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Active Compounds Variation in Grapevine Varieties Leaves Using GC-MS

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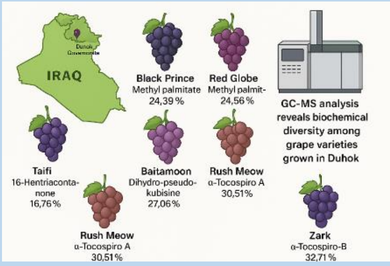
Abstract: This study aimed to recognize the major bioactive compounds in the leaves of seven grape (Vitis vinifera L.) cultivars viz., Black Prince, Red Globe, Taifi, Baitamoon, Tahlak, Rush Meow, and Zark, grown in the Duhok province (Kurdistan Region, Iraq) during the 2023 season. GC-MS analysis illustrates a total of 24 compounds in Black Prince, followed by Taifi (17), Red Globe (14), Baitamoon (13), and 10 each in Tahlak, Rush Meow, and Zark. The amplest compounds involved methyl palmitate in Tahlak (49.44%), Red Globe (24.56%), and Black Prince (24.39%); 16-hentriacontanone in Taifi (16.76%); dihydro-pseudokubisine in Baitamoon (27.08%); α-tocospiro A in Rush Meow (30.51%); and α-tocospiro B in Zark (32.71%). These findings highlight the chemodiversity among varieties and advocate that predominant constituents can compose as variant markers. Further research on limits of detection (LoD) and quantification (LoQ) is recommended to validate the analytically precisions of GC-MS profilings.



Keywords: Agronomic sustainability, chemodiversity, north environment, genotypes, technique analysis, GC-MS.

Introduction

Grapes are one of the most common fruit trees in the world [1]. More than one-third of the world's fruit is produced as grapes, with about 47% of that amount being used as fresh table grapes. Over 70% of the world's grapevine area is in Europe alone. Italy, France, Russia, Spain, and America are the world's leading grape-producing nations [2]. Iraq has the second-largest grape-growing area in the Arab world. Iraq has about 70 different types of grapes, the most of which are found in the country's northern areas [3]. In the Arab world and in Iraq, particularly in the northern and central parts of our nation where the climate is conducive to grape growth, there is a chance to expand the area planted with grapes and improve their productivity [4]. In traditional Chinese and Indian medicine, grapes have long been valued for their nutritional and medicinal properties, and their extracts have been incorporated into various treatments since ancient times. Sugars like glucose and fructose, which are quickly absorbed and digested, are present in grapes in good amounts.

The berries also include a good amount of minerals like K, Ca, Na, and others, and are high in vitamins A, B6, C, and E. Additionally, grapes offer therapeutic benefits because they contain a compound called resveratrol, an antioxidant that has been shown to reduce stiffness of the arteries by directly and visibly lowering cholesterol levels, which lowers the risk of heart disease. Grapes are a good anti-cancer agent because they contain specific acids that help to reduce the accumulation of free radicals. Additionally, because of their high calcium level, grapes can reduce osteoporosis.

Recipes with young grape leaves are used to treat joint diseases, stomach acidity, headaches, and tumor inflammations. Additionally, dried leaves and grape peel can be utilized in a variety of formulations to treat burns, inflammations, hemorrhoids, and scorpion stings. While grape seed oil is used to treat gynecological disorders, grape ash is utilized to promote hair growth in burn situations. [5,6,7,8]. Whole plants or their parts, such as roots, leaves, bark, or seeds, are the source of the active compounds utilized in traditional medicine [9]. The extraction of physiologically active substances is dependent on the extraction solvent, temperature, and combination with other classical methods including steam distillation, maceration, and Soxhlet extraction [10]. Antioxidant chemicals, such as vitamins, flavonoids, and other substances, are primarily responsible for the protection that fruits and vegetables offer to people, particularly against cancer and heart disease.

Higher antioxidant activity has been found in certain flavonoids, particularly the peroxyl radical's capacity to scavenge free radicals, which has a greater impact than vitamins C and E. The phrase "free radical scavengers" refers to antioxidant compounds that have taken a lot of interest, particularly in recent years, because they guard the body from the risks caused by free radicals, which can cause cancer and other diseases, as well as hasten the aging process of cells [11]. Flavonoids, which are found in many fruits and vegetables and comprise various substances like Proanthocyanidins, Quercetin, Rutin, Catechin, and Kaempferol, have received more attention in recent years. These substances are soluble in cellular fluid because of a class

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of flavanols known as glycosides that are bound to glucose at the C3 location [12,13]. Seeds of grapes had contained Saponin, tannins, alkaloids, flavonoids and terpenoids [14]. Many studies referred to difference in grapes phytochemicals depending on cultivars [15,16]. Grapes (Vitis vinifera L.) are represented as one of the most universally planted and economically important fruit crops worldwide, accounting for over one-third of total thorough fruit productivity. Approximately 47% of these are freshly intaken, with more than 70% of global vineyard areas located in Europe-particularly in Italy, France, Russia, Spain, and the United States [17]. Within the Arab world, Iraq achieves the second-largest grape cultivation area, with more than 70 known cultivars, mainly densified in the northern regions such as the Kurdistan Region. This agro-climatically proper zone offers significant potential for prolonging grape production and enhanicing the selection of cultivar.

Furthermore, Grapes are identified for their nutrient and curing properties. wealthy in glucose, fructose, and essential minerals (K, Ca, Na), grapes enhance extraordinary amounts of vitamins A, B6, C, and E. Among their bioactive compounds, resveratrol has been deeply studied for its antioxidant and cardioprotective impacts [18]. Grape leaves, traditionally exploited in folk medicine, had demonstrated effectiveness in curing joint pain, gastrointestinal ailments, inflammation, and even skin conditions. Other grape-derived products, such as seed oil and peels, have been indicated to heal wounds and possessed anti-inflammatory, and antioxidant properties.

various studies have highlighted the presence of diverse phytochemicals in grapes, including flavonoids, tannins, saponins, alkaloids, and terpenoids. However, the concentration and composition of these compounds are highly variety-dependent [19]. Extraction efficiency and compound profiles can also vary based on environmental conditions, geographic origin, and extraction methods.

as extraordinary research has been applied on grape phytoconstituents thoroughly, there is a marked lack of data on the phytochemical composition of grapevine leaves from Iraqi cultivars, particularly those grown in the Kurdistan Region. Most existing studies have focused on grape berries or seeds, with limited attention given to the bioactive efficacy of the leaves, which are widely exploited in local traditional remedies. Furthermore, no prior GC-MS-based comparative analysis had been published on multiple grape leaf varieties thrived in this geographic zone.

Given the diverse grape germplasms found in northern Iraq and the traditional medicinal use of grape leaves, the current study addresses a critical gap by employing Gas Chromatography-Mass Spectrometry (GC-MS) to identify and compare the bioactive chemical profiles of leaves from seven grape cultivars cultivated in Duhok, Kurdistan Region. By revealing the cultivar-specific distribution of major compounds such as Methyl palmitate, 16-Hentriacontanone, and α-Tocospiro isomers, this study enhances essential biochemical data that may aid in the selection of varieties for pharmaceutical, nutritional, or agricultural applications. It also sets the stage for future research on standardizing and validating the therapeutic properties of grape leaf extracts from this underexplored region.

It has been noted that the wide range of grapes available to us gives us the chance to select one with high concentrations of active components, all of which have positive and restorative qualities. The skin, seeds, leaves, cluster structure, stems, and other parts of grapes are all beneficial. Given that there are many different types of grapes and that they are among the oldest fruit crops grown in Iraq, the study's objective was to extract, segregate, determine, and assess the active chemical components from seven different grape varietals grown in Iraq's Kurdistan Region's Duhok Governorate.

Materials and Methods

Collection of leaves

Seven cultivars were considered in the study, including Black Prince, Red Globe, Taifi, Beitamooni, Tahlak, Rush Meow and Zark, grown in the two Bare-buhar villages close to Zawita, located between longitude 43005'49.34 E and latitude 36052'08.81 N, and according to the Agrometeorological Station, the vineyard is 754 m above sea level, and Bagera, which is located between latitudes 36057'41N and 43010'01E, and the vineyard is located in the Duhok governorate in the Kurdistan region of Iraq, 873 m above sea level. On April 25, 2023, a sample of leaves was taken during the spring growing season, with 50 leaves per replicate, between leaf positions 8-12, when the leaf is fully extended before berry ripening. After collecting the leaves were dried, ground and sieved using a sieve with 0.016 micron, then kept in the refrigerator in dark glass bottles until needed. The historical information of given varieties are illustrated in table 1.

