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Phytochemical Diversity in Seven Grapevine Varieties Using GC-MS.

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ABSTRACT

The aim of the current study was to extract the active compounds of seven grape varieties cultivated in Duhok Governorate, Kurdistan Region of Iraq, for the 2023 season. The varieties included (Hejazi, Tre rash, Eayn althawr, Superior seedless, Moon drop, Crimson, Samarkandi). The results indicated that the leaves of grape varieties contain many active compounds, and the number and concentrations of these active compounds vary among the studied varieties. The number of active compounds according to the sequence was Hejazi 61 with highest compound of Methyl linolenate (6.71%), Superior seedless 36, Moon drop 33, Tre rash and Crimson 22, Eayn althawr 14, and Samarkandi 13 with 22.50 of α-Tocospiro-B . The compound Methyl palmitate appeared at its highest levels in the varieties (Tre rash, Eayn althawr, Superior seedless, Moon drop, Crimson), with values of (16.02%, 26.53%, 21.39%, 18.29%, 32.16%), respectively. GC-MS analysis confirmed that there is a biochemical diversity in the given grapevine varieties. Therefore, these varieties could distinguish via the highest compound appeared in GC-MS analysis.

***Keywords*:** Phytochemical diversity, grapevine, varieties, active compounds, GC-MS.

INTRODUCTION

Grapes *Vitis vinifera* L. occupy a prominent position among various fruit trees in the world, with approximately 10,000 grape varieties worldwide [1]. The quantity of grape production exceeds one-third of global fruit production, with approximately 47% of the total production consumed as fresh table grapes. Europe ranks first in terms of grape cultivation area, followed by Asia, then America, Africa, and Australia. Europe alone occupies more than 70% of the grapevine area worldwide. In terms of production, Europe ranks first, followed by America and Asia. The most important grape-producing countries in the world are Italy, France, Russia, Spain, and the United States [2]. Iraq ranks second in the Arab world in terms of grape cultivation area, with productivity barely meeting local consumption. There are approximately 70 grape varieties in Iraq, most of which are concentrated in the northern regions of the country [3]. There is a possibility of increasing the area planted with grapes and increasing its productivity in the Arab world and in Iraq, especially in the northern and central regions of our country, where the climatic conditions are suitable for grape cultivation. Thus, it is possible to meet local needs and export the surplus production outside the country, especially table grapes and raisins. It is worth noting that Iraq is one of the leading countries in terms of the length of the consumption period for fresh grapes, which starts from May and lasts until the end of November [4].

Grapes are considered a fruit with good nutritional and therapeutic value, known to the Chinese and Indians since ancient times, where they have been used in many remedies. Grapes contain a good proportion of sugars such as glucose and fructose, which are quickly absorbed and easily digested. Additionally, the berries are rich in vitamins such as A, B6, C, and E, as well as containing a good proportion of minerals like potassium, calcium, sodium, and others. Grapes also have therapeutic effects due to the presence of a compound known as Resveratrol, which is an antioxidant substance known for its positive effect in reducing arterial stiffness by its direct and noticeable role in lowering cholesterol levels, thus reducing the risk of heart diseases. Grapes contain certain acids that play a role in preventing the accumulation of free radicals, making them a good anti-cancer agent. Additionally, grapes contribute to the treatment of osteoporosis due to their high calcium content. Green grapes are used to treat pharyngitis, tonsillitis, and headaches as astringents, while young grape leaves are used in recipes to treat headaches, tumour inflammations, gastric acidity, and joint diseases. Grape leaf tea is used as a diuretic and astringent, as well as for treating diarrhea and dysentery. Furthermore, grape peel and dried leaves can be used in various formulations to treat hemorrhoids, inflammations, burns, and scorpion stings. Grape ash is used to stimulate hair growth in cases of burns, while grape seed oil is used in treating gynecological conditions. It is evident from the above that grapes are beneficial for everyone, including the healthy, the sick, athletes, and laborers, as they help them regain their strength, muscle flexibility, and energy [5], [6], [7], [8].

