Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(4):126-133

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Isolation and Characterization of the Phytoconstituents in the Aerial Parts of Wild and Home Planted *Artemisia vulgaris* by Gas Chromatography-Mass Spectrometry

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ABSTRACT

Several phytochemical compounds inthe aerial parts of Artemisia vulgaris were isolated and identified. The investigation included home planted (fresh and dried) and the dry wild plant. A unique assay was used to demonstrate the extraction and identification. Methanol was used as the extraction solvent and gas chromatography coupled with mass spectrometry was used in separation and identification. Among others, camphor, piperitone, D-limonene and vulgarin are the major constituents of the extract of the dry wild plant. While Artimisia ketone, 1,8-Cenol, D-Fructose and Artinniun b are the major constituents of the extract of the home plant. Concentrations of the detectable phytoconstituents are calculated as relative proportions among extract populations. The major compounds are found in considerable percentages calculated from peak areas in the GC chromatograms. The aerial plant components of Artemisia vulgaris have important applications in traditional medicine.

Keywords: *Artemisia vulgaris*; Phytoconstituents; GC/MS

INTRODUCTION

Artemisia vulgaris is a plant belongs to the family Asteraceae which grows in different countries around the world [1,2]. Traditionally, this plant is widely used in medicine for the treatment of diabetes and extracts of the whole plant are used for epilepsy and in combination for psychoneurosis, depression, irritability, insomnia, anxiety, and stress [3]. In traditional European medicine, it is commonly used as a choleretic and for amenorrhoea anddysmenorrhoea [4]. The aerial parts of *Artemisia vulgaris* are being used as an anthelminthic, an antispasmodic, an antisepticand for various disorders including hepatosis [5]. Its crude extract has an antibacterial activity and showed efficacy in the correction of breech presentation [6]. Moreover the extract has traditionally an antimalarial effectsand antitumor activity [7]. The paste of the leaves is applied over skin diseases. *Artemesia vulgaris* has a well-documented history of herbal uses, including the flavoring of food and drinks [8]. Its extracts are recommended to women for a wide variety of gynecological problems [9]. The most common homeopathic use of *Artemisia vulgaris* is for the treatment of irregular menstruation and relief of menopausal ailments [8].

The flavonoid chemistry of *Artemisia vulgaris* used as anemmenagogue in traditional medicine, in conjunction with an evaluation of its estrogenicactivity was investigated. In that study the most abundant compounds were eriodictyol and luteolin [10]. Different parts of *Artemisia vulgaris* are also used for a multitude of other medicinal purposes including as antibacterial, anti-inflammatory, antiseptic, diaphoretic, emmenagogic, or stimulatory agents [11]. The antispasmodic effects of *Artemisia vulgaris* were evaluated and mediated through dual inhibition of muscarinic receptors and Calcium influx, which justify its traditional use in the gastrointestinal and airways hyperactivity [12].

Several trials were performed to identify the phytocomponents of the *Artemisia vulgaris* plant. For example, volatile allelochemicals were identified and characterized from fresh leafs. The volatile constituents were trapped and analyzed via gas chromatography coupled with mass spectrometry [13]. Pires et al. carried out phytochemical screening tests of hydroalcohol extracts of *Artemisia vulgaris*, in order to find a possible relation among the phytochemical constituents andthe observed effects [14]. Another study was conducted to investigate the essential oil compositions and antimicrobial and antioxidant activities of the essential oils and methanol extracts [15,16].

The chemical composition of the aerial and root oils in *Artemisia vulgaris* were investigate by using gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), and ^{13}C nuclear magnetic resonance (NMR) analysis [17]; this published work was extended to scan the antimicrobial activity; and to clarify the influence of plant organs specification and storageon essential oil yield and composition.

The chemical composition of the essential oil wing in Northern Lithuania has been studied by GC/MS. The major compounds that are Germacrene D, trans-Thujone, Chrysanthenyl acetate, 1,8-Cineole [2,18]. Five known flavonoids and three coumarins as inhibitors of mouse brain monoamine oxidase (MAO) enzyme have been isolated from 80% aqueous ethanol extracts of whole body of *Artemisia vulgaris* (Mugwort), and their structures were assigned using various spectroscopic methods [19].

