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Phytochemical Analysis and Bioactivity Screening of Primary and Secondary Metabolic Products of Medicinal Plants in the Valleys of Medina Region Saudi Arabia

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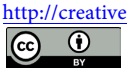

Abstract

Medicinal plants are highly valued for their active compounds. These plants can be used in various fields and preservation of these plants in their environment. The present study aimed to screen medicinal plants used in traditional medicine in Medina valleys for the presence of metabolites, and to answer the following question: is the ethnomedicinal importance of medicinal plants used in Medina valleys conform to their primary and secondary metabolite content. Eight plants (*Pulicaria incise*, *Heliotropium arbainense*, *Commicarpus grandiflorus*, *Rumex vesicarius*, *Senna alexandrina*, *Rhazya stricta*, *Withania somnifera* and *Asphodelus fistulosus*) were collected from the Medina valleys and were biochemically analyzed to determine the different compounds after leaves extraction analyzed statistically to clarify the content of primary compounds. The chemical compounds in the most active fraction were determined using quantitative phytochemical and gas chromatography-mass spectrometry (GC/MS) analytical methods, comparing the mass spectra of the GC/MS identified compounds with those of the Center of Excellence in Environmental Studies (CEES) database library. The result showed 16 aroma compounds representing the GC/MS analysis revealed the presence of various compounds like 4,4-Dimethyl octane, 5H-1-Pyrindine and 1,3-Cyclopentadiene, 1,2,5,5-tetramethyl- in the ethanolic extract of *Pulicaria incisa*. The most prevalent plants were *Pulicaria incisa*, *Senna alexandrina* and *Heliotropium arbainense* the study plants have high content of protein. There is a need to focus phytochemical screening on ethnobotanical studies to complete research into traditional medicine which leads to the discovery of new drugs.

Keywords

C Phytochemical, Medicinal Plants, GC-MS Analysis, Primary and Secondary Metabolic

1. Introduction

Plants are rich in active compounds or secondary metabolites such as alkaloids, steroids, tannins, glycosides, which are present in their organs such as leaves, flowers, seeds, etc [1]. They are widely distributed in Central Asia and Southern Africa. In Saudi Arabia, they are grown in the Wadi Najran, Taif region, and Al Madinah Al Munawwarah (Abyar Al-Mashy) [2]. The Saudi Arabian flora comprises about 2250 plant species that are distributed throughout the Kingdom [3]. It is well known that Saudi Arabia and the Arabian Peninsula are rich in natural and cultivated plants. The flora of Saudi Arabia contains a total of 2250 plant species belong to 142 families [4].

Some of these plants were used and still by native people who gain an accumulated experience in the uses of these plants in folk medicine to cure many different diseases. But unfortunately, there is a gap in transferring the indigenous knowledge between old and new generation further to the lack and information scarcity on their uses, further to the difficulties that may be found in identifying the wild medicinal plants as well. However, the gap is still wider between traditional herbal medicine and modern medicine manufacturing. Few studies on medicinal and Ethnomedicinal plants have been done in Saudi Arabia including Al-Baha region among these, studies conducted by Collenette, [5] who described twenty plant species traditionally used as a medicine with their main chemical constituents. Different types of primary and secondary metabolic compounds are used usually divided according to their chemical composition. Active compounds in medicinal plants can be used in medicinal drugs and move society to invest in crop production [6]. And it can be used to treat toxic and bacterial diseases [7]. Medical plants are considered an important economic resource of natural biodiversity [8]. Further, an initial survey on the medicinal plant diversity in the flora of the Kingdom of Saudi Arabia has been made covering seven families [9]. The active compounds can be extracted from medicinal plants this enables and preserving them from human intervention. Medicinal plants have many benefits in health uses and with this study it can prove the validity of their importance. Analysis of some medicinal plants biochemically and find out its medicinal very important to carry out the necessary that help in separating the important primary and secondary metabolic compounds helps in prove their value [10].

The major goal of this study was to investigate the chemical composition, screen and identify the medicinal plant diversity in Medina valleys to discuss their environmental distribution and work on conservation of these valuable plants to increase their bioavailability. The identified of some the active com-

pounds in the composition of these plants to determine its medicinal and nutritional value. Encouraging the conservation of these important plants and increasing their cultivation in their appropriate environment. Use of these plants in the health and therapeutic field because of their medicinal and nutritional value and can use in the commercial and cosmetic industries due to their useful metabolic compounds.

2. Materials and Methods

2.1. Study Area

Medina region is in the central part of the western sector of the kingdom of Saudi Arabia within the Hijaz Mountain range (Figure 1), and the southern outskirts

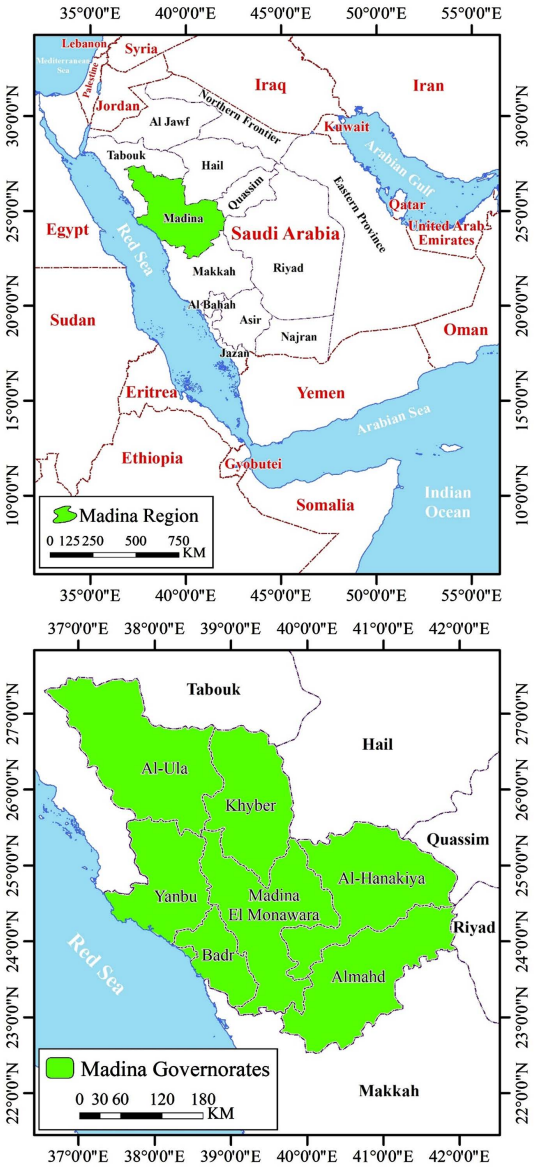

Figure 1. Map of the Kingdom of Saudi Arabia and the location of the Medina region and its governorate.

of Medina are located at the 20 - 24 North latitude and thus it is very close to the northern ends of the tropical zone with thermal surplus and as a result the heat in intense in summer and warm in winter. The annual temperature range is 13.8 degrees, and the average maximum temperature is about 42 degrees [11]. Samples were collected in January 2021 from three valleys (Wadi Al-Baidha, Al-Aqiq, Al-Fora 'a) in Medina, (**Figure 2**).

2.2. Maintaining the Integrity of the Specifications

Samples Collection

Samples were collected from eight plant species from different location, including 3 valleys from the Medina region during the winter season, and they were placed in plastic bags for preservation. The plant samples were divided into fresh samples that were preserved in ice and samples that were air-dried, milled, and preserved in plastic boxes until carry out the necessary analysis [12].