Active ingredients used in traditional medical treatment are obtained from whole plants or their parts such as roots, leaves, bark, or seeds [9]. The extraction of active compounds biologically depends on the extraction solvent used and the extraction temperature or mixing with other techniques, which can be considered classical, such as Soxhlet extraction, maceration, and steam distillation [10].

The protection provided by fruits and vegetables to humans, especially against heart diseases and cancer, is largely attributed to antioxidant compounds, including vitamins, flavonoids, and other compounds. It has been observed that

some flavonoids have high antioxidant activity, especially the free radical scavenging ability of the peroxyl radical, which affects more than vitamins C and E. The term "free radical scavengers" is used to describe antioxidant compounds that have received significant attention, especially nowadays, as they protect the human body from the dangers of free radicals, which causes many diseases including cancer, and accelerate the aging process of cells [11].

In recent years, attention has been directed towards flavonoids, which include several compounds such as Proanthocyanidins, Quercetin, Rutin, Catechin, and Kaempferol, widely found in fruits and vegetables. There is a group of flavanols in the form of glycosides bonded with glucose at the C3 site, making these compounds soluble in cellular juice [12], [13].

It has been observed that the abundance of grape varieties allows us the opportunity to choose a variety that contains high levels of active components, all of which have beneficial and healing properties. Grapes offer benefits from the skin to the seeds, leaves, cluster structure, stems, etc. Since grapes are one of the oldest fruit crops cultivated in Iraq and due to the presence of numerous varieties. The goal of the study was to extract, separate, identify, and quantify the active chemicals compounds from seven grape varieties cultivated in Duhok Governorate, Kurdistan Region of Iraq

2. Materials and Methods

Vineyard and leaves collection

The study included seven varieties namely Hejazi, Tre rash, Eayn althawr, Superior seedless, Moon drop, Crimson and samples Samarkandi, cultivated in the two villages of Bare-buhar village near Zawita, situated between the latitude 36052’08.81 N, longitude 43005’49.34 E, and the elevation of the vineyard is 754 m above sea level (Agrometeorological station), and Bagera situated between the latitude 36057’41N, longitude 43010’01E, and the elevation of the vineyard is 873 m above sea level, Duhok governorate, Kurdistan region, Iraq. Leaf samples were collected during the spring growing season on the 25th of April, 2023, at a rate of 50 leaves per replicate, ranging between leaf positions 8-12, at the stage of fully expanded leaves before berry ripening. The leaves were collected, their stems removed, and then dried. After drying, they were ground and sieved through a sieve with openings of 0.016 microns, then stored in dark glass bottles in the refrigerator until use.

Plant extraction process

Leaf samples processing

The target samples of species were collected on the 25th of April, 2023 and then cleaned from the suspended dust particles and impurities. Each of the given plant samples was dried in a well-ventilated room at a temperature of 25°±2. After each sample entirely dried, pods and leaves of each species were separated from each other. Next, the dry leaves were finely grinded using electrical grinder (Germany) then put in sealed polyethylene bags. The sealed bags were incubated at 25°c in dark circumstances far from light and moisture until they were used to prepare extracts [14], [15].

Plant extraction process

The preserved sealed bags of each species sample were taken to conduct the extraction process. 50g of each leaves powder of each species was weighed, then 200 ml of methanol solvent was poured on the sample in a flask (500 ml) under room temperature. The flasks of extract preparation were transferred into a water bath ultrasonicator to enhance the extraction process for 40 minutes. After this operation, the flasks were taken and then left overnight. The extracts of each species' leaves were filtered via Buchner funnel with filter paper (0.45 µm) connected to a vacuum pressure pump. After the filtration was done, the filtrate of each species leaves was translocated onto a magnetic hot plate stirrer using starting temperature of 150°c for 5 minutes, then switched to 45-55°c. the concentrated supernatant was transferred into sealed tubes (10 ml) after cooled. These tubes were saved in the refrigerator at 4°C±2 until they would be used [16].