Table (1): some information on studied grape varieties.

Varieties	Origin	Pedigree	Description
Beitamouni	Lebanon	natural cross between Samanci Cekyrdeksyzy x unknown variety	The white grape variety. Synonyms are Asba el Arus, Bairuti, Baitamouni, Beita Mouni, Betamuni, Beytamouni, Foga, Nychato, Rich Baba Sam, Safadi and Zeini Sheik.
Black Prince	South America		Synonyms are Criolla Mediana 2, Dolcino, Laurenzana, Rosa del Perú, Rose of Peru, San Francisco, Santa Chiara, Uva Criolla San Francisco and Uva San Francisco. dark purple with a thick blue bloom, offers a juicy, sweet flavour. Known for long bunches and large, oval berries.
Red globe	USA	Emperor variety in 1980.	very large, seeded red grapes with firm flesh used mainly as a table grape. It can be grown outdoors in very warm areas with long growing seasons such as California, Chile or Australia.
Rash miow (black berry)	Indigenous	Commonly in Iraq	Improved in iraq, conical bunches with shoulders. Black or purple Oval berries medium regular seting. Fleshy juicy sweet taste, used for raisin. Grown in areas that irrigated by rains.
Taifi	Central Asia and the Middle East	Arabian variety grown in Saudia Arabia	Taifi grapes is growing in medium to large, loose, conical bunches. The large, oval to round grapes average 2 to 3 centimeters in diameter and are connected to the bunch by a single yellow-green stem. The grapes have thick, smooth, and taut skin and range in color from a saturated red-pink or light pink to green, depending on the variety. The skin is also covered in a waxy coating, sometimes showcasing small dots. Underneath the surface, the flesh has a red or green translucent hue and is juicy and dense with a crisp, crunchy consistency.
Tahlak	Indigenous	Old Common variety in Iraq	Medium to large black bunches with shoulders. Black Spherical berries covered by light waxy caover. Fleshy, juicy. Bitter taste. Grown in areas that irrigated by rains.
Zarek	Indigenous	Riesling x common variety from Dohuk northern Iraq	Conical bunches with small shoulders, white-yellowish filling spherical berries with medium waxy coating, fleshy juicy pulp. Tart taste. Grown in areas that irrigated by rains.

Processing of leaf samples

On April 25, 2023, the target species samples had been collected and they were washed to remove the dust particles. Each plant sample was dried at a room temperature of 25°±2 with adequate ventilation. After the samples had completely dried, an electrical grinder (Germany) was used to finely grind the dry leaves, then placed in plastic bags that are sealed. Before using them to make extracts, the sealed bags had been kept at 25°C in a dark environment away from light and moisture [17,18].

Procedure for extracting plants

To do the extraction procedure, each species sample's preserved sealed bags were taken. After weighing 50g of each species' leaf powder, 200 ml of methanol solvent was added to the sample in a 500 ml flask and allowed to sit at room temperature. To improve the extraction process, the extract preparation flasks were placed in a water bath ultrasonicator for forty minutes. Following this procedure, the flasks were taken out and left overnight. Each species' leaf extracts were filtered using a Buchner funnel fitted with 0.45 µm filter paper and a vacuum pressure pump. Following filtration, the filtrate of each species' leaves was transferred onto a magnetic hot plate stirrer, which was initially set at 600°C for five minutes before being adjusted to 45–55°C. After cooling, the concentrated supernatant was transferred into 10 ml sealed tubes. Until they were used, these tubes were stored in a refrigerator at 4°C±2 [19].

Analysis of phytoconstituents

Using gas chromatography combined with electron impact mass spectrometry analysis (GC-MS), the phytochemical profiles of the methanolic leaf extract were investigated. A GC-MS-QP2010 plus device (Shimadzu, Kyoto, Japan) had a 5 ms capillary column with 30 × 0.25 mm dimensions and a 0.25 µm film thickness, as well as an autoinjector. He-gas was made available as the carrier gas at a flow rate of 1.15 mL min-1. The 70eV ionizing system was used to perform mass spectroscopic analysis. After starting at 80° C for two minutes, the primary temperature was progressively raised at a rate of 10° C per minute for five minutes, reaching 280° C. At 250° C, the sample was injected using split mode. The recovered chemical compounds were identified by comparing their mass spectra with two reference mass spectral databases based on retention durations and mass spectrum data: Wiley 10th/NIST 2014 mass spectral library (W10N14) and the National Institute of Standards and Technology (NIST14). Plotting the peak regions against five distinct concentrations (µg mL-1) of each standard allowed for the establishment of each calibration curve. Additionally, for every calibration level, five duplicates were used [20].

Statistical analysis

Originpro 2024b was used to construct PCA biplot and color triangular plot from area% of various GCMS profiles identified in seven grapes varieties. All chromatograms are fixed using software above.

Results and Discussion

Black Prince variety leaves analysis

GC-MS analysis of the methanolic extract of variety Black Princess leaves reveals 24 bioactive components (Fig. 1), according to the results reported in Table 2. The strongest ingredient in the extract, Methyl palmitate, was present at 24.39%, followed by Nonadecane at 10.55%, Triacontane at 9.63%, and α-Tocospiro B at 9.02%. In contrast, cis-3,3a,4,5,6,7-Hexahydro-7-(2-thienyl)cyclohexa[c]isoxazole had the lowest percentage (0.41%).

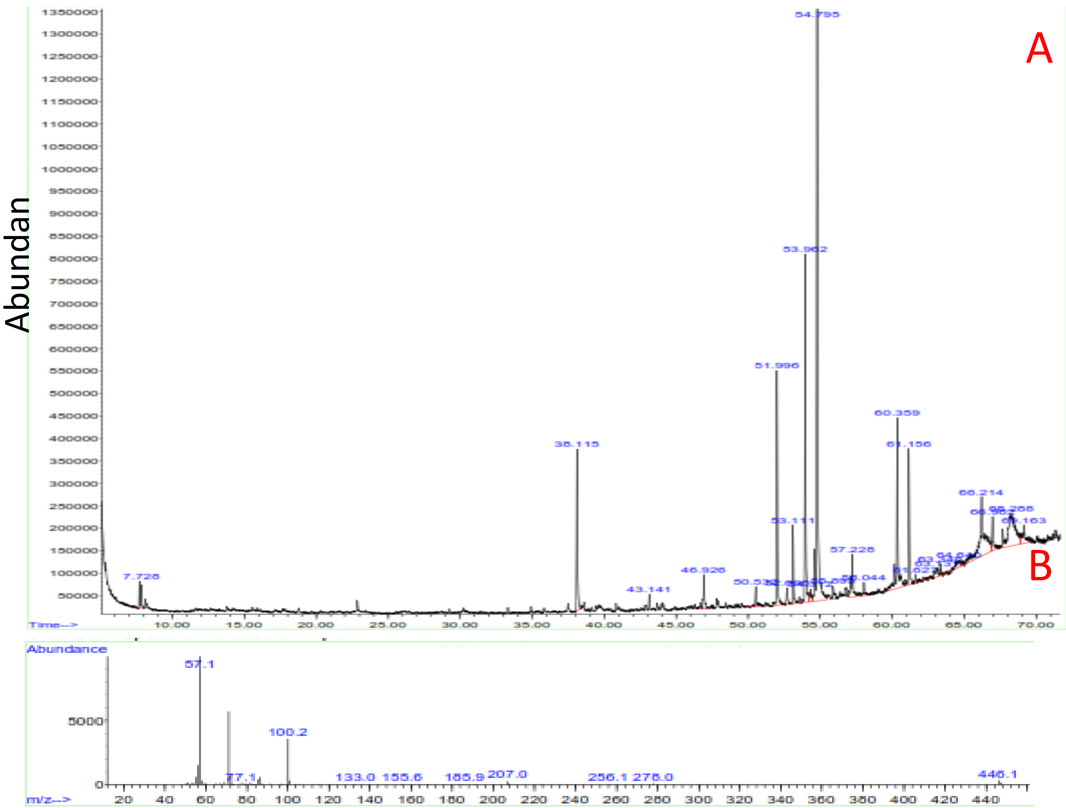

Figure (1): TIC chromatogram of twenty-four profiles characterized in black prince leaves extract (A) and unknown spectrum (B).

Table (2): GC-MS profilings of the grape leaves from Black Princess variety.