In Tafila city in Jordanthe plant is widely used in tradition medicine as antibacterial and antifungal, and it can be used for a wide range of digestive problems due to its action as a stomachic and cholagogue. This plant was chosen in the study because there is a difference in the therapeutic activity between wild and home planted plant. This motivates the group to complete this study and find the difference in phytoconstituents in the two types. It is also widely used as antineoplastic, inhibiting the growth of cancer cells, and is a mild sedative. Moreover, This work extendsthe investigation of the phytochemical compoundsin medicinal plants that performed in our laboratory [20,21].

EXPERIMENTAL SECTION

Collection and Preparation of the Plant Samples

Artemisia vulgaris samples were collected from Tafila city in Jordan. Three types of species were used; the dry wild plant (collected dry from the field), the fresh home planted and the dried home planted samples. Parts of different samples were separated and used without further treatment [20].

Preparation of the Dried Home Planted Samples

Leaves and stems of the fresh home planted *Artemisia vulgaris* (10.0 g each) were accurately weighed and put separately in two 500 mL beakers. The two pars were dried at a temperature of 35 ± 1°C for 5 days. The resulted dry samples were grinded to fine powder and inserted separately in two 50 mL volumetric flasks and covered with methanol to the mark. The flasks containing the powder were manually shaken, and left to stand for 24 h in dark at room temperature. The methanolic extracts were filtered, collected in new clean volumetric flasks and stored in refrigerator under a temperature of 5°C ± 1 until use.

Reagents

Acetonitrile, methanol, (HPLC grade) and potassium phosphate were purchased from Aldrich and used without further purification.

Method of Extraction

10.0 g of leaves and stems from each type of species are accurately weighed using of analytical balance. The samples are inserted in separate 50 mL volumetric flasks and covered with methanol to the mark. The flasks were manually shaken, and left to stand for 24 h in dark at room temperature. The methanolic extracts were filtered, collected in new clean volumetric flasks and stored in refrigerator under a temperature of 5°C ± 1 until use [20].

Spectrophotometric Measurements

A spectrophotometer type Shimadzu UV-1800 was used to perform the absorbance measurements.

Gas Chromatography/Mass Spectroscopy

The gas chromatograph was Agilent Technology type 7890 GC equipped with a mass spectrometer type 5975C Inert MSD triple axes detector [20]. The carrier gas was Helium (99.999%) with a pressure of 18.3 psi. The makeup gas for the mass spectrometer was highly pure argon (99.999%), at a flow rate of 19 mL/min. A column of 5% divinyl 95% dimethyl siloxane, 30 m, 0.25 μm was used. The column oven temperature was programmed as follows: start

temperature at 80°C; increased to 295°C with a ramp of 15 ºC/min, the temperature was held at 295°C for 5 min until elution was complete. After 15 s the split valves were opened for 3 min to purge the injector. All injections (2 μL) were made with a 10 μL Hamilton syringe [20].

RESULTS AND DISCUSSION

Motivations

The phytoconstituents of *Rubus Fruticosus* and *Convolvulus arvensis* plants were successfully characterized in our lab [20,21]. *Artemisia vulgaris* was chosen in this study because it is widely used in tradition medicine in Tafila as antibacterial and antifungal, and can be used for a wide range of digestive problems due to its action as a stomachic and cholagogue. This plant was chosen in the study because there is a difference in the therapeutic activity between wild and home planted plant. This motivates the group to complete this study and find the difference in phytoconstituents in the two types. The gas chromatograph/mass spectrometer was chosen to perform the analysis.

