IDENTIFICATION OF CHEMICAL COMPOSITIONS IN SOME MEDICINAL PLANTS BY GC/MS ANALYSIS

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ABSTRACT

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Medicinal plants used in treatment of diseases earlier times, are potential sources of new drugs. In this study, chemical compositions of Echium khuzestanicum, Marrubium anisodan and Echinops cephalotes were identified by Gas Chromatography-Mass Spectrometry (GC/MS). M.anisodan and E.cephalotes aerial organs and E.khuzistanicum flowers were extracted by maceration method. According to the results, 46 kinds of chemical compounds found in methanol extract of E.khuzestanicum. There are mucilage, fatty acids, flavonoid and diterpenes in the flower of E.khuzestanicum. Seventy six kinds of chemical compounds were found in methanol extract of E.cephalotes including aldehydes (7.9%), phenols (7.5%), fatty acids (5.8%) and furfural (5.4%) and 86 kinds of chemical compounds found in M.anisodan extract. Furfural, steroids, vitamin B and flavonoids are the main compounds of M.anisodan.

Keywords: Echinops cephalotes, *Marrubium anisodan*, *Echium khuzestanicum*, coniferol.

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INTRODUCTION

Plants have the ability to produce a variety of chemicals so that new compounds always are discovered and extracted from plants. Each of these compounds may have a therapeutic effect (Edeoga *et al.*, 2005). *Echium khuzistanicum* is a biennial plant of Boraginaceae which grows in the southwestern area of Iran (Khatamsaz, 2002). This plant is widely used in traditional medicine and is a potential source of valuable compounds such as shikonin and unsaturated fatty acids (Mohammadi and Piri., 2014). Boraginaceae is one of the most well- known source of gamma-linoleic acid that has a high nutritional and medicinal value (Horrobin, 1992). In about 150 species of Boraginaceae, naphthoquinonone pigments such as alkanin and shikonin derivatives exist in roots. Alkanin (S enantiomer) and shikonin (R enantiomer) and their derivatives have a lot of medicinal properties like anti-allergic, antibacterial, antiviral, antifungal, antioxidant, anti-inflammatory and wound healing (Gao *et al.*, 2011, Kim *et al.*, 2012, Lee *et al.*, 2014, Li *et al.*, 2015, Papageorgiou *et al.*, 1999, Skrzypczak *et al.*, 2015, Yang *et al.*, 2014). Shikonin plays an important role in the treatment of the obesity, intestinal ulcers, skin diseases, cancers and AIDS (Andujar *et al.*, 2013, Chen *et al.*, 2003, Deng *et al.*, 2013, Fan *et al.*, 2015, Gwon *et al.*, 2015, Kontogiannopoulos *et al.*, 2011, Lee *et al.*, 2011, Yang *et al.*, 2014, Zhang *et al.*, 2015).

Marrubium anisodan is a plant of the Lamiaceae. This genus has various properties such as being antioxidant and anti-inflammatory (Edziri *et al.*, 2012, Weel *et al.*, 1999). *Echinops cephalotes* belongs to Asteraceae family. This genus is very important in terms of chemical composition. The presence of alkaloids, saponins, plant sterols, polyphenols, and carotenoids has been detected in different parts of the *Echinops* genus (Hymete *et al.*, 2005). Dozens of alkaloid types were extracted from various parts of this genus that are used in industry, agriculture and medicine (Sheidai *et al.*, 2000).

The stages of extraction are important in the study of medicinal plants. Each extraction method is unique for each plant (Ingle *et al.*, 2017). The extraction methods mostly used are maceration, digestion, soxhlet extraction and ultrasound assisted extraction (Sharifi *et al.*, 2017).

GC/MS is actually a combination of two powerful analytical techniques i.e. gas chromatography and mass spectrometry. Gas chromatography separates the mixture components in the specified time interval. To identify the components, mass spectrometry gives information about the structure of each component. Mass spectrometer is a device that measures the ratio of mass-to-charge of ions in the gas phase as well as indicates the frequency of each of these ions. There are peaks in the mass spectra that relate to ionic compounds and molecules. The interpretation of mass spectra shows the type and quantity of compounds (Sparkman *et al.*, 2011). There are no previous references in literature about the extract compositions of these plants. In this study, chemical compositions of *Echium khuzestanicum*, *Marrubium anisodan* and *Echinops cephalotes* have been identified by GC/MS analysis for the first time.