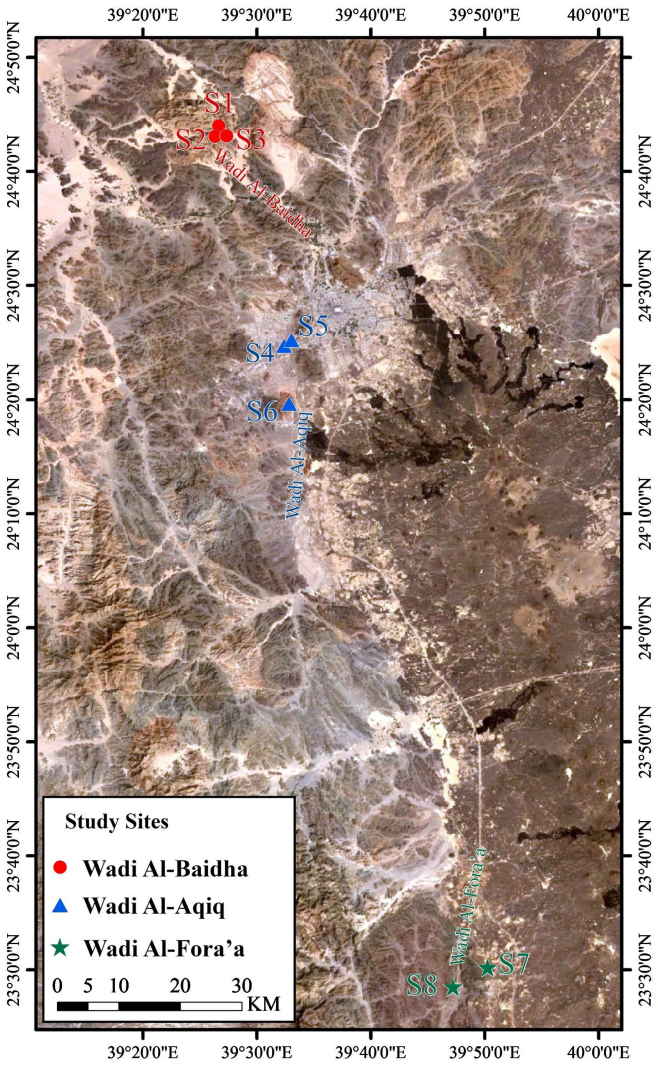

Figure 2. Map of Study Area in three valleys (Wadi Al-Baidha, Al-Aqiq, Al-Fora 'a) in Medina region.

2.3. Plant Material

Eight plants (*Pulicaria incise*, *Heliotropium arbainense*, *Commicarpus grandiflorus*, *Rumex vesicarius*, *Senna alexandrina*, *Rhazya stricta*, *Withania somnifera* and *Asphodelus fistulosus*) as show in Figure 3 were identified according to Saudi Arabia flora and authenticated by the method of Chaudhary plant Protection. Plants were collected, dried and deposited at the herbarium of Jeddah university [13] [14] [15].

2.4. Aqueous Extracts of Plant for Primary Metabolic Analysis

A sample of the fresh plant was taken from each plant species 1:20 and then ground into a porcelain mortar using distilled water; the volume was completed in a standard flask, and the extract was kept at very low temperature until perform the necessary analysis The extracts were filtered through a filter paper several times and kept at 4˚C in the dark until use [16].

2.5. Ethanolic Extraction for Secondary Metabolic Analysis

Dried plant at flask with about 1:15 of ethanol 75% after the first filtration, the Ethanolic extract was kept in the refrigerator. Repeat the same steps 2 day. Then samples were heating the extract using water bath under condenser. The extracts were filtered through a filter paper several times after 24 hours and kept at 4˚C in the dark until use [17].

2.6. Tests of Primary Compounds

2.6.1. Protein

0.2 ml of fresh plant extract was added to 5 ml Coomassie brilliant blue using an electric stirrer and measured in a spectrophotometer (NOVA SPCC II SECTROPHOTOMETER) [18].

Figure 3. Pictures and scientific names of the plant study. Photos by mobile iPhone 7 Model: A1778.

2.6.2. Proline

0.5 ml of plant extract with 2.5 ml of sulfosalicylic acid with 2 ml ninhydrin with 2 ml of glacial acetic acid, the closed with a pulp and placed in water bath for 1 hour [19].

2.6.3. Amino Acids

Take 2 ml of plant extract with 1 ml of ninhydrin 1% with 2.4 ml of glycerin with 0.4 ml of buffer [20].

2.6.4. Carbohydrates

0.4 ml of plant extract mixed with 3.6 ml of carbohydrate reagent and immediately put in a boiling water bath covered with pulp for 30 seconds [21].

2.7. Tests of Secondary Compounds

2.7.1. Phytosterols

Drops of each of the plant extract in a test tube was added drops of concentrated H_2SO_4 Reddish brown color in chloroform layer indicates the presence of phytosterols [22].

2.7.2. Triterpenoids

Plant extracts were treated with few drops of acetic anhydride, boil and cool. Red color indicated the presence of triterpenoids [23].

2.7.3. Saponins

Small amount of the plant extract with little quantity of water and shaken vigorously. Appearance of foam persisting for 10 min indicated presence of saponins [24].

2.7.4. Alkaloids

Little of plant extract were dissolved in few drops of conc. HCL and adding few drops of Mayer's reagent. Appearance of white precipitate indicated presence of alkaloids [25].

2.7.5. Flavonoids

Extract mix with few drops of ferric chloride. Appearance of blackish red color indicated presence of flavonoids [26].

2.7.6. Coumarins

Small amount of the plant extracts with little drops of NaOH 10% and chloroform. Appearance of yellow color indicated presence of coumarin [27].

2.7.7. Tannins

Few drops of ferric chloride were added to plant extract. Appear of black precipitate gives positive result [28].

2.7.8. Gas Chromatography-Mass Spectrometry (GC/MS) Analysis

Plant extracts were selected for gas chromatography-mass spectroscopy (GC-MS) analysis in order to identify the active compound present. Analysis was conducted using the database of Center of Excellence in Environmental Studies

(CEES), with the spectrum of the unknown components being compared with the spectrum of known components stored in the (CEES) library. Before identifying the compounds in the extracts 0.1 ml of each plant extract was mixed with 1ml of ethanol (HPLC grade) (WINLAB, UK) and filtered using a 0.22 μ Millipore filter (Millex-HV) to obtain a crystal-clear solution. The identification of the compounds was done by injecting 1¼ l of crystal-clear sample into an RTx -5 columns (the initial temperature of the column oven was 250°C. A HP 5 ms, 30 m capillary column was used (30 m × 0.25 mm i.d. × 0.25 m, 5% phenylmethylsiloxane, RTx -5MS)) with (30 × 0.32 nm) of GC-MS model (Perkin Elmer, Clarus 500, USA) and helium (3 ml/min) was used as a carrier gas. The following temperature gradient program was used (75˚C for 2 min followed by an increase from 75˚C to 175˚C at a rate of 50˚C per min and finally 7 min at 175˚C). The fractions were compared with those in the mass spectrum library of the corresponding organic compounds. The chemical components of the plant extracts were analyzed in the central laboratory of (CEES) [29].

3. Result

The result in Table 1 shows the different types of plants with their families that were counted in the three valleys (Wadi Al-Baidha, Al-Aqiq, Al-Fora ʻa) of Medina region from which the study plants were collected (*P. incise*, *H. arbainense*, *C. grandiflorus*, *R. vesicarius*, *S. alexandrina*, *R. stricta*, *W. somnifera* and *A. fistulosus*). Showed prevalent plants were (*S. alexandrina*, *P. incise*, *H. arbainense*).

A sample. The results of phytochemical screening of aqueous and ethanol plant extract showed the existence of Protein, Proline, Amino acids and carbohydrates (Table 2) for Primary Compounds and Phytosterols, Triterpenoids, saponins, alkaloids, flavonoids, coumarins and tannins (Table 3) for secondary compounds.

Table 2 shows the statistical results of the primary metabolic compounds in the form of the mean and standard deviation. There is a high protein content in

Table 1. Inventory of some medicinal plants in valleys of medina region (Wadi 1-Al-Baidha, 2-Al-Aqiq, 3-Al-Fora ʻa) presence (+) and absence (−) of plant study.

Plant species	Families	Life form	Life growth	Sites		
				1	2	3
Asphodelus fistulosus	Asphodelaceae	Herb	Annual	−	+	−
Commicarpus grandiflorus	Nyctaginaceae	Herb	Perrenial	−	−	+
Heliotropium arbainense	Boraginaceae	Herb	Perrenial	−	+	+
Pulicaria incisa	Asteraceae	Herb	Annual	+	−	+
Rhazya stricta	Apocynaceae	Shrub	Perrenial	−	+	−
Rumex vesicarius	Polygonaceae	Herb	Annual	−	+	−
Senna alexandrina	Leguminosae	Shrub	Perrenial	+	+	+
Withania somnifera	Solanaceae	Shrub	Perrenial	+	−	−

Table 2. The averages and standard deviations of the primary compounds in the study plants (mg/g f.wt.) n = 3.