Pk#	RT (min)	%Area	Compounds	Functional groups
1	7.728	1.08	3-Hexanone	Ketone
2	38.116	5.45	4-methylidenecyclohexa-2,5-dien-1-one	Quinones
3	43.140	0.51	Hexadecane	Alkane
4	46.923	1.73	Heptadecane	Alkane
5	50.530	1.00	Octadecane	Alkane
6	51.999	7.69	Hexahydrofarnesyl acetone	Terpene
7	52.696	0.72	Isobutyl octadecyl phthalate	Organic compound
8	53.113	2.57	Z-5-Nonadecene	Alkene
9	53.965	10.55	Nonadecane	Alkane
10	54.313	0.93	7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	Lactone
11	54.793	24.39	Methyl palmitate	Fatty methylester
12	55.897	0.83	Phthalic acid, butyl 2-(2-methoxyethyl)hexyl ester	Organic compound
13	57.228	2.69	Eicosane	Alkane
14	58.045	0.97	Methyl margarate	Fatty methylester
15	60.360	8.18	Heneicosane	Alkane
16	61.155	5.08	Methyl stearate	Fatty methylester
17	61.629	0.41	Cis-3,3a,4,5,6,7-Hexahydro-7-(2-thienyl)cyclohexa[c]isoxazole	Alkaloid
18	63.132	1.39	2-Chloro-3-(4-methoxyphenyl)but-2-enenitrile	phenylpropenoid
19	63.344	0.45	11-Methylnonacosane	Alkane
20	64.641	1.08	5-Methyl-2-phenylindole	Indole
21	66.213	9.63	Triacontane	Alkane
22	66.984	1.72	Methyl arachidate	Fatty methylester
23	68.287	9.02	α-Tocospiro B	Triterpenoid
24	69.162	1.95	5-Methyl-2-phenylindole	Indole

Red Globe variety leaves analysis

The information in Table 3 indicated that 14 bioactive components were found in the methanolic extract of variety Red globe leaves by GC-MS analysis (Fig. 2). Methyl palmitate accounted for 24.56% of the extract, with α-Tocospiro B and α-Tocospiro A at 21.47 and 18.05%, respectively. However, Nonadecane and Longiborneol had the lowest percentages, at 0.56 and 0.75%, respectively.

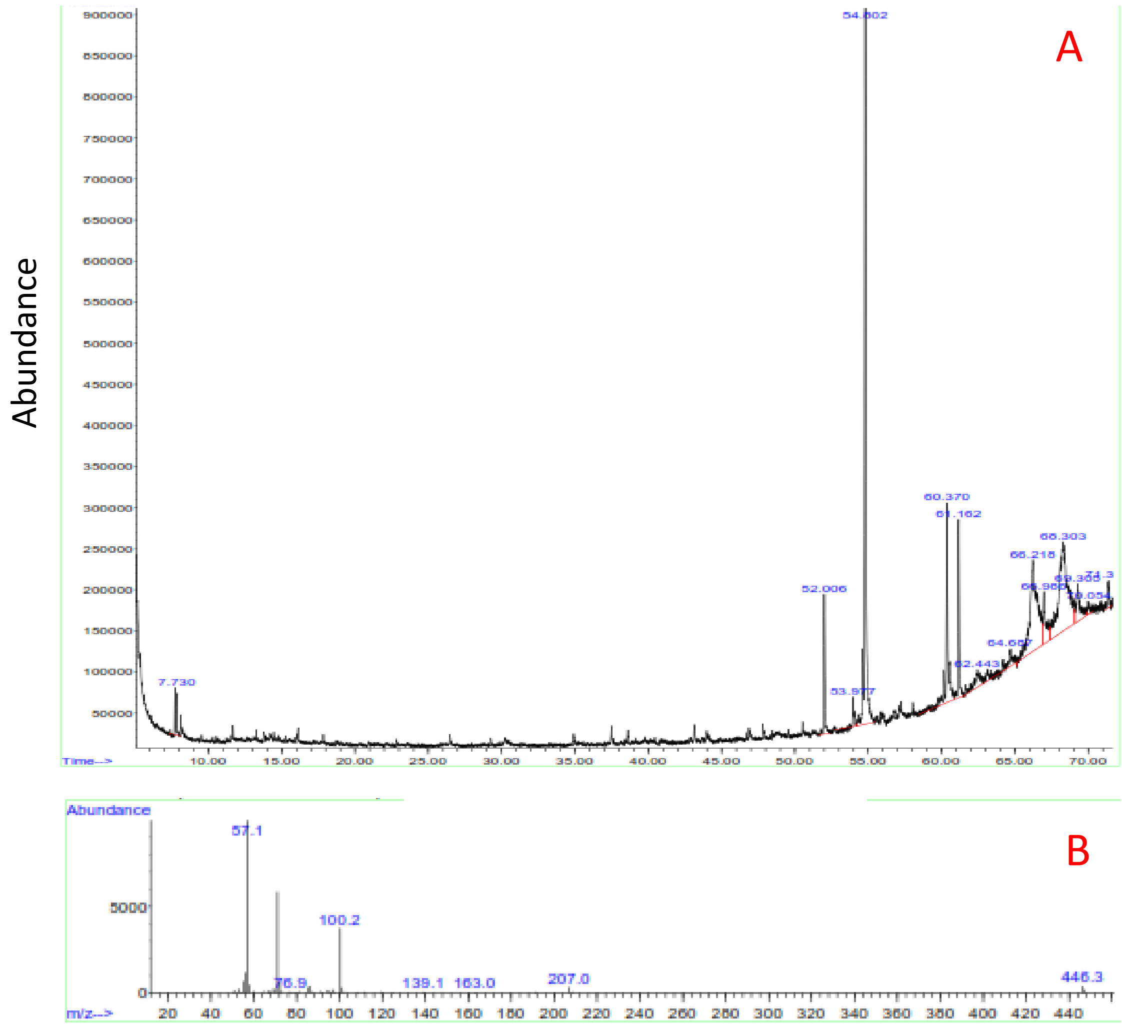

Figure (2): TIC chromatogram of twenty-four profiles characterized in Red Globe leaves extract (A) and unknown spectrum (B)

Table (3): GC-MS profilings of the grape leaves from Red Globe variety.

Pk#	RT(min)	%Area	Compounds	Functional groups
1	7.728	1.35	3-hexanone	Ketone
2	52.004	4.08	Hexahydrofarnesyl acetone	Terpene
3	53.976	0.56	Nonadecane	Alkane
4	54.805	24.56	Methyl palmitate	Fatty methylester
5	60.372	9.54	Methyl elaidate	Fatty methylester
6	61.160	5.21	Methyl stearate	Fatty methylester
7	62.441	2.67	Behenyl behenate	Fatty docosylester
8	64.687	2.87	Tricyclo[9.3.1.1(4,8)]hexadeca-1(15),4,6,8(16),11,13-hexaene-5,14-dimethanol	Diterpenoid
9	66.218	18.05	α-Tocospiro A	Triterpenoid
10	66.984	3.71	Methyl arachidate	Fatty methylester
11	68.304	21.47	α-Tocospiro B	Triterpenoid
12	69.305	3.65	13-Methylhentriacontane	Fatty methylester
13	70.053	0.75	Longiborneol	sesquiterpenoid
14	71.362	1.52	Methyl lignocerate	Fatty methylester

Taifi variety leaves analysis

17 bioactive compounds have been determined by GC-MS analysis in the methanolic extract of variety Taifi leaves (Fig. 3), according to the results listed in Table 4. The compound with the largest percentage, 16.76%, was 16-Hentriacontanone, followed by α-Tocospiro B, α-Tocospiro A and Methyl palmitate, with respective percentages of 15.96, 13.96, and 11.43%. Additionally, the lowest percentages of compounds were Hexadecane and Octadecane, which were 0.44 and 0.68%, respectively.