Selection of Chromatographic System

In order to identify the phytocomponents in the methanolic extract of *Artemisia vulgaris*, good separation is necessary. The separation was performed by using a modern gas chromatograph connected to mass spectrometer under the conditions described above. A quadruple mass analyzer was used. The resulted chromatograms have a peak for each component in the extract. Mass spectrum of each component shows number of signals. The mass of the whole molecule can be assigned at the peak at highest m/z. Signals with lower m/z show the fragmentation pattern of the molecular ion and can provide some structural information. The base peak appears at 100% abundance. Split mode was used in the analysis and one split less injection was performed as a test whether new components were appeared or not. The mass spectra fragmentation patterns for peaks in the chromatograms in Figures 1 and 2 and were compared with those stored in the computer library and with other sources for matching components in the extract such as National Institute of Standards Technology (NIST08s), Wiley Registry of Mass Spectral Data's, New York (Wiley 8) and Fatty Acid Methyl Esters Library version 1.0 (FAME library). In the present study, the acquired mass spectra provided structural information to identify many compounds in the extract mixture. By screening the mass spectra and fragmentation with the library, each spectrum matched with one structure with high probability >96% [20].

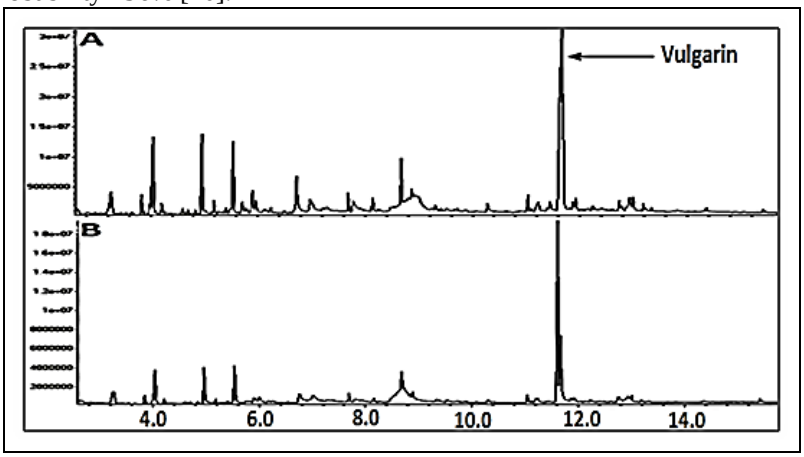

Figure 1: GC/MS chromatograms for the extract of the dry wild plant A) leaves B) stems

Wild Dry *Artemisia vulgaris*

The GC-MS analysis proved that fifteen individual, well resolved and readily quantified peaks are presented in the two extracts of the aerial parts of Wild *Artemisia vulgaris*. The mass spectra of the peaks were matched with the corresponding components in the libraryby percentages > 96% (Table 1).

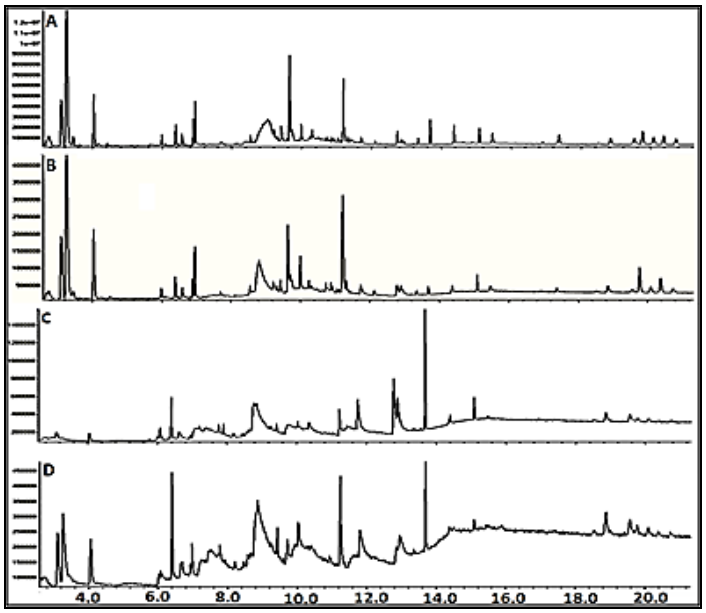

Figure 2: GC/MS chromatograms for the extract of the home plant A) dry home leaves B) fresh home leaves C) dry home stems D) fresh home stems