Plant species	Protein	Proline	Amino acids	Carbohydrates
Pulicaria incisa	$3.23^{d} \pm 0.02$ (0.01)	$0.03^{g} \pm 0.0$ (0.0)	$0.01^{e} \pm 0.0$ (0.0)	**$0.04^{e} \pm 0.01$ (0.003)**
Heliotropium arbainense	$3.62^{a} \pm 0.02$ (0.01)	$0.12^{d} \pm 0.0$ (0.0)	$0.03^{e} \pm 0.0$ (0.0)	**$0.10^{c} \pm 0.0$ (0.0)**
Commicarpus grandiflorus	$3.54^{b} \pm 0.02$ (0.01)	$0.12^{d} \pm 0.0$ (0.0)	$0.03^{e} \pm 0.0$ (0.0)	**$0.07^{d} \pm 0.01$ (0.003)**
Rumex vesicarius	$3.03^{e} \pm 0.01$ (0.01)	$0.36^{a} \pm 0.01$ (0.0)	$3.15^{b} \pm 0.28$ (0.16)	**$0.03^{f} \pm 0.01$ (0.003)**
Senna alexandrina	$2.86^{f} \pm 0.02$ (0.01)	$0.11^{e} \pm 0.0$ (0.0)	$7.69^{a} \pm 0.28$ (0.16)	**$0.19^{b} \pm 0.01$ (0.003)**
Rhazya stricta	$3.06^{e} \pm 0.02$ (0.01)	$0.13^{c} \pm 0.0$ (0.0)	$2.17^{c} \pm 0.08$ (0.04)	**$0.03^{ef} \pm 0.0$ (0.0)**
Withania somnifera	$3.07^{e} \pm 0.03$ (0.02)	$0.21^{b} \pm 0.01$ (0.0)	$2.0^{c} \pm 0.14$ (0.08)	**$0.04^{ef} \pm 0.0$ (0.0)**
Asphodelus fistulosus	$3.34^{c} \pm 0.01$ (0.0)	$0.04^{f} \pm 0.0$ (0.0)	$1.42^{d} \pm 0.28$ (0.16)	**$0.64^{a} \pm 0.01$ (0.01)**
F (p)	**628.698* (<0.001*)**	**3920.0* (<0.001*)**	**614.698* (<0.001*)**	**4444.33* (<0.001*)**

Table 3. List of presence (+) and presence in high concentration (++) and absence (−) of secondary compounds in plant study.

Plant species	Phytosterols	Triterpenoids	Saponins	Alkaloids	Flavonoids	Coumarins	Tannins
Pulicaria incisa	+	+	+	+	+	+	+
Heliotropium arbainense	+	+	+	+	−	+	+
Commicarpus grandiflorus	+	+	+	+	−	+	+
Rumex vesicarius	+	+	−	+	++	+	+
Senna alexandrina	+	+	+	+	+	+	+
Rhazya stricta	+	+	−	+	−	+	+
Withania somnifera	+	+	+	+	−	+	+
Asphodelus fistulosus	+	+	−	+	+	+	+

the study samples. The changes in the soluble protein content of the different plant species shows in Table 2, and they retained the largest amount of it significantly and the highest of protein is the plant *H. arbainense*, and the lowest plant is *S. alexandrina*.

The saponins sowed little content for *R. stricta*, *R. vesicarius* and *A. fistulosus* but, moderate level was showed for *P. incise*, *H. arbainense*, *C. grandiflorus* and *S. alexandrina*. Also *R. vesicarius* extract showed a high content of flavonoids, but *P. incisa*, *S. alexandrina* and *A. fistulosus* have the moderate content and the little amount was observed in *R. stricta*, *C. grandiflorus* and *H. arbainense*. All plant extract has moderate content of alkaloids, Triterpenoids, coumarins and tannins respectively.

The proposed chemical structures of these compounds are indicated in Figures 4-11. The names of the probable bio molecules present with their RT, Retention Time, Values, and peak areas are indicated in Table 4. GC-MS analysis the results of GC-MS analysis of ethanol extracts of *P. incisa* are given in Table 4 and Figure 4, the GC-MS analysis of *P. incisa*, the compounds found were -

Table 4. GC/MS analysis of plant study Ethanolic extracts.

Compounds	RT (min)	Area (mAU*s)	Height
Pulicaria incisa			
2-Propanone, 1,1-dimethoxy-	3.342	357,977	133,535
1,2,4-Benzenetricarboxylic acid, 1,2-dimethyl ester	3.483	51,547	9336
3-Hexanol, 2-methyl-	4.315	30,558	13,107
2-Propenoic acid, butyl ester	5.147	144,967	39,482
2-Butenoic acid, 2-methyl-, (Z)-	5.263	228,272	49,700
5H-1-Pyrindine	11.525	3,032,157	440,220
Acrylic acid, (5-cyclopropylidenepentyl) ester	12.575	47,653	10,055
1,3-Cyclopentadiene, 1,2,5,5-tetramethyl-	13.461	914,482	273,337
1,3-Cyclopentadiene, 1,2,5,5-tetramethyl-	13.544	2,706,387	773,527
2-Hydroxy-2,6-dimethyl-hept-6-en-3-one	16.641	59,827	15,683
Heptane, 4,4-dimethyl-	3.144	1,462,217	976,610
Ethylbenzene	4.439	18,637	8779
Ethanone, 1-cyclobutyl-	4.733	17,488	2260
4,4-Dimethyl octane	5.197	6,939,195	3,301,821
Pentane, 2,2-dimethyl-	12.641	32,434	11,081
Heptane, 4,4-dimethyl-	3.144	1,462,217	976,610
Heliotropium arbainense			
Butanoic acid, anhydride	3.131	9464	5471
2-Propanone, 1,1-dimethoxy-	3.326	125,502	52,314
Acetic acid, butyl ester	3.45	57,268	15,950
2-Propenoic acid, butyl ester	5.155	212,791	55,756
1,3-Cyclopentadiene, 1,2,5,5-tetramethyl-	13.529	2,570,446	717,552
1,3-Dioxolane-2-methanol	15.289	37,964	10,627
Bicyclo[2.2.1]heptane, 2-(2-propenyl)-	16.076	44,160	10,578
1-Undecene, 9-methyl-	16.44	54,827	17,210
Isophthalaldehyde	17.191	15,120	5174
4,6-Heptadienoic acid, 3,3,6-trimethyl-, ethyl ester	17.892	359,433	62,759
Heptane, 4,4-dimethyl-	3.148	1,517,641	1,024,671
Ethylbenzene	4.443	11,865	7807
Undecane	5.2	7,194,793	3,497,076
3-Hexanone, 2,5-dimethyl-	12.643	21,693	8316
Phenol, 3-(1,1-dimethylethyl)-4-methoxy-	16.465	2,787,397	193,202