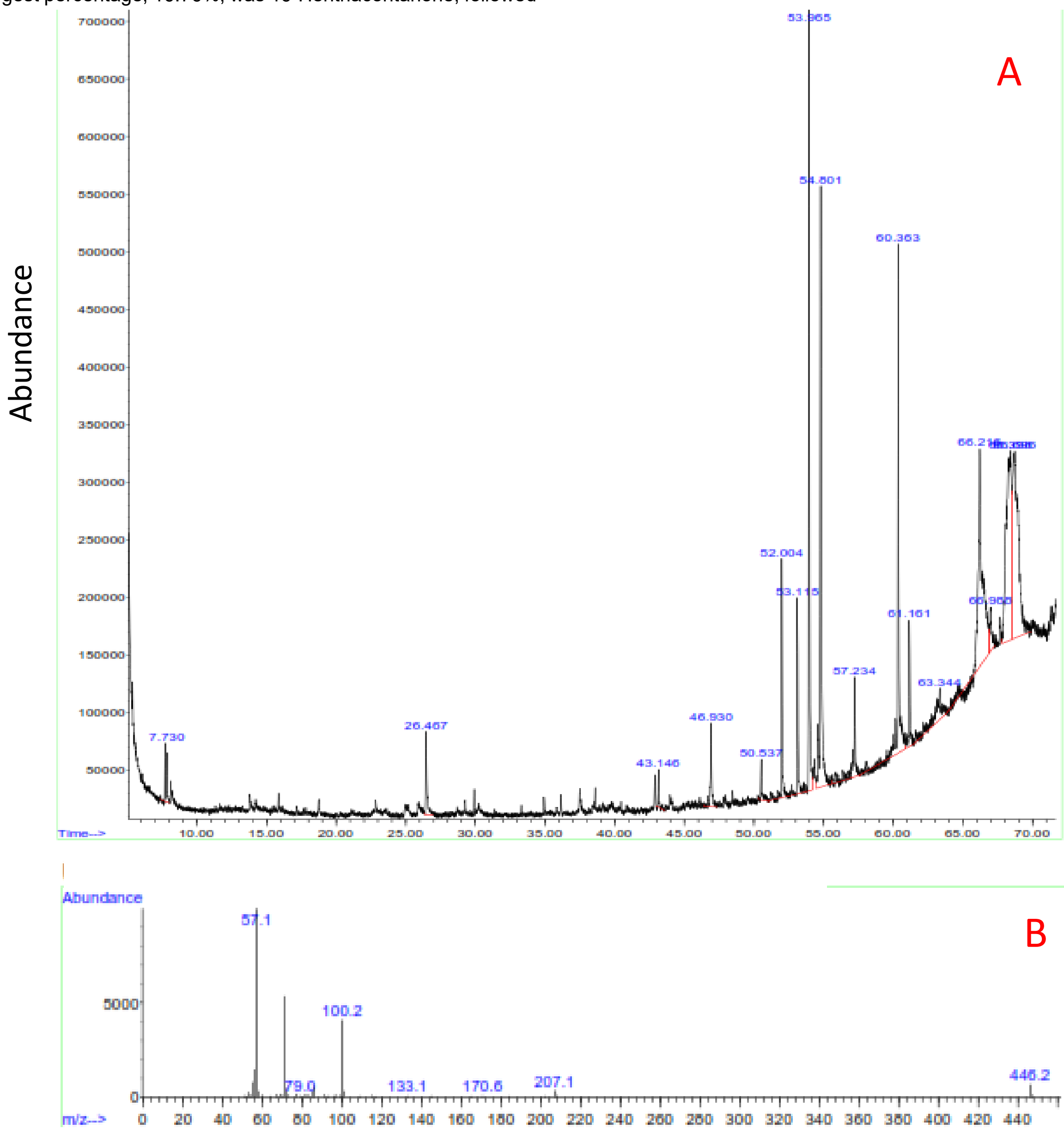

Figure (3): TIC chromatogram of twenty-four profiles characterized in Taifi leaves extract (A) and unknown spectrum (B).

Table (4): GC-MS profilings of the grape leaves from Taifi variety.

Pk#	RT(min)	Area%	Compounds	Functional groups
1	7.728	0.98	3-hexanone	Ketone
2	26.468	1.67	Verbenone	Monoterpene
3	43.146	0.44	Hexadecane	Alkane
4	46.929	1.70	Heptadecane	Alkane
5	50.535	0.68	Octadecane	Alkane
6	52.004	3.85	Hexahydrofarnesyl acetone	Sesquiterpenoid
7	53.113	3.13	1-Nonadecanol	fatty alcohol
8	53.965	10.41	Nonadecane	Alkane
9	54.799	11.43	Methyl palmitate	Fatty methylester
10	57.234	2.82	Eicosane	Alkane
11	60.366	9.45	Heneicosane	Alkane
12	61.160	2.48	Methyl stearate	Fatty methylester
13	63.343	2.99	Eicosyl isopropyl ether	Ether
14	66.218	13.96	α-Tocospiro A	Triterpenoid
15	66.990	1.30	22-Dehydrocholesterol	Steroid
16	68.396	15.96	α-Tocospiro B	Triterpenoid
17	68.699	16.76	16-Hentriacontanone	Ketone

Beitamouni variety leaves analysis

Table 5 showed that 13 bioactive were identified by GC-MS analysis in the methanolic extract of Baitamoon leaves (Fig 4). The extract's strongest compound, Dihydro-pseudokubisine was 27.08%, followed by 2-(3-Benzyloxyprop-1-en-2-yl)-1-(tert-butoxylcarbonyl)-3-butyl-1H-indole and Methyl palmitate which were 20.51 and 19.44%, respectively. Furthermore, had the lowest percentages, at 1.20, 1.82 and 1.88%, respectively with the components 3-Hexanone, 1-Decyl-1H-imidazole-2-methanol and Methyl 2-(4-(tert-butyl)phenoxy) acetate.

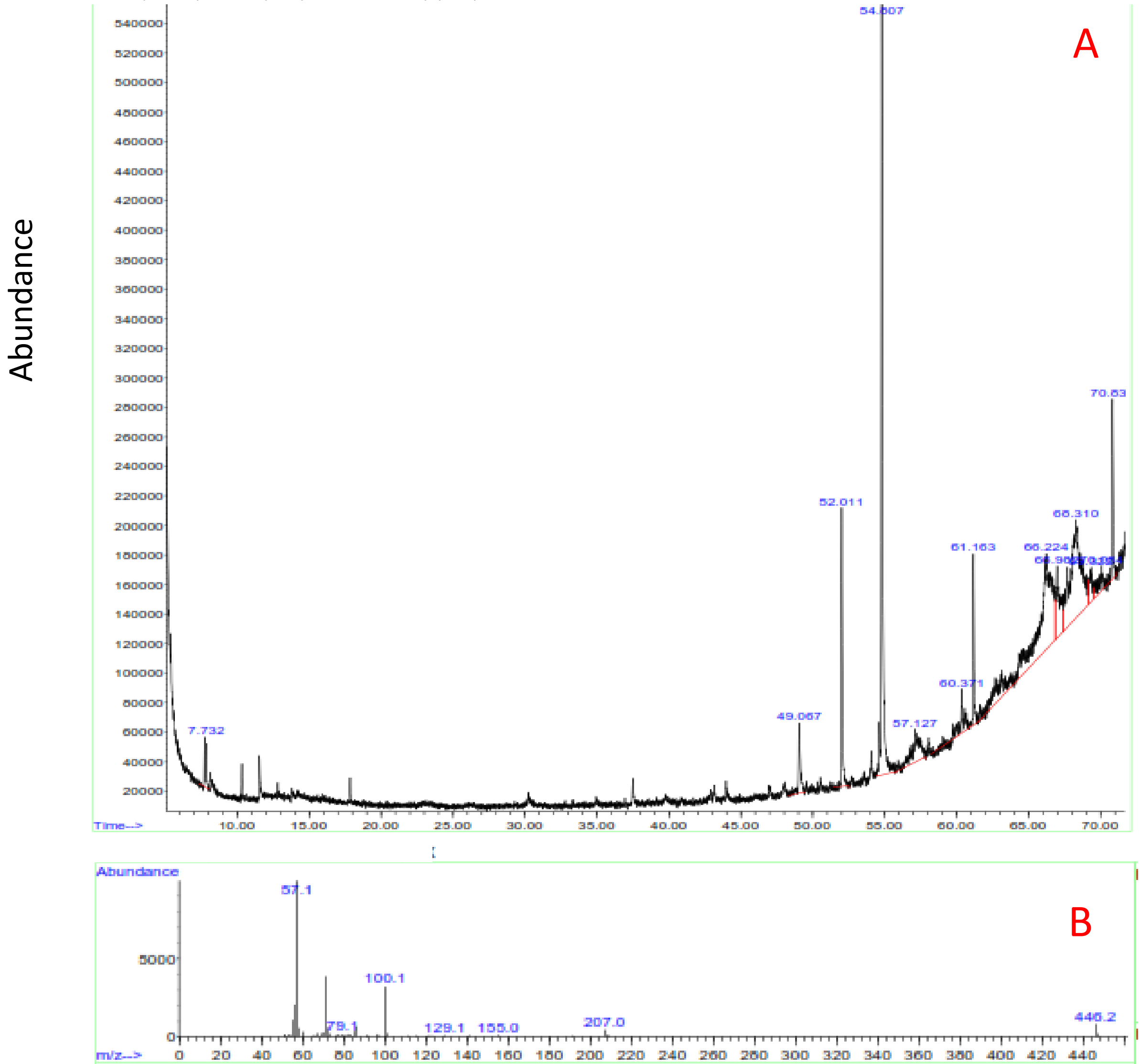

Figure (4): TIC chromatogram of twenty-four profiles characterized in Baitamoon leaves extract (A) and unknown spectrum (B).