Table 1: Detail description of the components found in the wild dry plant extracts. The components with percentages less than 0.2% are not listed in the table

Retention Time (min)	Phyto-constituent	Composition in Leaves %	Composition in Stems %
3.269	1,5-heptadien-4-one	2.31	1.4
3.852	2-cyclohexen-1-one	1.38	0.56
4.015	Camphor	11.53	4.9
4.202	Endo-borneol	0.37	0.35
4.959	piperitone	11.53	4.9
5.598	D-Limonene	10.6	4.9
6.725	1-Propenoic acid	5.77	1.4
7.798	Piperidine	1.38	0.7
8.142	Jasmonate	0.46	0.35
8.7	6-ethoxy-6-methyl-2-cyclohexenone	5.77	3.5
8.9	1-propene	0.46	0.45
11.039	t-oxoeudesma-2,4-dien-11-beta-H-1,2,6-alpha—lide	1.38	0.7
11.225	4-Quinolinol (2,8-dimethyl)	0.69	0.7
11.633	Vulgarin	46.13	70.03
12.746	9-octadecenamide	0.69	0.7

The Major Phytoconstituents in the Wild Dry *Artemisia vulgaris*: Camphor, Piperitone, D-Limonene and Vulgarin

Camphor oil (Figure 3A) is an effective stimulant, which boosts the activity of the circulatory system, metabolism, digestion, secretion, excretion and found to be a good anesthetic [22]. It also reduces the severity of nervous disorders and convulsions, epileptic attacks, and chronic anxiety. It is widely used as antispasmodic, antineuralgic, anti-inflammatory and sedative [23]. Camphor has also been used to treat fungal infections of the toenail, warts, cold sores, hemorrhoids, and osteoarthritis [24]. Camphor is used topically to increase local blood flow and as a "counterirritant," which reduces pain and swelling by causing irritation [25].

Piperitone (Figure 3B) increases nitrofurantoin susceptibility in members of Enterobacteriaceae. The activity of nitroreductase was reduced by piperitone produced in *E. cloacae*. Chinese studies reported a central sedative action by piperitonewhen given orally [26].

D-limonene (Figure 3C) is one of the most common terpenes in nature. Being an excellent solvent of cholesterol, d-limonene has been used clinically to dissolve cholesterol-containing gallstones. Because of its gastric acid neutralizing effect and its support of normal peristalsis, it has also been used for the relief of heartburn. D-limonene has achemopreventive activity against many types of cancers. A clinical trial shows a partial response in a patient with breast cancer and stable disease for more than six months in three patients with colorectal cancer [27]. Vulgarin

(Figure 3D) is the green parts of wild *Artemisia* [28]. In this work, Vulgarin has a peak in the leaves and the stems dry extracts chromatograms at a retention time of 11.633 min with percentages of 46.13% and 70.03% respectively. Medically, Vulgarin exhibited a significant antihepatotoxic activity by reducing the elevated levels of serum enzymes such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate oxaloacetate transaminase (SGPT) and alkaline phosphatase (ALP). The total protein (TP) levels were increased when compared with standard drug silymarin against CCl4-induced toxicity in Wister rats. These biochemical observations were also supplemented by histopathological examinations of the liver sections [29]. *Artemisia vulgaris* exhibits combination of anticholinergic and Ca^{2+} antagonist mechanisms, which provides pharmacological basis for its folkloric use in the hyperactive gut and airways disorders, such as abdominal colic, diarrhea and asthma [30].