Continued

Commicarpus grandiflorus			
2-Propanone, 1,1-dimethoxy-	3.136	102,048	37,944
2-Propenoic acid, butyl ester	5.035	33,535	12,867
2-Butenoic acid, 2-methyl-, (Z)-	5.178	323,558	57,313
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	7.547	29,364	8863
5H-1-Pyrindine	11.508	2,996,333	446,081
1,3-Cyclopentadiene, 1,2,5,5-tetramethyl-	13.534	61,177	21,525
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	14.305	256,448	42,365
(S)-(+)-1-(2-Pyrrolidinylmethyl)-pyrrolidine	16.45	61,558	9967
1-Butanol, 3-methyl-, acetate	16.837	323,177	32,881
12-Hydroxy-3-keto-bisnor-4-cholenic acid	17.889	361,988	59,190
Heptane, 4,4-dimethyl-	3.153	1,603,394	1,044,918
Benzene, 1,3-dimethyl-	4.445	50,368	25,880
4,4-Dimethyl octane	5.202	7,576,894	3,621,189
8-Hydroxy-2,2,8-trimethyldeca-5,9-dien-3-one	12.63	64,037	22,688
1,3-Cyclopentadiene, 5,5-dimethyl-2-ethyl-	13.533	2,937,389	917,337
Rumex vesicarius			
2-Propanone, 1,1-dimethoxy-	3.159	120,361	59,949
2-Propenoic acid, butyl ester	5.049	45,284	18,451
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	7.563	43,099	12,345
5H-1-Pyrindine	11.5	393,080	105,109
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	14.313	372,536	62,756
1-Heptanol, 3-methyl-	16.45	83,592	10,852
Octane, 1-ethoxy-	16.6	221,363	15,146
1-Butanol, 3-methyl-, acetate	16.992	617,214	38,696
9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, trans-	17.198	462,205	44,485
Sulfurous acid, hexyl 2-propyl ester	17.475	51,454	6142
Heptane, 4,4-dimethyl-	3.159	1,645,515	1,120,801
Undecane	5.206	7,634,942	3,654,061
Butane, 2,2-dimethyl-	12.64	36,668	11,532
n-Tridecan-1-ol	16.537	814,164	42,294
5,8-Decadien-2-one, 5,9-dimethyl-, (E)-	17.895	387,599	71,229

Continued

Senna alexandrina			
Furan, 2-butyltetrahydro-	3.153	11,528	8192
2-Propanone, 1,1-dimethoxy-	3.349	203,673	78,579
Acetic acid, butyl ester	3.467	39,942	9713
2-Propenoic acid, butyl ester	5.145	182,112	49,726
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	7.606	25,143	9151
Propanoic acid	8.055	38,578	15,228
1-Pentene, 4,4-dimethyl-	9.604	62,706	9678
Benzeneacetaldehyde	9.98	49,835	15,902
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	14.347	96,211	17,843
Octane, 2-chloro-	16.454	87,489	18,523
2-Hexanone	3.043	50,170	17,244
Heptane, 4,4-dimethyl-	3.197	2,060,280	1,357,310
Benzene, 1,3-dimethyl-	4.477	23,469	12,631
4,4-Dimethyl octane	5.229	9,288,316	4,340,129
Butane, 2,2-dimethyl-	12.645	40,835	14,779
Rhazya stricta			
2-Propanone, 1,1-dimethoxy-	3.133	25,949	13,582
2-Propanone, 1,1-dimethoxy-	3.171	37,558	21,506
Acetic acid, butyl ester	3.311	28,369	9479
2-Propenoic acid, butyl ester	5.057	51,816	19,997
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	7.564	62,619	15,279
Acetic acid, pentyl ester	12.475	99,794	8983
Diazene, bis(1,1-dimethylethyl)-	12.55	38,618	6718
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	14.315	326,506	59,466
Urazole	14.517	19,727	4249
Diisopropyl(methoxy)silane	15.054	44,535	12,398
Heptane, 4,4-dimethyl-	3.218	2,326,418	1,594,435
Ethylbenzene	4.317	4953	7110
Benzene, 1,3-dimethyl-	4.492	25,204	13,065
Undecane	5.242	10,019,914	4,839,199
Pentane, 2,2-dimethyl-	12.644	44,962	15,107

Continued

Withania somnifera			
2-Propanone, 1,1-dimethoxy-	3.298	166,182	69,608
Acetic acid, butyl ester	3.411	28,083	10,727
2-Propenoic acid, butyl ester	5.117	165,834	55,952
Pyridine, 3-ethyl-	6.883	34,106	9710
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	7.595	27,639	9230
Propanoic acid	8.048	30,208	11,163
Formic acid, 2-propylpentyl ester	9.576	57,843	12,413
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	14.332	154,754	30,031
Acetate, 2-hydroxy-2-(3-chloro-4,5-dihydro-5-isoxazolyl)-, ethyl ester	15.072	39,594	12,349
1-Heptanol, 3-methyl-	16.458	37,852	13,249
Heptane, 4,4-dimethyl-	3.159	1,616,307	1,104,565
p-Xylene	4.45	22,523	11,159
Undecane	5.205	7,482,419	3,490,731
Pentane, 2,2-dimethyl-	12.637	39,647	11,939
Phenol, 5-methyl-2-(1-methylethyl)-, acetate	16.478	176,498	11,942
Asphodelus fistulosus			
2-Propanone, 1,1-dimethoxy-	3.346	294,890	93,973
3-Furanmethanol	4.529	133,436	26,256
2-Propenoic acid, butyl ester	5.134	202,046	54,975
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	7.59	60,182	15,462
Formic acid, 2-propylpentyl ester	9.565	100,012	17,130
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	14.347	299,297	40,393
Phosphonofluoridic acid, methyl-, 3,3-dimethylbutyl ester	14.562	147,540	24,060
dl-Erythro-O-methylthreonine	15.892	105,398	12,480
1H,5H,7H,11H-Dipyrazolo[1,2-a:1',2'-d][1,2,4,5] tetrazine, tetrahydro-	16.11	78,817	9909
1-Heptanol, 3-methyl-	16.454	68,031	16,568
Heptane, 4,4-dimethyl-	3.19	1,949,351	1,309,283
p-Xylene	4.47	25,669	11,960
Undecane	5.223	8,546,527	4,089,618
Pentane, 2,2-dimethyl-	12.641	44,432	13,159
(SS)- or (RR)-2,3-hexanediol	15.29	13,811	5761

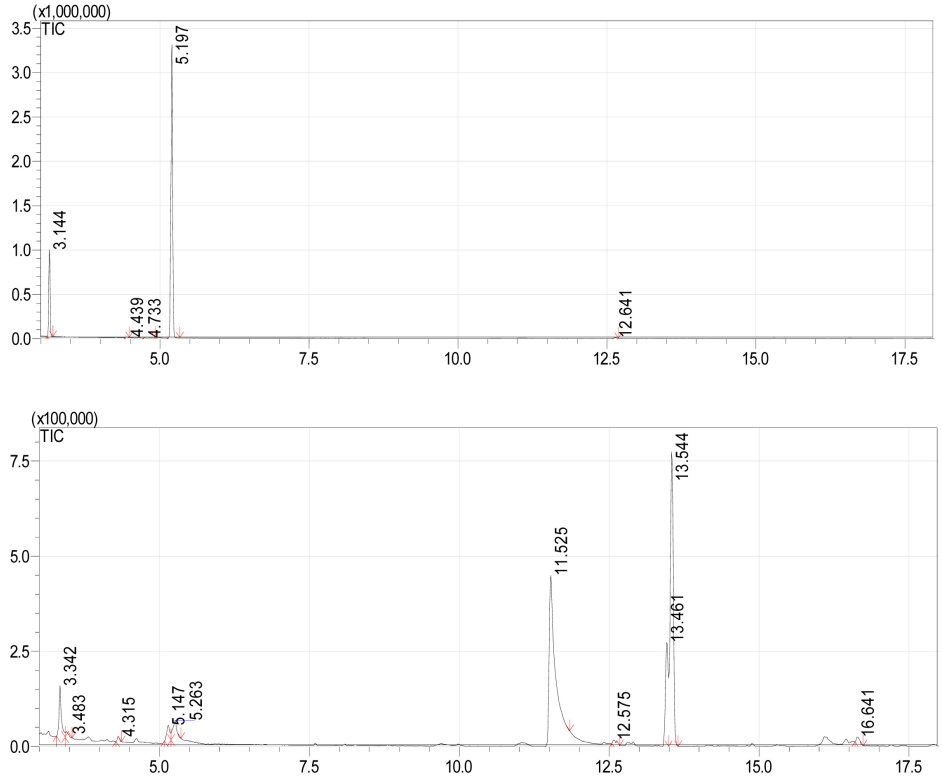

Figure 4. Chromatogram of the chemical compounds identified via GC-MS of *Pulicaria incise*.