Phytoconstituent characterization

The phytochemical profiles of the methanolic leaf extract were studied via gas chromatography with electron impact mass spectrometry analysis (GC-MS). A GC-MS-QP2010 plus instrument (Shimadzu, Kyoto, Japan) was equipped with an autoinjector and 5 ms capillary column of 30 × 0.25 mm dimensions with 0.25 µm film thickness. He-gas availed as the carrier gas at 1.15 mL min-1 flow rate. Mass spectroscopic analysis was applied via 70eV ionizing system. The primary temperature was initialized at 80° C for 2 min and gradually elevated at a rate of 10° C per min up to 280°C for 5 min. The sample was injected according to split mode at 250° C. based on retention times and mass spectrum data, the extracted bioactive compounds were identified by comparing their mass spectra with two reference mass spectral databases: National Institute of Standards and Technology (NIST14) and Wiley 10th/NIST 2014 mass spectral library (W10N14). Each calibration curve was established by plotting the peak areas against five different concentrations (µg mL-1) of each standard. Furthermore, five replicates were applied for each calibration level [17].

3. Results and Discussion

Hejazi variety leaves analysis

The results shown in Table 1 indicate that GC-MS analysis identifies 61 bioactive compounds in methanolic extract of variety Hejazi leaves. Where, Methyl linolenate was the highest compound in extract of 6.71% then Methyl palmitate of 5.87%, docosane of 5.30% and Cyclomethicone 6 of 4.81%. whereas, 2-Hexanone recorded the lowest percentage at 0.26%.