Table (5): GC-MS profilings of the grape leaves from Beitamouni variety.

Pk#	RT(min)	%Area	Compounds	Functional groups
1	7.734	1.20	3-Hexanone	Ketone
2	49.067	2.51	cis-1,2-Diphenylcyclobutane	Benzenoid
3	52.010	5.59	Hexahydrofarnesyl acetone	Terpenoid
4	54.805	19.44	Methyl palmitate	Fatty methylester
5	57.125	4.52	2-Isopropyloxirane	Ether
6	60.372	2.33	Petroselaidic acid	Fatty acid
7	61.166	3.62	Methyl stearate	Fatty methylester
8	66.224	27.08	Dihydro-pseudokubisine	Diterpenoid
9	66.984	5.11	Ethyl 6-amino-5-cyano-4-(5-cyano-2,4-dimethyl-1H-pyrrol-3-yl)-2-methyl-4H-pyran-3-carboxylate	Alkaloid
10	68.310	20.51	2-(3-Benzyloxyprop-1-en-2-yl)-1-(tert-butoxylcarbonyl)-3-butyl-1H-indole	Alkaloid
11	69.322	1.82	1-Decyl-1H-imidazole-2-methanol	Alkaloid
12	70.082	1.88	Methyl 2-(4-(tert-butyl)phenoxy)acetate	Ether
13	70.831	4.39	2,4,6-Triphenyl-1-hexene	phenylpropanoid

Tahlak variety leaves analysis

Table 6 shows that 10 bioactive components were identified by GC-MS analysis in a methanolic extract of Tahlak leaves (Fig 5). The highest compound in the extract, methyl palmitate, was 49.44%, followed by Methyl linolenate at 15.42%. In addition, methyl Myristate and Methyl arachidate had the lowest percentages at 1.14 and 1.82%, respectively.

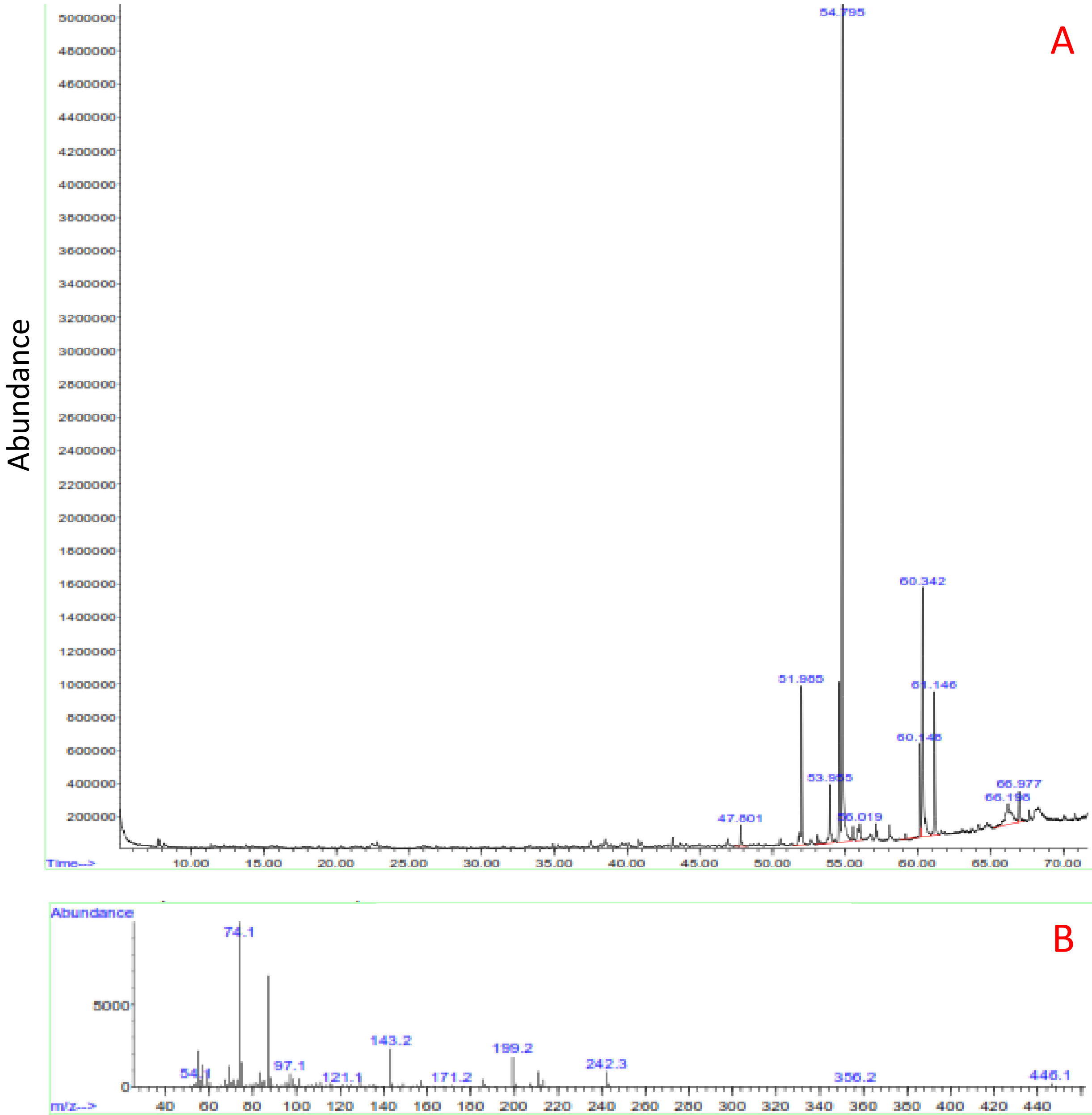

Figure (5): TIC chromatogram of twenty-four profiles characterized in Tahlak leaves extract (A) and unknown spectrum (B).

Table (6): GC-MS profilings of the grape leaves from Tahlak variety.

Pk#	RT(min)	%Area	Compounds	Functional groups
1	47.798	1.14	Methyl Myristate	Fatty methylester
2	51.987	7.55	Hexahydrofarnesyl acetone	Terpenoid
3	53.953	4.16	Nonadecane	Alkane
4	54.793	49.44	Methyl palmitate	Fatty methylester
5	56.017	2.11	Y-Heptalactone	Lactone
6	60.149	4.04	Methyl linoleate	Fatty methylester
7	60.343	15.42	Methyl linolenate	Fatty methylester
8	61.143	7.30	Methyl stearate	Fatty methylester
9	66.196	7.02	α-Tocospiro A	Triterpenoid
10	66.979	1.82	Methyl arachidate	Fatty methylester

Rush Meow variety leaves analysis

Based on the data reported in Table 7, GC-MS analysis of the methanolic extract of the variety Rush Meow leaves reveals 10 bioactive components (Fig 6). α-Tocospiro A had the greatest values (30.51%), followed by α-Tocospiro B (27.78%), however, (R)-2-(Hydroxyethyl)-5,5,8a-trimethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalene having the lowest values which was (1.37%).