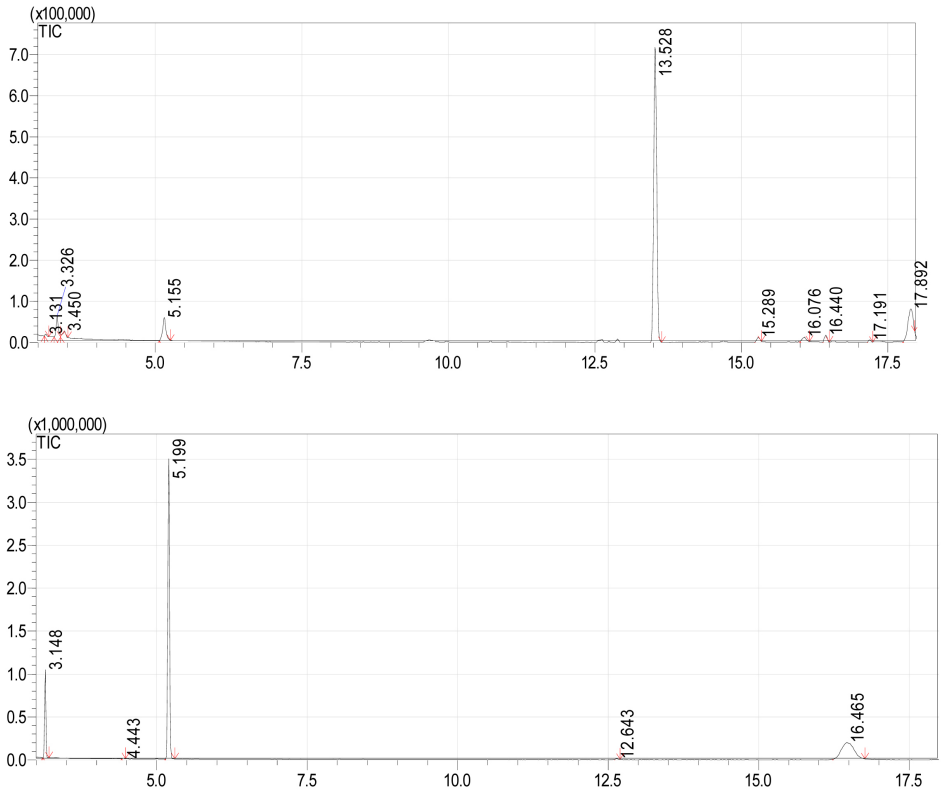

Figure 5. Chromatogram of the chemical compounds identified via GC-MS of *Heliotropium arbainense*.

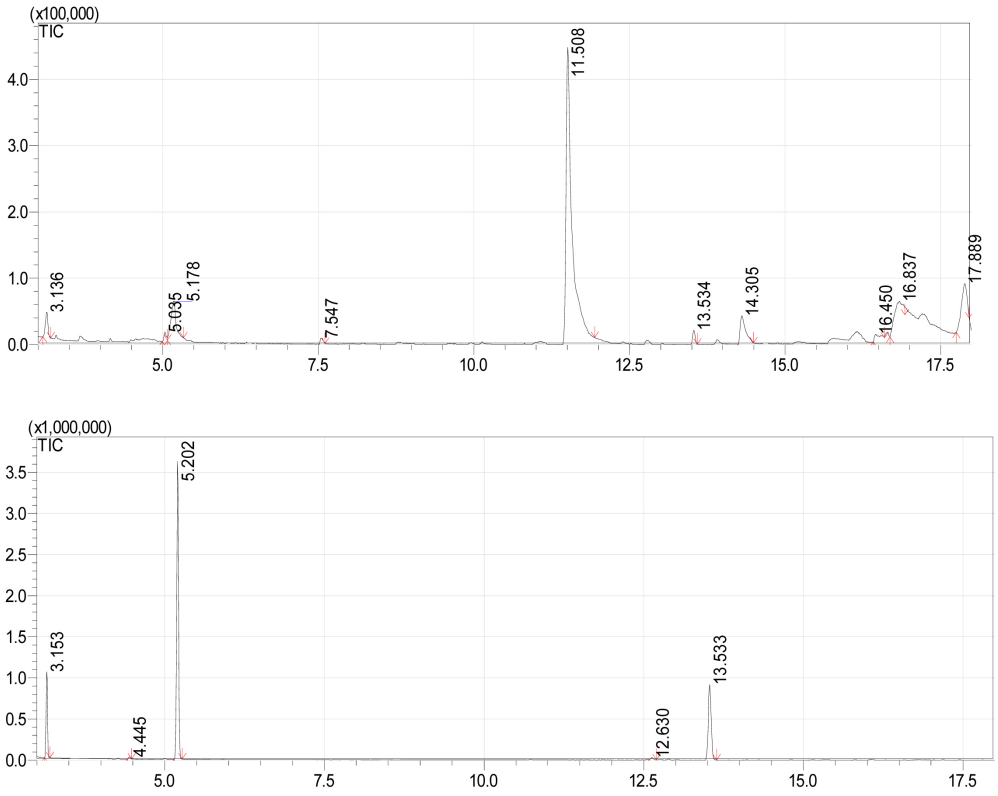

Figure 6. Chromatogram of the chemical compounds identified via GC-MS of *Commicarpus grandiflorus*.

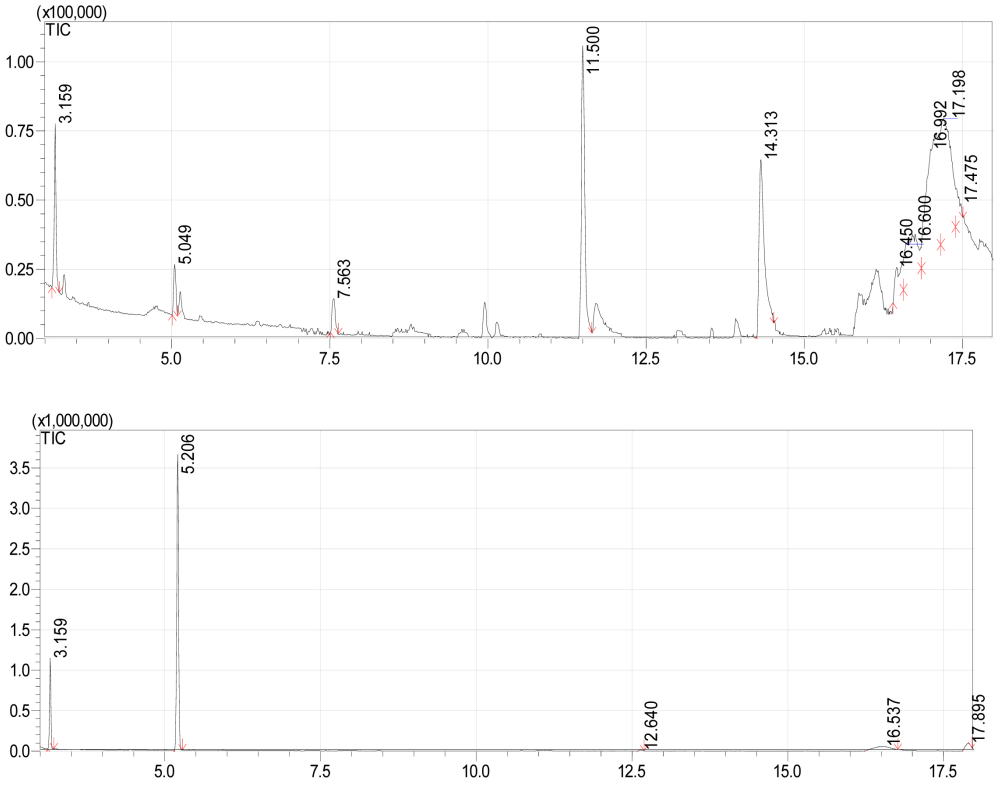

Figure 7. Chromatogram of the chemical compounds identified via GC-MS of *Rumex vesicarius*.

