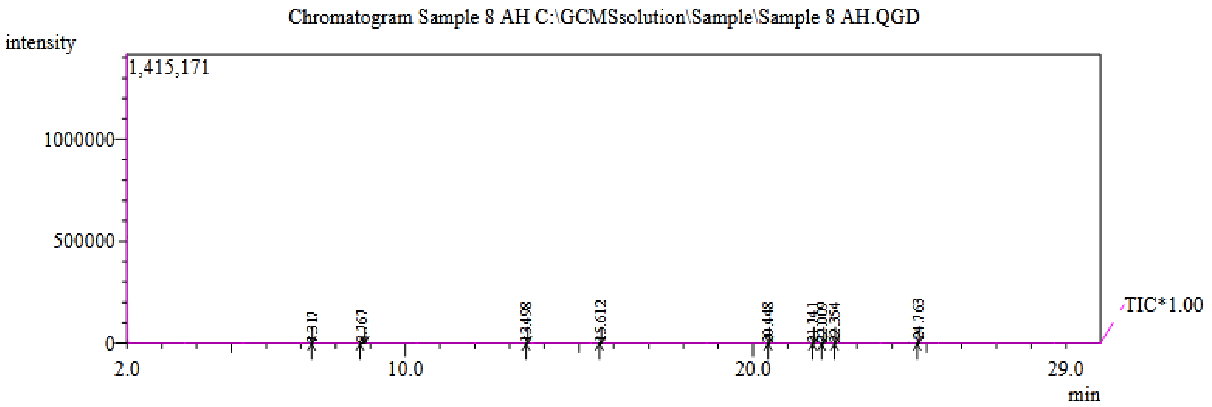
Al-Khalis



Bani Saad

Conclusion

According to obtained results the varying environmental locations affect the oil content of milk thistle seeds, with the Khanaqin location exhibiting the highest oil content was 32.68 % Low salt soil . Furthermore, its biochemical contents varies depending on the chemical properties of the soil in which it naturally grows, specifically its salt and organic matter content, as well as rainfall levels. .

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