Table (1): GC-MS analysis of the grape leaves Hejazi variety

Pk#	RT	Area %	Identity	group
1	7.819	0.26	2-Hexanone	ketone

2	25.817	4.81	Cyclomethicone 6	Organosilicone
3	32.047	5.87	Methyl palmitate	Fatty acid ester
4	32.641	1.27	Caryophyllene	sesquiterpene
5	33.235	0.45	Oleic acid	Fatty acid
6	35.224	0.96	Y-Methyl-Y-caprolactone	lactone
7	35.699	1.24	Cyclomethicone 7	Organosilicone
8	37.893	0.42	1-Nonadecene	alkene
9	38.888	1.79	Meadowlactone	lactone
10	39.717	1.03	2,4-Di-tert-butylphenol	phenol
11	42.294	2.47	cis-9-Hexadecenal	Fatty aldehyde
12	42.791	1.98	Elaidic acid	Fatty acid
13	43.672	0.96	Myristaldehyde	Fatty aldehyde
14	44.026	0.49	4-Amino-7-(diethylamino)coumarin	aminocoumarin
15	44.397	0.75	1,13-Tetradecadiene	alkene
16	45.026	1.14	5-(O-Tetrahydropyranyloxy) bicyclo[2.2.1]heptan-2-one	diterpene
17	46.066	0.58	(7R,8R)-cis-syn-trans-Tricyclo [7.3.0.0(2,6)]dodecan-7,8-diol	triterpene
18	46.306	0.53	Petroselaidic acid	Fatty acid
19	46.884	1.48	2-Pentadecanone	ketone
20	47.529	2.11	methyl oleyl ether	Fatty alcohol
21	48.850	1.49	Methyl (7E,10E,13E)-hexadeca-7,10,13-trienoate	Fatty ester
22	49.073	0.72	Cysteamine S-sulfate	Amine
23	49.553	2.01	Methyl lineoleate	Fatty ester
24	49.987	6.71	Methyl linolenate	Fatty ester
25	50.364	3.37	Methyl oleate	Fatty ester
26	51.181	0.91	2-Hexadecyloxirane	Ether
27	51.650	0.96	Methyl stearate	Fatty ester
28	51.982	1.46	Phytone	ketone
29	53.176	1.02	Eicosane	alkane
30	53.405	0.62	Oleic acid	Fatty acid
31	54.016	2.34	2-Heptadecanone	ketone
32	54.331	1.58	Ethyl 3-hydroxydodecanoate	Aliphatic alcohol
33	54.776	3.48	Methyl palmitate	Fatty ester
34	55.548	0.59	2-Pentadecanol	Fatty alcohol
35	55.822	1.26	Docosane	alkane
36	56.234	1.00	Plamitic acid	Fatty acid
37	57.188	1.66	Olealdehyde	Fatty aldehyde
38	57.966	0.30	(3R,3aS,4R,6aS)-3-Methyl-3-(phenylthio)-4-tridecyldihydrofuro[3,4-b]furan-2,6-dione	lactone
39	59.726	1.20	9-Octadecenoic acid	Fatty acid
40	60.349	2.94	Methyl vaccenate	Fatty ester
41	60.629	3.84	Tricosane	alkane
42	61.138	0.99	Methyl stearate	Fatty ester
43	61.595	0.75	Cis-palmitvaccenic acid	Fatty acid
44	61.903	0.53	propyl 2-ethylphenylcarbamate	carbamate
45	62.921	0.60	t-butyl palmitate	Fatty ester
46	63.149	0.80	1-Nonadecene	alkene
47	63.355	1.08	N-(5-Oxo-tetrahydro-furan-2-ylmethyl)-acetamide	lactone
48	64.212	0.58	(+)-Pseudocordatolide C	coumarin
49	64.795	3.33	1-Nonadecene	alkene
50	63.355	1.08	Lauric acid	Fatty acid
51	64.212	0.58	1,2,4,5-tetraethylcyclohexane	alkane
52	64.795	3.33	Lignocerane	alkane
53	66.184	2.77	Eicosane	alkane
54	66.441	1.06	Amyl elaidate	Fatty ester
55	66.790	1.77	Methyl 2-hydroxyicosanoate	Fatty ester
56	67.784	2.81	18-Nonadecenoic acid	Fatty acid
57	68.619	5.30	Docosane	alkane
58	69.362	2.22	Eicosane	alkane
59	69.853	1.46	monoheptyl phthalate	Phthalate ester

60	70.191	1.12	stearic acid	Fatty acid
61	71.317	0.93	Heptadecyl methoxyacetate	Fatty ester

Tre rash variety leaves analysis

The data in Table 2 showed that GC-MS analysis identifys 22 bioactive compounds in methanolic extract of variety Tre rash leaves. Where, Methyl palmitate was the highest compound in extract of 16.02% followed by Eicosane and 2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene at 11.82 and 11.17%, respectively. On the other hand, 2-Methyl-3-phenyl-1H-indole and 4-Phenylpyrido [2,3-d] pyrimidine indicated the lowest of 0.90 and 0.97%, respectively

Table (2): GC-MS analysis of the grape leaves Tre rash variety

Pk#	RT	Area%	Compound	Group
1	7.728	1.23	3-hexanone	Ketone
2	52.010	4.12	Hexahydrofarnesyl acetone	Ketone
3	52.610	11.82	Eicosane	Alkane
4	54.811	16.02	Methyl palmitate	Fatty ester
5	57.217	3.88	6-nitro-7-hydroxycoumarin	Coumarin
6	60.372	2.61	Methyl elaidate	Fatty ester
7	60.663	0.97	4-Phenylpyrido[2,3-d]pyrimidine	Alkaloid
8	61.166	3.79	Methyl stearate	Fatty ester
9	62.046	1.69	Cysteamine S-sulfate	Ethylamine
10	62.703	1.38	2,4-dichloro-6-nitrophenol	Nitrophenol
11	62.966	0.90	2-Methyl-3-phenyl-1H-indole	Indole
12	63.647	2.72	2-Chloro-3-(4-methoxyphenyl)but-2-enenitrile	Nitrophenol
13	64.144	4.06	Hexadecyl 3-methylbutanoate	Fatty ester
14	64.458	2.59	8-Hexyl-8-pentylhexadecane	Fatty alkane
15	64.978	1.84	Sclareolide	Lactone
16	65.350	9.76	Heptacosane	Alkane
17	66.990	11.17	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	Phenol
18	67.504	4.88	2-Hydroxy-6-methyl-4-(4'-nitrophenyl)-5-(phenylthio)-3,4-dihydropyridine-3-carboxamide	Alkaloid
19	67.996	3.13	14α-Cheilanth-12-enic Methyl Ester	Terpenoid
20	68.379	7.98	Fumaric acid, 2-chlorophenyl dodecyl ester	Organochlorine
21	68.958	1.25	Decarboxynorimbricaric acid	Polyketide
22	69.333	2.20	2-Methyl-3-phenylindole	Indole