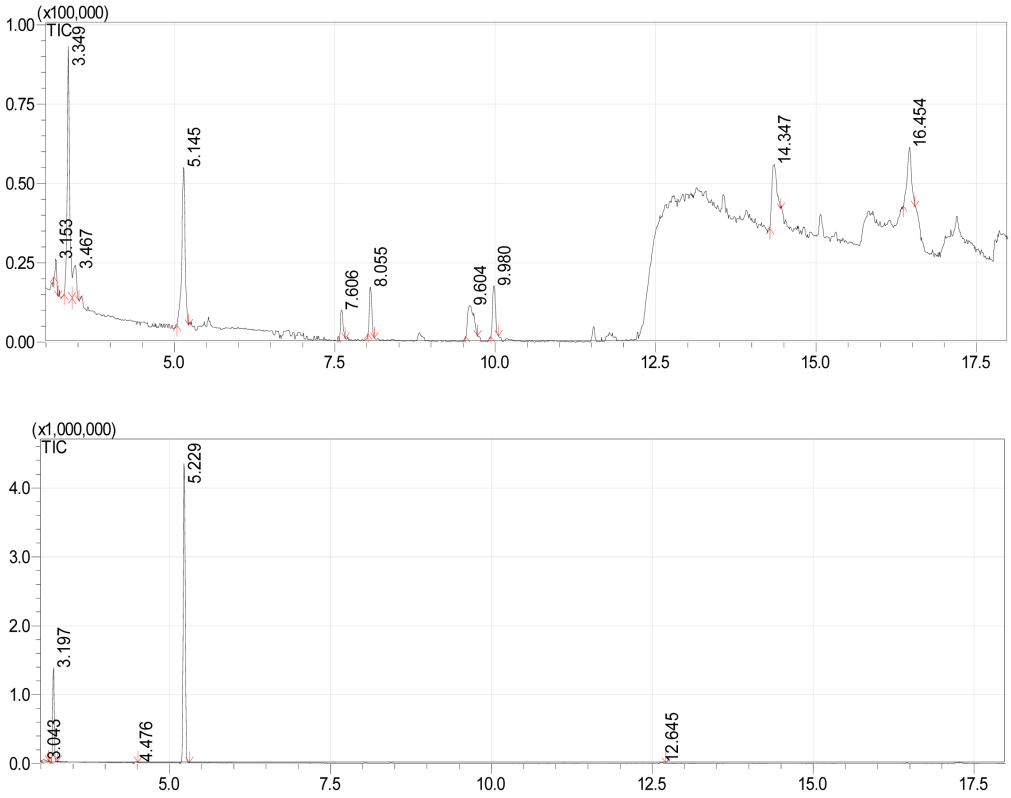

Figure 8. Chromatogram of the chemical compounds identified via GC-MS of *Senna alexandrina*.

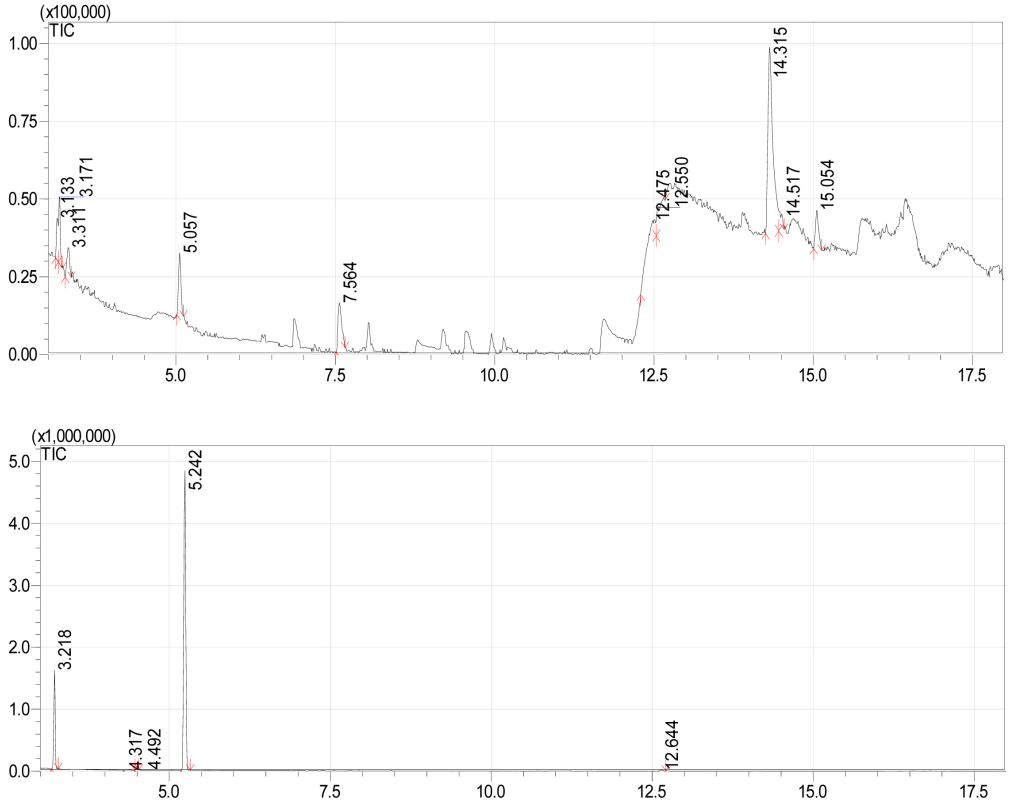

Figure 9. Chromatogram of the chemical compounds identified via GC-MS of *Rhazya stricta*.

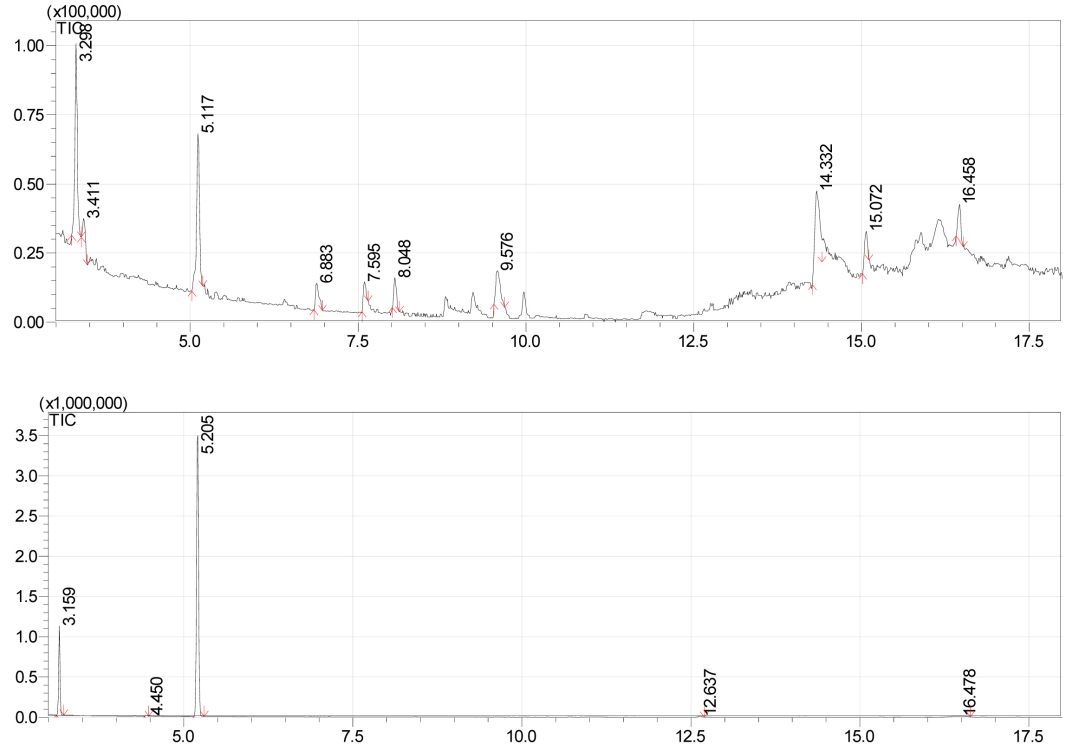

Figure 10. Chromatogram of the chemical compounds identified via GC-MS of *Withania somnifera*.