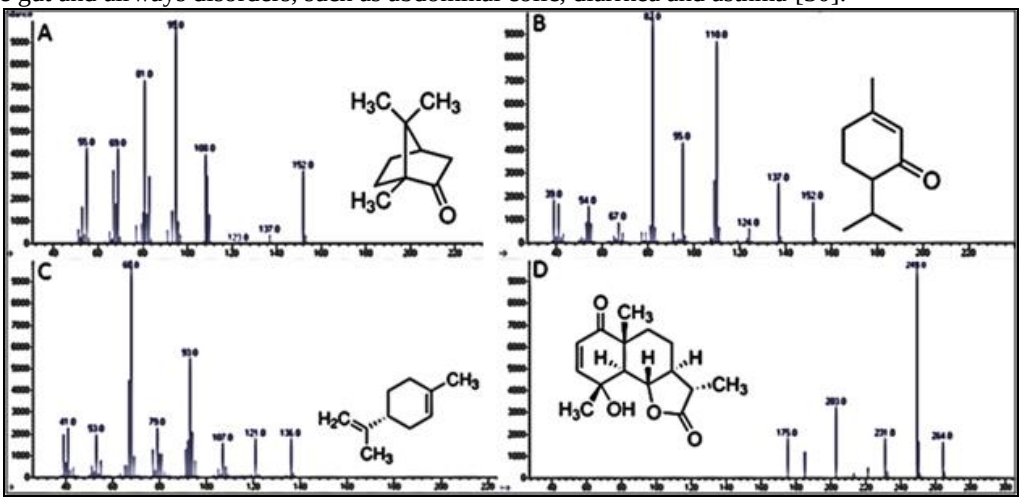

Figure 3: Mass spectra of the major compounds in the wild dry *artemisia vulgaris*: (A) camphorat t_R 4.015 min (B) piperitoneat t_R 4.959 min (C) D-limonene at t_R5.598 min (D) vulgarin at t_R11.633 min

Fresh Home Plant and Dried Home Planted *Artemisia vulgaris*

The phytoconstituents in theaerial parts of the fresh home planted *Artemisia vulgaris* were extracted directly after collection. More samples of fresh home planted *Artemisia vulgaris* were dried and thentreated similarly. The GC-MS analysis proved that twenty seven individual, well resolved and readily quantified peaks are presented in the four extracts of the aerial parts of fresh *Artemisia vulgaris*. The mass spectra of the peaks were matched with the corresponding components in the library adapted with GC/MS by percentages >96% (Table 2).

The Major Compounds in the Fresh/Dried Home Plant *Artemisia vulgaris:* Artimisia Ketone, 1,8-Cenol, D-Fructose and Artinniun b

Artemisia ketone (Figure 4A) is distinguishedin*Artemisia* species; it gives the fragrant property of *Artemisia vulgaris.* Numerous studies were focused onthe oil containing Artemisiaketone to test the antibacterial and antifungal activity [31]. Tests were carried out both on the whole oil and on its separate components such as artemisia ketone,camphor, 1,8-cineoland α-pinene, [32]. Artemisia ketone is the component of the oil that has the greatest antimicrobial activityand it always turns out to be effective against bacteria and some fungi (C. albicans and A. fumigatus) at very low concentrations (range 0.07–10 mg/mL) [33].

Fructose (Figure 4C) has been used widely for intravenous feeding in medicine and surgery. The metabolism of fructose is largely insulin-independent. The ultimate fate of fructose carbons is determined by the presence or the absence of insulin. Clinically, fructose may exhibit useful effects for patients with mild and well-balanced diabetes [34]. 1,8-Cenol (Figure 4B) was found in the home plant *Artemisia vulgaris* and has a peak in the leaves and the stems dry extracts chromatograms at a retention time of 3.111 min with percentages around 8% in leaves and stems. When dried, *Artemisia vulgaris* stems has no 1,8-cenol. This phyto-compound is a distinguished chemical component with strong therapeutic effects that have been well investigated. It has a strong healing potential,airborne antimicrobial [35], Analgesic [36,37] anti-inflammatory [38], antibacterial [39] and antioxidant [39].

Arteannuin-B (Figure 4D) and artemisinin are highly synergistic against a chloroquine resistant strain of Plasmodium falciparum. Artenannuin-B is the effective molecule giving this synergy that has a synergetic action in the presence ofartemisinin in a large series. The production ofnitric oxide NO is strongly inhibited by arteannuin-B. Moreover, Arteannuin-B has a strong inhibitory effect on the pro-inflammatory interleukines IL-1β, IL-6, TNF-α,

whileArtemisinin, DHA, artemisinic acid show none. Arteannuin-B has also a strong inhibitory effect against the pro-inflammarory IL-6 and IL-8 which is very poor in artemisinin but rich in arteannuin-B [40].