Eayn althawr variety leaves analysis

B The results shown in Table 3 indicate that GC-MS analysis identifies 14 bioactive compounds in methanolic extract of variety Eayn althawr leaves. Where, Methyl palmitate recording the highest percentage of compound at 26.53% then dodecyl allyl carbonate, Linolenic acid and 4,6-cholestadienol of 15.06, 10.54 and 10.43%, respectively. Furthermore, 3-hexanone and Methyl 15-methylhexadecanoate were the lowest percentage of compound in the extract at 0.99 and 1.17%, respectively.

Table (3): GC-MS analysis of the Eayn althawr grape leaves Eayn althawr variety

Pk#	RT	Area%	Compound	Group
1	7.728	0.99	3-hexanone	Ketone
2	52.004	5.92	Hexahydrofarnesyl acetone	Terpene
3	54.62	3.08	2-Heptadecanone	Ketone
4	54.799	26.53	Methyl palmitate	Fatty ester
5	57.217	4.89	2-Heptadecenal	Aldehyde
6	58.051	1.17	Methyl 15-methylhexadecanoate	Fatty ester
7	60.366	10.54	Linolenic acid	Fatty acid
8	61.160	4.10	Methyl stearate	Fatty ester
9	64.824	3.13	9-Bromononanoic acid	Halogenated fatty acid
10	66.218	10.43	4,6-cholestadienol	Steroid
11	66.996	5.63	Methyl arachidate	Fatty ester
12	67.693	4.76	2,5-diethoxy-2-methyl-2,5-dihydrofuran	Lactone
13	68.573	15.06	dodecyl allyl carbonate	Carboxylic ester
14	70.082	3.77	Eicosane	Alkane

Superior seedless variety leaves analysis

The results as shown in Table 4, indicate that GC-MS analysis identifies 36 bioactive compounds in methanolic extract of variety Superior seedless leaves. Where, Methyl palmitate was the highest compound in extract of 21.39% then Methyl linolenate of 10.57%, while, Hexadecane was the lowest of 0.41%