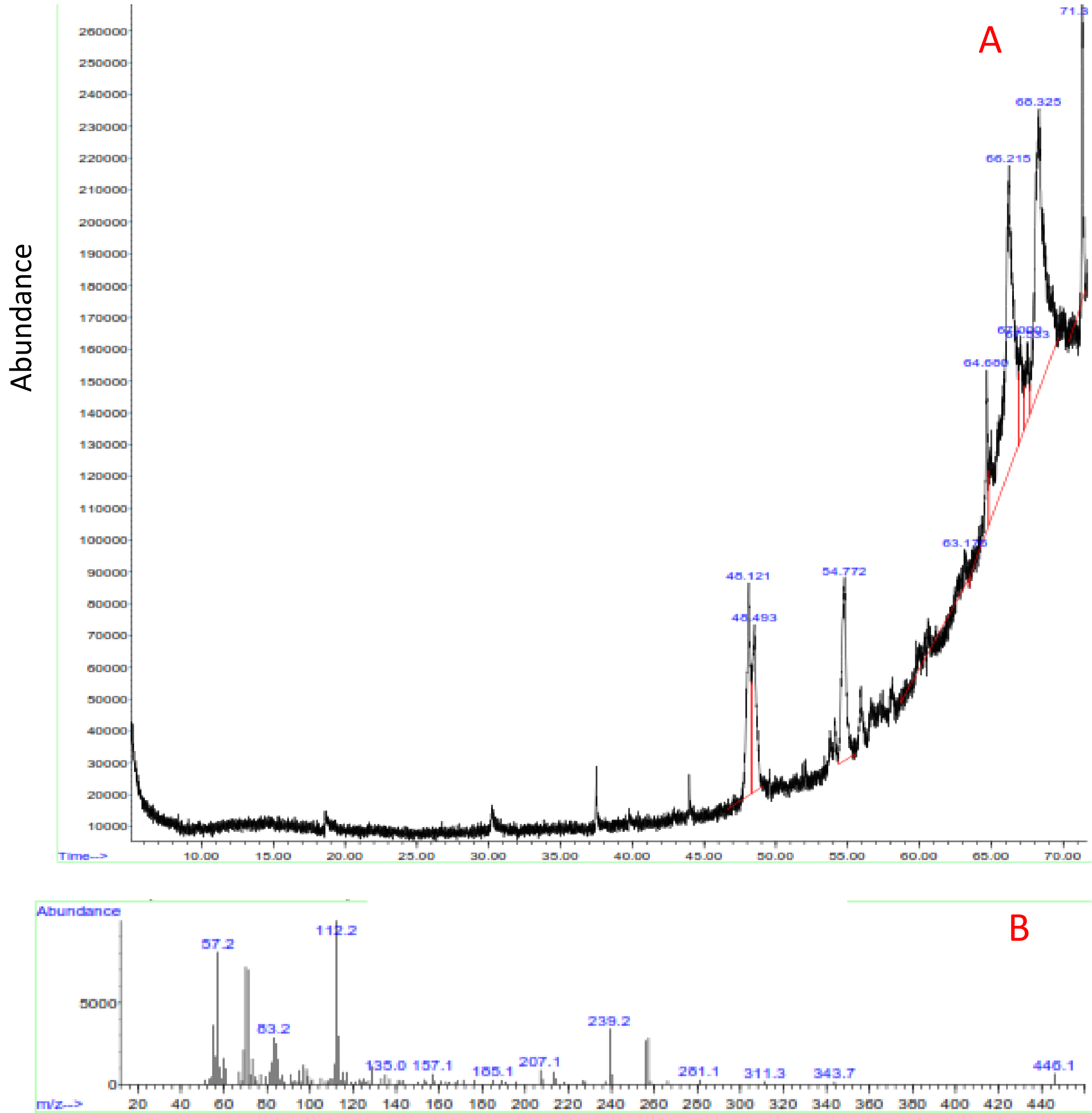

Figure (6): TIC chromatogram of twenty-four profiles characterized in Rush Meow leaves extract (A) and unknown spectrum (B).

Table (7): GC-MS profilings of the grape leaves from Rush Meow variety

Pk#	RT(min)	%Area	Compounds	Functional groups
1	48.118	8.63	3-(hydroxymethyl)-1-methylpyrrolidin-2-one	alkaloid
2	48.495	7.34	2-ethylhexyl epoxystearate	Fatty ester
3	54.771	9.01	Methyl behenate	Fatty methylester
4	63.178	1.37	(R)-2-(Hydroxyethyl)-5,5,8a-trimethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalene	sesquiterpenoid
5	64.681	4.25	Methyl tricosanoate	Fatty methylester
6	66.213	30.51	α-Tocospiro A	triterpenoid
7	67.001	3.90	Thymol	phenol
8	67.533	2.72	2-Methyl-3-phenylindole	indole
9	68.327	27.78	α-Tocospiro B	triterpenoid
10	71.351	4.49	Methyl lignocerate	Fatty methylester

Zark variety leaves analysis

According to Table 8, the GC-MS analysis of the methanolic extract of Zark leaves indicates 10 bioactive components (Fig 7). The greatest compounds in the extract were Tocospiro-B, α-Tocospiro-A, and methyl palmitate which were 32.71, 24.62 and 15.09%, respectively. Additionally, the lowest percentages at Methyl linoleate, 2-(3-Benzyloxyprop-1-en-2-yl)-1-(tert-butoxylcarbonyl)-3-butyl-1H-indole and 2-Heptadecanone which were 1.16, 2.92 and 3.86%, respectively.

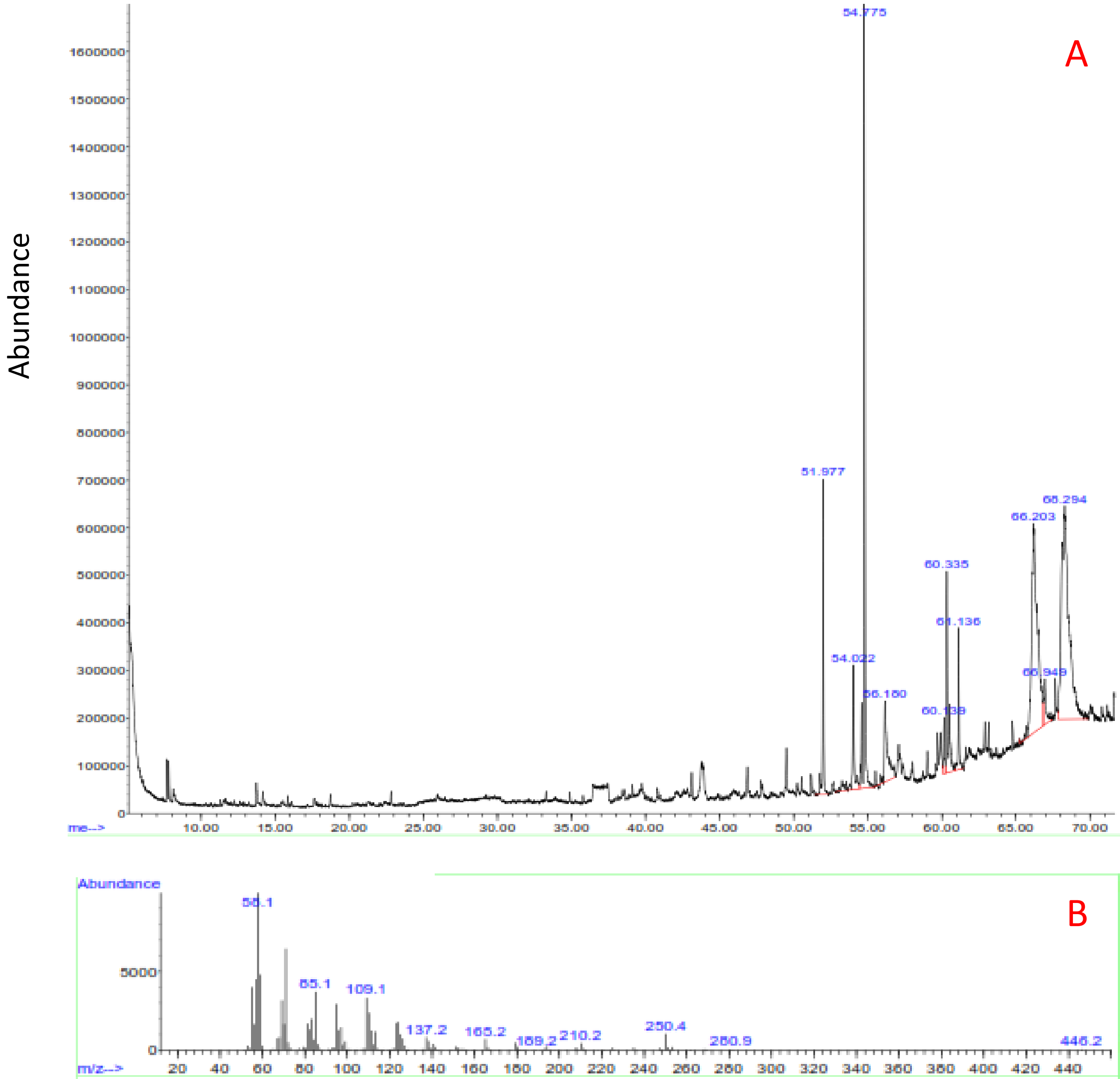

Figure (7): TIC chromatogram of twenty-four profiles characterized in Zark leaves extract (A) and unknown spectrum (B).