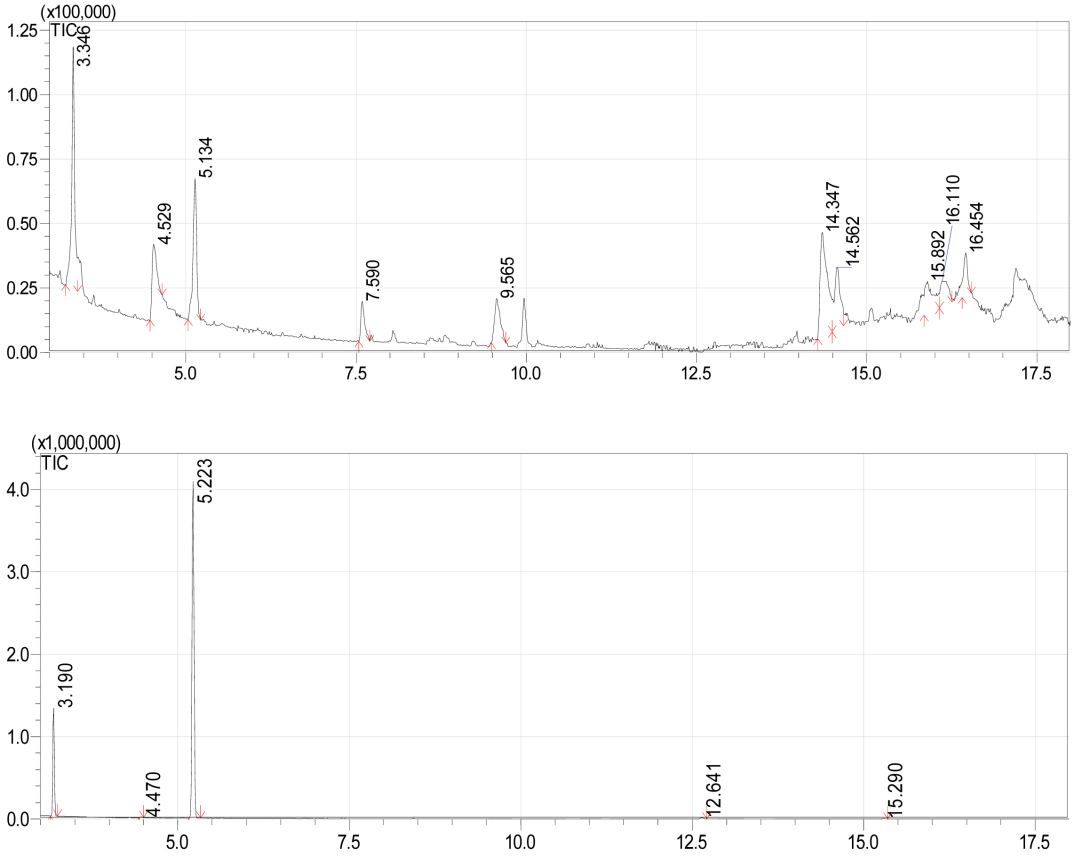

Figure 11. Chromatogram of the chemical compounds identified via GC-MS of *Asphodelus fistulosus*.

Propanone, 1,1-dimethoxy-, 1,2,4-Benzenetricarboxylic acid, 1,2-dimethyl ester,3-Hexanol, 2-methyl-, 2-Propenoic acid, butyl ester, 2-Butenoic acid, 2-methyl-, (Z)-, 5H-1-Pyrindine, Acrylic acid, (5-cyclopropylidenepentyl) ester, 1,3-Cyclopentadiene, 1,2,5,5-tetramethyl-, 1,3-Cyclopentadiene, 1,2,5,5-tetramethyl-, 2-Hydroxy-2,6-dimethyl-hept-6-en-3-one, Heptane, 4,4-dimethyl-, Ethylbenzene, Ethanone, 1-cyclobutyl-, 4,4-Dimethyl octane, Pentane, 2,2-dimethyl- and Heptane, 4,4-dimethyl.

The GC-MS analysis of *H. arbainense*, was shown in Table 4 and Figure 5 the compounds found were Butanoic acid, anhydride, 2-Propanone, 1,1-dimethoxy-Acetic acid, butyl ester, 2-Propenoic acid, butyl ester, 1,3-Cyclopentadiene, 1,2,5,5-tetramethyl, 1,3-Dioxolane-2-methanol, Bicyclo [2.2.1]heptane, 2-(2-propenyl), 1-Undecene, 9-methyl, Isophthalaldehyde, 4,6-Heptadienoic acid, 3,3,6-trimethyl-, ethyl ester Heptane, 4,4-dimethyl, Ethylbenzene, Undecane, 3-Hexanone, 2,5-dimethyl and Phenol, 3-(1,1-dimethylethyl)-4-methoxy.

Fifteen compounds (2-Propanone, 1,1-dimethoxy-, 2-Propenoic acid, butyl ester, 2-Butenoic acid, 2-methyl-, (Z)-, 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, 5H-1-Pyrindine, 1,3-Cyclopentadiene, 1,2,5,5-tetramethyl, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, (S)-(+)-1-(2-Pyrrolidinylmethyl)-pyrrolidine, 1-Butanol, 3-methyl-, acetate, 12-Hydroxy-3-keto-bisnor-4-cholenic acid, Heptane, 4,4-dimethyl, Benzene, 1,3-dimethyl, 4,4-Dimethyl octane, 8-Hydroxy-2,2,8-trimethyldeca-5,9-dien-3-one, 1,3-Cyclopentadiene, 5,5-dimethyl-2-ethyl-) ware appear from *C. grandiflorus* extract, the data reported in Table 4 and Figure 6.

Results revealed in Table 4 and Figure 7 the identification of 15 compounds (2-Propanone, 1,1-dimethoxy-, 2-Propenoic acid, butyl ester, 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, 5H-1-Pyrindine, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 1-Heptanol, 3-methyl-, Octane, 1-ethoxy-, 1-Butanol, 3-methyl-, acetate, 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, trans-, Sulfurous acid, hexyl 2-propyl ester, Heptane, 4,4-dimethyl, Undecane, Butane, 2,2-dimethyl-, n-Tridecan-1-ol, 5,8-Decadien-2-one and 5,9-dimethyl-) in *R. vesicarius* extract. Previous phytochemical investigation revealed the presence of anthraquinones, flavonoids and minerals.

The GC/MS data and spectrum of the chemical constituents in ethanol extract of *S. alexandrina* are shown in Table 4 and Figure 8 The chromatogram showed that ethanol leaf fraction contained 15 compounds and they are Furan, 2-butyl-tetrahydro-, 2-Propanone, 1,1-dimethoxy, Acetic acid, butyl ester, 2-Propenoic acid, butyl ester 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, Propanoic acid, 1-Pentene, 4,4-dimethyl, Benzeneacetaldehyde 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, Octane, 2-chloro, 2-Hexanone, Heptane, 4,4-dimethyl, Butane, Benzene, 1,3-dimethyl, 4,4-Dimethyl octane, 2,2-dimethyl.

We detected 15 peaks associated with segregated constituents by GC–MS in the *R. stricta* extract. Composites are listed in Table 4 and Figure 9. 2-Propanone, 1,1-dimethoxy, 2-Propanone, 1,1-dimethoxy, Acetic acid, butyl ester, 2-Propenoic acid, butyl ester, 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, Acetic

acid, pentyl ester, Diazene, bis(1,1-dimethylethyl), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, Urazole, Diisopropyl (methoxy)silane, Heptane, 4,4-dimethyl, Ethylbenzene, Benzene, 1,3-dimethyl, Undecane and Pentane, 2,2-dimethyl, were the major components present in the extract.

The GC/MS data and spectrum of the chemical constituents in ethanol extract of *W. somnifera* are shown in **Table 4** and **Figure 10** respectively.

4. Discussion

Widely distributed in Central Asia and Southern Africa. In Saudi Arabia, they are grown in the Wadi Najran, Taif region, and Al Madinah Al Munawwarah (Abyar Al-Mashy) [13]. Teucrium polium *L. occurs* throughout the Mediterranean and Irano-Turanian regions [14]. In Saudi Arabia, it is widely distributed in Aljuf, Hail, and Al Madinah Al Munawwarah (Gabal Al-aquiq) [14]. Many of these Species that are distributed throughout the Kingdom have been used by the local communities for the treatment of a large number of ailments [15]. This result sagest the report of Migahid (16). Primary metabolic compounds are important compounds in the processes of transporting and storing energy, regulating growth and respiration, and they are the primary compounds to produce secondary metabolic compounds [30].