Table (4): GC-MS analysis of the grape leaves Superior seedless variety

Pk#	RT	Area%	Compound	Group
1	6.745	2.26	Di-sec-butyl sulfide	Alkane
2	7.842	1.05	2-hexanone	ketone
3	8.134	0.83	Hexylsilane	silane
4	15.872	1.00	Stearoylethanolamide	Fatty amide
5	17.181	0.54	5-(chloromethyl)-2-oxolanone	Chlorinated lactone
6	17.673	1.34	D-Limonene	monoterpene
7	18.758	0.73	Octahydropentalene	terpene
8	22.439	1.54	Dimethyl malate	Fatty ester
9	22.839	1.22	N-isovaleryl-L-valinate	Amino acid ester
10	32.241	5.38	Triacetin	triglyceride
11	37.505	1.05	Cyclomethicone 7	siloxane
12	38.585	1.29	Docosane	alkane
13	43.134	0.41	Hexadecane	alkane
14	46.872	1.17	Tetradecane	alkane
15	51.993	4.81	Hexahydrofarnesyl acetone	terpene
16	53.976	1.93	Nonadecane	alkane
17	54.302	0.86	Methyl β-(Z)-2-Dimethylphenylsilylacrylate	alkene
18	54.793	21.39	Methyl palmitate	Fatty ester
19	55.536	0.64	2-Nonadecanone	ketone
20	56.026	0.58	citronellol Tetrahydropyranyl ether	ether
21	56.777	0.73	Ethyl margarate	Fatty ester
22	57.114	0.99	dideutri-pinane	terpene
23	58.040	0.77	Methyl 14-methylhexadecanoate	Fatty ester
24	60.349	10.57	Methyl linolenate	Fatty ester
25	60.903	2.81	2-Methylnonadecane	Fatty ester
26	61.155	3.49	Methyl stearate	Fatty ester
27	61.686	1.53	tridecyl 2-methylbutanoate	Fatty ester
28	63.075	3.10	13-Methylpentadec-14-ene-1,13-diol	Fatty alcohol
29	64.304	0.76	Z-22-Hentriaconten-2-one	Fatty ketone
30	64.818	1.24	Methyl elaidate	Fatty ester
31	66.430	8.71	ethyl 6,8-difluoro-4-hydroxyquinoline-3-carboxylate	alkaloid
32	66.984	2.03	Methyl arachidate	Fatty ester
33	67.356	2.04	Decarboxynorimbricaric acid	polyketide
34	67.681	1.80	4-methyl-5-pentyloxolan-2-one	lactone
35	68.179	7.97	Oleamide	Fatty amide
36	69.019	1.44	Actinolide	lactone

Moon drop variety leaves analysis

The data in Table 5 indicate that GC-MS analysis identifies 33 bioactive compounds in methanolic extract of variety Moon drop leaves, Methyl palmitate achieved highest compound in extract of 18.29% followed by β-Caryophyllene epoxide at 16.98%, Conversely, the lowest percentage were observed in the Propyl oleate, Octadecane and Methyl 14α-Cheilanth-12-enate of 0.44, 0.48 and 0.49%, respectively.

Table (5): GC-MS analysis of the grape leaves Moon drop variety

Pk#	RT	Area%	Compound	group
1	7.842	0.96	2-Hexanone	ketone
2	24.919	1.12	(+)-Borneol	terpene
3	26.468	1.04	(-)-Verbenone	terpene
4	32.264	0.85	Triacetin	triglyceride
5	41.631	0.95	Caryophyllene oxide	sesquiterpene
6	42.900	16.98	β-Caryophyllene epoxide	sesquiterpene
7	43.986	1.99	Humulene oxide II	sesquiterpene
8	45.169	2.31	Guaiol	sesquiterpene
9	45.689	2.50	α-Cadinol	sesquiterpene
10	46.443	2.88	3-(3-Butenyl)-2-cyclohepten-1-one	terpene
11	46.923	1.10	Heptadecane	alkane
12	47.152	1.04	Calarenepoxide	terpene
13	50.530	0.48	Octadecane	alkane
14	51.999	4.11	Hexahydrofarnesyl acetone	terpene

15	52.696	0.56	Phthalic acid, hexyl 2-methoxybenzyl ester	ester
16	53.113	2.04	Z-5-Nonadecene	Fatty acyl
17	53.965	8.88	Nonadecane	alkane
18	54.336	0.60	2,2-Dimethyl-3-heptyne	alkyne
19	54.793	18.29	Methyl palmitate	Fatty ester
20	57.228	1.70	Eicosane	alkane
21	60.360	7.41	Heneicosane	alkane
22	61.160	3.97	Methyl stearate	Fatty ester
23	62.046	2.44	Hexahydroxyiminocyclohexane	nitrobenzene
24	63.349	1.00	Eicosyl isopropyl ether	Fatty ether
25	63.824	0.44	Propyl oleate	Fatty ester
26	64.184	0.49	Methyl 14α-Cheilanth-12-enate	terpenoid
27	65.407	2.34	2-Ethylacridine	heterocycle
28	65.893	3.20	16-Hentriacontanone	Fatty ketone
29	66.213	1.57	Octacosane	alkane
30	66.716	2.76	Heptadecyl pentanoate	Fatty ester
31	66.978	1.18	Methyl arachidate	Fatty ester
32	68.213	2.18	Isopropyl octacosyl ether	Fatty ether
33	71.374	0.63	Methyl lignocerate	Fatty ester