Table (8): GC-MS profilings of the grape from Zark leaves variety.

Pk#	RT(min)	Area%	Compound	Functional groups
1	51.976	5.85	Hexahydrofarnesyl acetone	terpenoid
2	54.022	3.86	2-Heptadecanone	ketone
3	54.776	15.09	Methyl palmitate	Fatty methylester
4	56.182	5.36	Palmitic acid	Fatty acid
5	60.137	1.16	Methyl linoleate	Fatty methylester
6	60.337	6.31	Methyl vaccenate	Fatty methylester
7	61.137	2.92	Methyl stearate	Fatty methylester
8	66.201	24.62	α-Tocospiro-A	terpenoid
9	66.950	2.13	2-(3-Benzyloxyprop-1-en-2-yl)-1-(tert-butoxylcarbonyl)-3-butyl-1H-indole	indole
10	68.293	32.71	α-Tocospiro-B	terpenoid

Discussion

The findings demonstrate the presence of a wide range of secondary metabolites, which differ in type and concentration between grapevine varieties. These variances are most likely due to genetic variability, environmental effects, and epigenetic alterations such as DNA methylation, which regulate the expression of genes involved in metabolic pathways. The GC-MS profiles captured in this study are persistent with previously recorded researches on the phytochemical configuration of Vitis vinifera leaves. Ordinarily, constituents recorded such as methyl esters of fatty acids, hydrocarbons, and tocopherol derivatives were also preeminent in our samples, alongside variety-specific secondary compounds that present new intuitions into the metabolic diversity of grapevine foliage.

Compounds such as methyl palmitate, α-tocospiro A/B, dihydro-pseudokubisine, and 16-Hentriacontanone were found to be prominent in distinct cultivars, indicating cultivar-specific metabolic specialization. This is consistent with earlier research showing that grapevine types have distinct chemical fingerprints driven by genotype and agroecological circumstances [21, 22, 23, 24, 25]. For Fatty Acid Esters (e.g., Methyl Palmitate, Methyl Linolenate, Methyl Stearate), methyl palmitate was an outstanding constituent in five of the seven studied varieties, particularly Tahlak (49.44%) and Red Globe (24.56%). This is consistent with [26], who recorded methyl palmitate as a primary compound in Egyptian grapevine leaves extracts and concomitant it with antioxidant and anti-inflammatory efficacies. The existence of methyl linolenate and methyl stearate, also familiar in studied samples, enhanced by [27], where very much alike esters were related to bioactivities involved antimicrobial and anticancer properties.

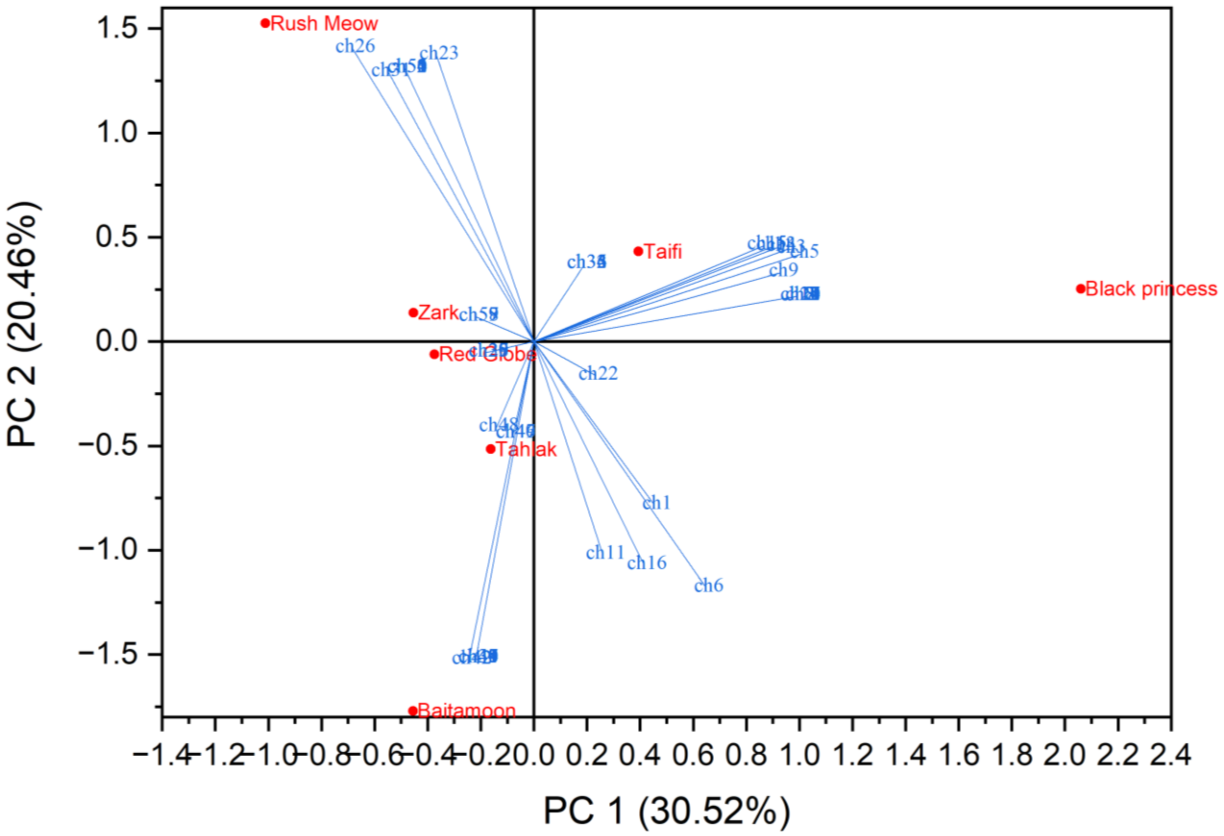

Figure (8): PCA analysis of various phytochemicals identified in seven grape varieties

For Tocopherol Derivatives (α-Tocospiro A and B), α-Tocospiro A and B were peculiarly highly in Red Globe, Zark, and Rush Meow. These findings are proportionate to [31], who differentiated tocopherol-related compound in Portuguese grapevine foliage and featured their antioxidative activity, supposing their efficient advantage in functional foods and nutraceuticals. The tables mentioned above demonstrate that many chemical groups were determined by GC-MS analysis. Several studies have mentioned this variation based on genetic material, environmental factors, and leaves [20,25]. On the other hand, the expression of genes that encode the bioactive compounds was influenced by epigenetic factors such as methylation and demethylation [27]. Consequently, these factors help enhance the manufacture of biosynthesis of certain phytochemicals. The bioactive components that result from this biosynthesis are different [28]. As a result, these chemical compounds will have lots of applications in human healthy [29,30,31]. The PCA analysis found that Rush Meow, Taifi, and Black Princess have the largest chemical variability, accounting for 50.90% of overall variance and so serving as important "gene boxes" for future grape breeding efforts (Fig. 8). Furthermore, correlations between cultivars such as Red Globe, Rush Meow, and Taifi, as well as Tahlak and Black Princess, indicate that shared metabolic features are most likely impacted by similar biosynthetic gene expression (figure 9). Methyl palmitate has been demonstrated to have antiparasitic, antiviral, and cytotoxic properties [32, 33], whereas α-Tocospiro A has antituberculosis potential in other plant species such Pourthiaea arguta and Ficus microcarpa [34, 35]. Furthermore, the inclusion of numerous bioactive classes-terpenoids, lipids, ketones, and phenolics-indicates potential applications in the pharmaceutical, nutraceutical, and food sectors. The PCA and correlation analyses applied in this study also align with previous studies [36,37] that exploited multivariate statistics for chemotaxonomic classifications and differentiations of grapevine varieties. These tools demonstrated efficient in disclosing metabolic similarity and dissimilarity among the given varieties.