Table 2: Detail description of the components found in the fresh home plant and fresh dried home plant *Artemisia vulgaris*. The components with percentages less than 0.2% are not listed in the table

Retention Time (min)	Phyto-constituent	Composition in Fresh Leaves %	Composition in Fresh Stems %	Composition in dried Leaves %	Composition in dried Stems %
2.686	Sabiene	1.2	0.74	2.17	-
3.111	1,8-Cinol	8	8.92	8.65	-
3.263	Artimisia ketone	24.24	11.89	27.17	-
3.455	Artimisia alcohol	0.85	-	1.36	-
4.038	Camphor	8.73	7.43	8.65	1.75
5.985	Alpha-Copaene	0.73	-	0.68	2.45
6.457	Germacrene D	1.33	14.86	1.63	7.7
6.527	Oxalic acid	0.73	1.04	0.76	1.75
6.952	Beta-Selinene	6.06	1.49	4.89	-
8.957	D-Fructose	10.91	14.86	5.43	17.5
9.424	7,8-dihydro-1,2,4-triazolo[2,3.c]pyrimidin-5(6H)-ylidenecyanamide	0.73	0.59	1.63	0.245
9.645	Qinghao acid	7.27	0.59	13.58	-
9.983	5,7,8-trimethylflouro[b]tropone	3.03	1.49	1.22	0.53
10.403	Myo-Inositol	-	-	0.54	2.45
11.196	Artinniun b	10.9	13.37	1.63	3.5
12.868	N-tetradecanoic acid amide or octadecanamide	0.73	2.97	1.36	17.5
13.306	N,N-dimethyl-4-nitroso-3-trimethylsilyl)aniline	0.24	0.149	0.22	-
13.679	1,2-benzene dicarboxylic acid	0.73	13.37	2.72	35
14.355	1-Eicosinol (Arachic alcohol)	0.85	-	1.9	1.4
15.083	2,6,10,14,18,22-tetracosahexaene	1.45	0.3	1.63	2.45
15.485	4-triflouroacetoxytetradecane	0.49	-	1.63	-
18.849	E-23-ethylcholesta-5,22-dien-3-beta-ol	0.85	3	1.36	2.45
19.536	Stigmast-5-en-3-ol	0.49	1.49	1.36	2.1
19.909	Silikonfett (grease)	3.6	0.74	2.72	0.53
20.102	9,10-dihydro-9,10-(prop-11-eno)anthracene	0.73	0.74	1.36	0.7
20.358	3-keto-urs-12-ene	1.82	-	1.63	-
20.72	URS-12-ene-3-ol	6.06	-	1.36	-