Crimson variety leaves analysis

The results as shown in Table 6, indicate that GC-MS analysis identifies 22 bioactive compounds in methanolic extract of variety Crimson leaves. Methyl palmitate recording the highest values at 32.16%, then α-Tocospiro A and α-Tocospiro-B of 19.37 and 14.08%, respectively, while, 7-methyl-2-phenyl-1H-indole was the lowest of 0.21%.

Table (6): GC-MS analysis of the grape leaves Crimson variety

Pk#	RT	Area%	Compound	group
1	7.728	0.73	3-Hexanone	ketone
2	46.923	0.84	Heptadecane	alkane
3	51.993	6.32	Hexahydrofarnesyl acetone	ketone
4	53.113	0.39	1-Nonadecene	alkene
5	53.542	0.36	Cyclopentanemethanol	alcohol
6	53.965	1.73	Nonadecane	alkane
7	54.793	32.16	Methyl palmitate	Fatty ester
8	55.542	0.84	Isophytol	diterpene
9	57.114	1.28	Pyrrolizin-1-one, 7-hydroxy-	alkaloid
10	58.045	1.09	Methyl 14-methylhexadecanoate	Fatty ester
11	60.360	2.49	Heneicosane	alkane
12	61.155	7.01	Methyl stearate	Fatty ester
13	61.629	0.57	6-isopropyl-1-oxaspiro[2.5]octane	monoterpene
14	62.629	0.21	7-methyl-2-phenyl-1H-indole	indole
15	63.103	0.53	3-Phenyl-1,2-dihydro-1,2,4-triazin-6(5H)-one	Nitrogenous compound
16	63.806	0.44	Geranylgeraniol	diterpene
17	64.178	1.02	Ascorbyl Palmitate	Fatty ester
18	64.664	0.98	1-[4-(2-Thienyl)butyl]-3,3-dimethyl-2,3-dihydroindolene-1-spiro-2'-6'-nitro-3',4'-didehydrochroman	indole
19	66.218	14.08	α-Tocospiro-B	terpenoid
20	66.984	3.17	Methyl arachidate	Fatty ester
21	68.144	19.37	α-Tocospiro A	terpenoid
22	69.339	1.66	11-Methylnonacosane	Fatty ester

Samarkand variety leaves analysis

The results as shown in Table 7 GC-MS analysis identifies 13 bioactive compounds in methanolic extract of variety Samarkand leaves. Where, α-Tocospiro-A, α-Tocospiro-B and Methyl palmitate were the highest compound in extract of 22.64, 22.50 and 21.76%, respectively, Furthermore, 3-hexanone, Methyl arachidate, 5-Methyl-2-phenylindole and 1-Propene, 3-(2-cyclopentenyl)-2-methyl-1,1-diphenyl were the lowest of 1.01, 1.69, 1.76 and 1.90%, respectively.

Table (7): GC-MS analysis of the grape leaves variety Samarkand

Pk#	RT	Area%	Compound	group
1	7.734	1.01	3-hexanone	ketone
2	52.004	7.03	Hexahydrofarnesyl acetone	ketone
3	53.976	4.91	Eicosane	alkane
4	54.805	21.76	Methyl palmitate	Fatty ester
5	55.931	4.57	2-methyl-3-phenyl-3H-indole	indole
6	56.760	1.90	1-Propene, 3-(2-cyclopentenyl)-2-methyl-1,1-diphenyl	phenol
7	57.120	1.42	Nonadecyl trifluoroacetate	Fluorinated aliphatic
8	60.372	3.69	Methyl elaidate	Fatty ester
9	61.166	5.11	Methyl stearate	Fatty ester
10	63.138	1.76	5-Methyl-2-phenylindole	indole
11	66.218	22.50	α-Tocospiro-B	monoterpenoid
12	66.984	1.69	Methyl arachidate	Fatty ester
13	68.304	22.64	α-Tocospiro-A	monoterpenoid