The protein parts are used as therapeutic agents in combination with other chemicals, which makes them have a high medicinal value [30]. In **Table 2** it shows the changes in the soluble proline content of different plant species, the largest amount of which was retained significantly, and the highest plant was *R. vesicarius* it is very clear, and the lowest plant are *P. incisa* and *A. fistulosus*. Low levels of proline ensure that girls are in a favorable environment that is not subject to stress [31].

On the other hand, the result in **Table 2** shows the decrease in the amino acid content in *P. incise*, *H. arbainense* and *C. grandiflorus*, and then the observed increase in the plant sample *R. vesicarius* and *S. alexandrina* then the average decrease in the rest of the samples. Amino acids are the main component of protein, contribute to cellular digestion and osmotic pressure, and are an essential component in the synthesis of the alkaloid secondary metabolite, which gives it medicinal value in therapeutic uses [32]. Also, the result shows the decrease in carbohydrate content in the plant samples and the observed increase in the plant sample *A. fistulosus* then *S. alexandrina*. The carbohydrate content in the plant affects the plant's immunity, and it has sugar molecules that can be extracted and used to increase the plant's immunity and protect it from diseases [33].

The result in **Table 3** showed secondary compounds in the ethanol plant extract, the result clears that, phytosterol compound appeared in all plant extract, which this compound has types as sitosterol and stigmasterol usually abundant in many plant foods such as fruits and vegetables and has a common role in reducing harmful cholesterol (LDL) [34]. Thus, protects against many heart and cancer diseases [35]. All plant samples have triterpenoids compound. This compound has played several roles in immunity against bacteria and allergies, the

most important of which is regulating and controlling the proliferation of cancer cells [36]. The results are agreement with Pathak who suggested that quantitative phytochemical study indicated an appreciable number of polyphenols, flavonoids, flavonols, proanthocyanidins, tannins, saponins and alkaloids. The presence of these phytochemicals has been reported to exert profound stabilizing effect on lysosomal membrane while tannins and saponins bind cations, thereby stabilizing erythrocyte membranes and other biological macromolecules [37].

Alkaloids are known anti-inflammatory effects. Flavonoid and phenolic compounds are potent antioxidants which prevent oxidative cell damage and possess anti-inflammatory, anti-allergic and anti-thrombotic [38]. Proanthocyanidins are a type of bioflavonoid that has been shown to have very potent antioxidant activity [39]. Previous studies had also shown that plant extracts possessing anti-inflammatory properties may contain phytochemicals with antioxidant activity against deleterious chain reactions triggered by reactive oxygen species associated with inflammations [40].

Some plant samples haves saponins compound this benefit in role of lowering cholesterol and regulating glucose in the blood, which makes it anti-diabetic [41]. Plants differed in flavonoid content and were only in plant *R. vesicarius*, *P. incisa* and *S. alexandrina*. It protects against cell damage, cancer, and liver diseases, and helps in the dilation of blood vessel [42]. And has antioxidant activity [43]. The coumarin compound appeared in all the samples it's benefit as an anti-inflammatory and usually found in the peels of fruits; it gives role in protecting and preservation them from rotting [44]. Also gives the samples a distinctive aromatic smell upon concentration varies, and this effective compound is useful in the perfume industry [1]. The tannin compound has many uses in dyes and leather tanning [45], health benefits as antioxidant, anti-allergic, anti-helminthic and usually us as astringent [46]. There are a number of reports on the phytochemical constituents of *Pulicaria incisa*. The presences of Sixty-six compounds representing 89.4% of total oil were identified. The main components were α-Ocimene (15.17%), τ-Cadinol (6.79%), α-Cadinol (4.51%), Alloaromadendrene (4.45%) δ-Cadinene, (+) - (4.13% [29]. Some more reports indicate the presence of compounds such as dl-limonene, bcaryophyllene, 5, 30-dihydroxy-3, 7, 40, 50-tetramethoxy flavone, myricetin-7,30,40-trimethyl ether, and a di-C-glycosyl flavone, vicenin 3 [29]. For example, the study of Muhammad [46] a large number of extracts and bioactive constituents of different species of genus Heliotropium revealed significant biological activities such as antimicrobial, antitumor, antiviral, antiinflammatory, wound healing, cytotoxicity and phytotoxicity [47].

This is in accordance with the previous findings with respect sample, where had major quantitative changes in *C. digitatum* after processing; a special combinations of volatile compounds which gives the aroma of the processed *C. digitatum* have also other benefits as functional food ingredients; some of them with additional activity as natural food additives, analgesic or antioxidant which participate in the reasonably high antioxidant capacity of *C. digitatum* that were previously determined [48]. However, the hepatic protective activity of *R. vesi-*

carius extract was not previously studied [49]. Chemical composition: Senna contains anthraquinonoid compounds flavonoid, saccharide, naphthalene derivatives, phytosterols, essential oils, waxes, tannins, mineral salts, resins, and mucilage [50]. These values are considered high when compared to other plants, corresponding to a previous work [51]. Iqbal *et al.* have reported that the antioxidant capability of the methanolic extracts of *R. stricta* leaves was significantly elevated when compared to the α-tocopherol or the synthetic antioxidant BHA [51]. Another study conducted in Saudi Arabia has demonstrated the high TPC of *R. stricta* extract [52].

The chromatogram showed that ethanol leaf fraction contained 15 compounds and they are 2-Propanone, 1,1-dimethoxy-, Acetic acid, butyl ester, 2-Propenoic acid, butyl ester, Pyridine, 3-ethyl-2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one Propanoic acid, Formic acid, 2-propylpentyl ester, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-Acetate, 2-hydroxy-2-(3-chloro-4,5-dihydro-5-isoxazolyl)-, ethyl ester, 1-Heptanol, 3-methyl-, Heptane, 4,4-dimethyl-p-Xylene, Undecane, Pentane, 2,2-dimethyl-Phenol and 5-methyl-2-(1-methylethyl)-, acetate. The chemistry of Withania species has been extensively studied and several groups of chemical constituents such as steroidal lactones, alkaloids, flavonoids, and tannins have been extracted and identified [53]. A qualitative analysis of constituents present in ethanol extract was performed by GC/MS. As shown in **Table 4** and **Figure 11**, the full scan revealed the presence of several phenolic compounds, based on the over15 major peaks detected. 2-Propanone, 1,1-dimethoxy-, 3-Furanmethanol, 2-Propenoic acid, butyl ester, 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, Formic acid, 2-propylpentyl ester, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-Phosphonofluoridic acid, methyl-, 3,3-dimethylbutyl ester, dl-Erythro-O-methyl threonine, 1H,5H,7H,11H-Dipyrazolo [1,2-a:1',2'-d][1,2,4,5]tetrazine, tetrahydro, 1-Heptanol, 3-methyl, Heptane, 4,4-dimethyl, p-Xylene Undecane, Pentane, 2,2-dimethyl- and (SS)- or (RR)-2,3-hexanediol. In fact, different results obtained from lyophilized samples of A. tenuifolius harvested from septentrional Algerian Sahara [54].

5. Conclusion and Recommendation

Medicinal plants are of great medicinal and nutritional importance, as they highly contain content of proteins and secondary compounds of phytosterols, triterpenoids, tannins, etc. That extracted from leaves, enabling them to be used in several cosmetic, medicinal, and commercial fields. Alkaloid compound, which makes them anti-diseases and the most prominent role of them is to be a rich source for the manufacture of pharmaceutical drugs and vitamins. Phytochemical screening is an important step in the detection of the bioactive components existing in medicinal plants that are used in traditional medicine. There is a need to focus phytochemical screening on ethnobotanical studies to complete research into traditional medicine which leads to the discovery of new drugs. The flora of Kingdom of Saudi Arabia contains many medicinal plants, some of them were studied in research to discover their importance, and the field is still open for

many studies to identify the important components in these plants and how to use them in industry to produce medical and cosmetic materials that benefit humanity.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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