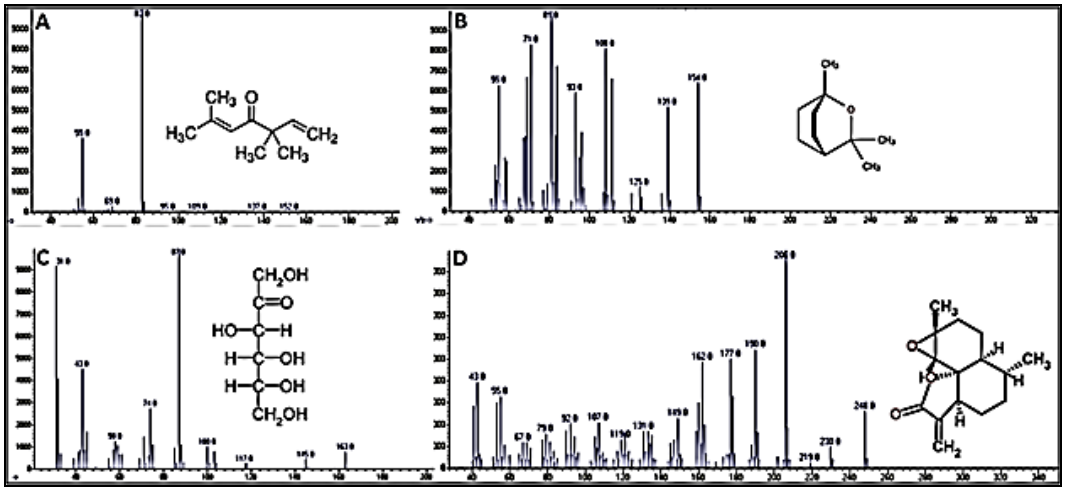

Figure 4: Mass spectra of the major compounds in the fresh home plant *Artemisia vulgaris:*(A) artimisia ketone at t_R3.263 min (B) 1,8-cenol at t_R3.111 min (C) D-fructose at t_R8.957 min (D) artinniun b at t_R11.196 min

1,2-Benzene Dicarboxylic Acid

Phthalic acid or (1,2-benzene dicarboxylic acid) has a peak in the dry extract chromatogram and all parts of the fresh plant extracts chromatograms at a retention time of 13.679 min. It was found to be a major component with high percentages as shown in Table 2. The mass spectrum of Phthalic acid is presented in Figure 5. In literature, the *in vitro* administration of phthalic acid causes less severe testicular injury [41].

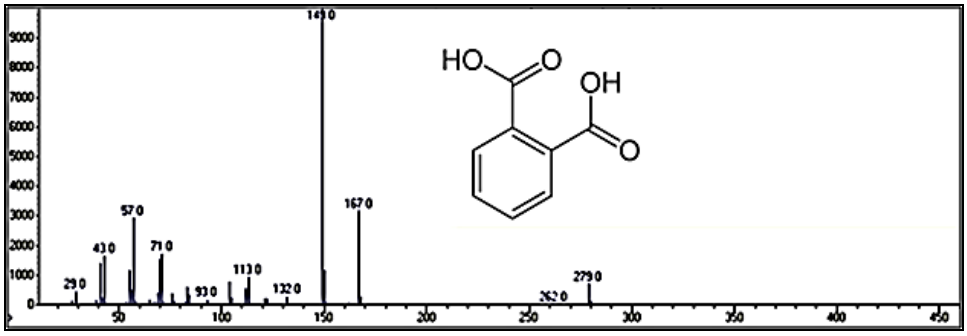

Figure 5: Mass spectra of Phthalic acid which has a peak in the GC chromatogram at 13.679 min

Comparison between Wild and Home Planted *Artimisia vulgaris*

The composition of phytochemical constituents of *Artimisia vulgaris* varies with harvesting conditions. Also different planting conditions lead to different phytochemical composition. In this study, the difference in extracts composition between wild and home planted *Artimisia vulgaris* can be easily recognized. Isolation, purification and full characterization of the crude extracts are essential to study the valuable bioactivity and toxicity effects of the two types. GC-MS plays a key role in the analysis of unknown components of plant origin and the concentrations of the detectable phytoconstituents are calculated as relative proportions among extract populations. In this work some new components other than those in literature are also discovered.

CONCLUSION

The obtained results in the present investigation showed that the methanolic extracts of home planted and wild *Artimisia vulgaris* contains many phytoconstituents in high concentrations. The identified compounds from the two types of the plant are traditionally widely used as antibacterial and antifungal as well as antispasmodic, antineuralgic, anti-inflammatory and Sedative. Moreover, *Artimisia vulgaris* oil is the unique source for endoperoxide lactone artemisinin that is used in the treatment of the chloroquine-resistant and cerebral malaria. More than fifteen compounds were identified the extract of the dry wild plant, the following compounds are major: camphor, piperitone, D-limonene and vulgarin. While more than twenty seven compounds were identified the extract of the home planted *Artimisia vulgaris* the following compounds are major: Artimisia ketone, 1,8-Cenol, D-Fructose and Artinniun b. Concentrations of the detectable phytoconstituents are calculated as relative proportions among extract populations.

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