Discussion

Cluster Analysis

It's been noted that the wide range of grape varietals gives us the chance to select one with high concentrations of active ingredients, all of which have positive and restorative qualities. The leaves of grapes is beneficial. The results showed that the leaves of grape varieties contain many active compounds, and the number and concentrations of these active compounds vary among the studied varieties. The number of active compounds according to the sequence was Hejazi 61 with highest compound of Methyl linolenate (6.71%), Superior seedless 36, Moon drop 33, Tre rash and Crimson 22, Eayn althawr 14, and Samarkandi 13 with 22.50 of α-Tocospiro-B. The compound Methyl palmitate appeared at its highest levels in the varieties (Tre rash, Eayn althawr, Superior seedless, Moon drop, Crimson), with values of (16.02%, 26.53%, 21.39%, 18.29%, 32.16%), respectively. GC-MS analysis confirmed that there is a biochemical diversity in the given grapevine varieties. This is consistent with findings reported by [18], [19], [20], [21]. From the tables above, it is illustrated that there are different chemical groups that were characterized in GC-MS analysis. Many studies referred to this difference based on leaves, environmental factor, genetic material [17], [22]. In contrast, epigenetic factors as methylation and demethylation took part in gene expression that encodes the bioactive compounds [23]. Therefore, these factors contribute on increase biosynthesis of certain phytochemicals. Consequently, this biosynthesis leads to bioactive constituents being divergent [24]. Therefore, these compounds will possess various uses in human life [25], [26], [27].

Conclusion

The study concludes that all grape varieties contained active compounds in their leaves, and the number, type, and concentrations of these active compounds varied among the studied varieties. The phytochemical analysis via GC/MS illustratred that there is a highly phytoconstituent diversity among certain grapevine varieties. Therefore, this diversity could use to identify these varieties. Furthermore, these varieties could be identified via the highest compound as GC/MS characterize

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التنوع الكيميائي النباتي لسبعة أصناف من العنب باستخدام GC-MS

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الخلاصة:
هدفت الدراسة الحالية إلى استخلاص المركبات الفعالة لسبعة أصناف من العنب المزروعة في محافظة دهوك التابعة لأقليم كردستان العراق للموسم 2023 وشملت الاصناف ***(Hejazi ، Tre rash ، Eayn althawr ، Superior seedless ، Moon drop ، Crimson و Samarkandi)***. بينت النتائج احتواء أوراق أصناف العنب على العديد من المركبات الفعالة وان عدد هذه المركبات الفعالة وتراكيزها يختلف مابين الاصناف المدروسة. وقد بلغ عدد المركبات الفعالة حسب التسلسل وظهر المركب ***(Hejazi 62، Superior seedless 36، Moon drop 33، Tre rash ، Crimson 22، Eayn althawr 14 و Samarkandi 13).***

Methyl palmitateبأعلى مستوياته في كل من الاصناف ***(Tre rash*** ، ***Eayn althawr***، ***Superior seedless***، ***Moon drop***و ***(Crimson***وبلغت القيم (16.02% ، 26.53% ، 21.39% ، 18.29% ، 32.16%) على التتابع. أكد تحليل ***GC-MS*** وجود تنوع كيميائي حيوي في أصناف العنب المعطاة. ولذلك أمكن لهذه الأصناف التمييز من خلال أعلى مركب ظهر في تحليل ***GC-MS.***

الكلمات المفتاحية : التنوع الكيميائي النباتي، العنب، الأصناف، المركبات الفعالة، ***GC-MS***