Table (9): Given phytoprofilings groups appeared in seven grapes varieties.

varieties	Ketone	phenolics	alkanes	Aromatic	Lactones	Terpenoids	steroids	lipids	alkaloids
black prince	1.08	8.39	34.29	2.57	0.93	16.71	0	32.16	3.44
Red Globe	1.35	0	0.56	0	0	47.22	0	50.86	0
Taifi	17.74	0	25.5	2.99	0	35.44	1.3	17.04	0
Baitamoon	1.2	4.39	0	8.91	0	32.67	0	25.39	27.44
Tahlak	0	0	4.16	0	2.11	14.57	0	75.12	0
Rush Meow	0	3.9	0	0	0	59.66	0	25.09	11.35
Zark	3.86	0	0	0	0	63.18	0	30.84	2.13

Terpenoids, for example, were more common in Zark (63.18%), but lipids predominated in Tahlak (75.12%). Notably, the Taifi cultivar demonstrated a varied chemical profile, with ketones accounting for 17.74%.

For hydrocarbons and long-chain ketones (e.g., Nonadecane, Triacontane, 16-Hentriacontanone), hydrocarbons such as nonadecane and triacontane were noted across varieties, particularly in Black Prince and Taifi. Similar alkanes have been formerly characterized in Vitis species by [38], where they were explained as participating to the waxy cuticle and abiotic stress tolerance. The detection of 16-hentriacontanone in Taifi (16.76%) coincides with [39], who found this compound domination in grapevine leaves adapted to arid climates.

For unique compounds (e.g., Dihydro-pseudokubisine, Indole Derivatives), the detection of dihydro-pseudokubisine as a prevailing compound in Beitamouni (27.08%) is particularly eminent, as it is scarcely recorded in grapevine investigations. Its occurrence may indicate to unparalleled biosynthetic tracks in this variety. Moreover, indole derivatives existed in Zark and Beitamouni offer earlier unrecorded phytochemical features that undertake further pharmacological and metabolomic explorations.

For variation among varieties, previous investigations had featured variety-dependent variability in phytochemical compounds due to genotypic and environmental factors [40,41]. The present results enhanve this sight, especially given the distinguished chemical profiles noted in Rash Miw, Taifi, and Beitamouni, supposing that genetic variability and possibly epigenetic regulation participate in their metabolic expressions.

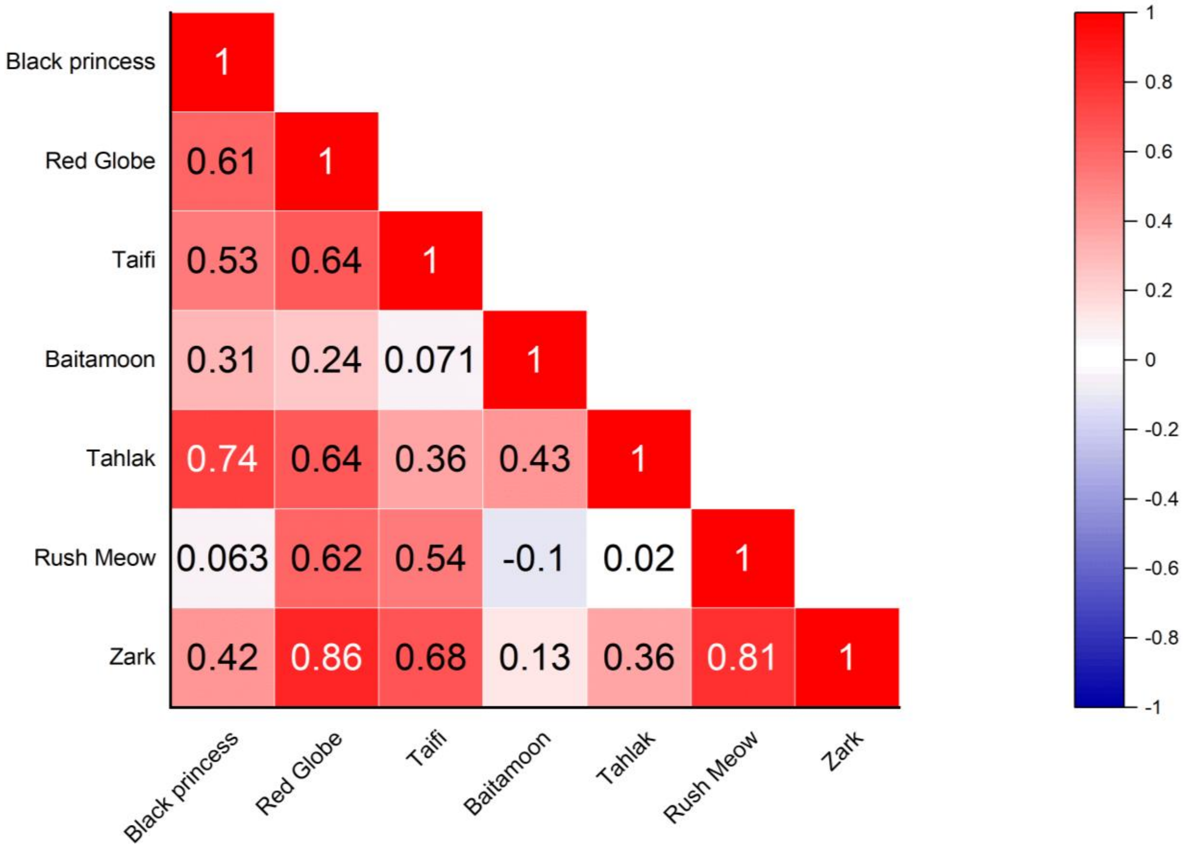

Figure (9): Pearson coefficients of correlation among seven grape varieties based on phytochemicals.

The identified bioactive chemicals are consistent with observations indicating grapevine leaf extracts have considerable antibacterial, antioxidant, and anti-inflammatory properties. Grape leaf ethanolic extracts have been found in studies to reduce microbial growth in food systems while also improving human health [42,43]. Grape pomace, a polyphenol-rich byproduct, inhibits biofilm formation by S. aureus and B. cereus [38], whereas grape leaves contain quercetin, rutin, and other phenolics with anti-inflammatory and antioxidant activities [44,45,46]. The active elements of grape leaves were extracted utilizing various safe and environmentally acceptable procedures in accordance with client requirements [47]. The phenolic components isolated from grape leaves were evaluated for antifungal activity [48]. The addition of pulverized grape leaves to pisticho calisson improved its quality and nutraceutical benefits due to quercetin and rutin [49]. Furthermore, these bioactives have a variety of pharmacological actions, including antiviral, anticataract, antiplatelet, antiobesity, anticancer, and wound healing properties [50,51,52,53]. Extracts from variations such as Fetească neagră have also shown promise in combating periodontal infections by lowering ROS and inflammatory cytokines [54,55,56]. The most plant part are used in folk medicine is leaves [57, 58]. These results are lined with [59] who mentioned that Positive antibacterial data may lead to the development of effective medications for managing and treating various bacterial infections [60,61,62]. Some plant extracts have enzyme inhibitory effect due to their content of active compounds [63,64], which based on solvent.

Conclusion

The GC-MS study of methanolic extracts from several grape leaf cultivars indicated a high diversity in both the amount and kinds of bioactive chemicals, showing extensive chemical variability among the six examined cultivars. Some cultivars, such as Black Princess, Rush Meow, and Taifi, have shown significant quantitative and qualitative diversity in bioactive components, making them interesting sources of pharmacological and nutraceutical chemicals. Methyl palmitate was the most abundant and prominent component in most cultivars, especially Tahlak, where it reached 49.44%. Rush Meow had the highest concentration of α-Tocospiro A (30.51%), while Baitamoon had the highest concentration of Dihydro-pseudokubisine (27.08%), indicating chemical variability due to genetic and environmental factors. This variation can be attributed to genetic makeup, environmental conditions, and epigenetic modifications such as DNA methylation, which can affect the expression of genes responsible for the biosynthesis of these active compounds. From an application standpoint, the presence of compounds belonging to classes such as terpenoids, lipids, phenolics, and ketones highlights the potential use of grape leaves in pharmaceutical industries as antimicrobial, antioxidant, and anti-inflammatory agents. Based on PCA analysis, cultivars Rush Meow, Taifi, and Black Princess demonstrated the highest chemical variability, qualifying them as potential "gene boxes" for breeding programs aimed at enhancing the bioactive properties of grape crops. As a result, the study suggests that additional research be conducted on other grape plant sections, as well as the use of modern analytical techniques to better detect bioactive components. Furthermore, biological experiments are required to determine the pharmacological properties of the isolated molecules. Studies on LoD and LoQ are required to know whether the GC/MS analysis are precise or randomized.

Disclosure Statement

- **Ethics approval and consent to participate:** Not applicable
- **Consent for publication:** Not applicable.
- **Availability of data and materials:** All collected data during this study was used to present the obtained findings in the body and illustrations of this manuscript.
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