MATERIALS AND METHODS

Plant Material

M.anisodan and *E.cephalotes* were collected from the medicinal plant garden of Hamadan (Natural Resources Department, Hamedan Natural Resources and Agriculture, Education and Research Center, Medicinal Plant Garden, Hamedan, Iran) and *E.khuzistanicum* was planted in a greenhouse. The plant's seeds were collected from Alhaii region around Ahwaz (the southwest of Iran). The plants were identified by the botanist, Dr Dinarvand (Faculty member, Natural Resources Department, Khuzistan Natural Resources and Agriculture, Education and Research Center, Ahvaz, Iran).

Extraction of plant material

The samples including *M.anisodan* and *E.cephalotes* aerial organs and *E.khuzistanicum* flower were dried at room temperature and in the dark and then reduced to powder. Ten grams of each plant powders were extracted in 100 ml of acidic methanol (85% V/V) solvent by maceration method (48hrs). After filtering, the extract was concentrated at a temperature below 40°C in an oven until it was dried and then the resulting extract was weighted and used for determining the compound composition by GC/MS analysis.

Gas Chromatography-mass Analyzer

To identify the compounds, Agilent 6890N gas chromatography coupled with Agilent 5973N mass detector with a mass range from m/z 2 to 800 was used in which the column was HP-5 with the following specification. A capillary column with a length of 30 m, diameter of 0.25 mm and stationary phase layer's thickness of 0.25 µm. One µl of each extract was injected. The injector temperature was 260˚C and the injection type was splitless. The analysis conditions were shown in Table 1. The solvent delay was 5 min and the compounds were identified by comparing their mass spectra with mass spectra database recorded in Wiley 7n and reported references.

Table 1. Temperature program of analysis.

Rate(˚C/min)	Temperature ˚C	Hold (min)
-	60.00	0.00
5.00	150.00	0.00
10.00	250.00	0.00

RESULTS AND DISCUSSION

After extraction, chemical composition of plants was identified by GC/MS. The percent and type of chemical compounds was achieved by comparing the data from

GC/MS with information for libraries (Mass spectra database recorded in Wiley 7n and reported references).

Chemical Composition of *Marrubium anisodan*

In methanol extract of this plant's aerial organs, 86 compounds were identified by GC/MS (Figure 1). The compounds present in this plant (with more than one percent), their retention time (RT), molecular formula, molecular weight (MW), and percent (peak area %) are presented in Table 2. Furfural, steroids, vitamin B and flavonoids are the main compounds of *M.anisodan*. According to the results, furfural is the highest compound (20.43%). Furfural is the natural product of lignocellulosic degradation. Also, furfural is obtained from dehydration of pentose sugars during cellulose depolimerization under acidic conditions (Martinez *et al.*, 2001). Furfural and its derivatives are the main flavors of foods. Furfural at low concentrations (1-12 mM) inhibits microorganisms (Boopathy *et al.*, 1993). About 13.26% of this plant extract is cyclopentane which is in steroid structure. The extract consists of lactose (9.53%) and inositol vitamin (8.55%). This plant extract has flavonoids such as 4H-pyran-4-one (5.42%). Fatty acids such as Dodecanoic acid (1.036%) and Pentadecanoic acid (1.55%), alkaloids such as Alpha-pyrrolidone (2.21%) and cyclic isoprenoids such as cyclotetradecan (2.32%). Previous studies have reported that there are some compounds such as diterpenes, sterol, derivatives of caffeic acid and flavonoids in this genus (Meyre-Silva andCechinel-Filho, 2010). The acylated flavonoid glycoside and two tetrasaccharidic phenylethanoid glycosides velutinosides I-II have been isolated from Marrubium velutinum shoot (Karioti *et al.*, 2003). Marrusidins A and B are two labdane-type diterpenes isolated from Chloroform extract of *Marrubium anisodon* along with polyodonine (Hussain *et al.*, 2010). Methanol extract of the plant showed 27.7% inhibitory activity of acetylcholine esterase used for the treatment of the disease Alzheimer. This inhibitory effect was attributed to the components that are functionally or structurally similar to tacrine (Gholamhoseinian *et al.*, 2009). According to the results of GC / MS, it is possible that this effect of the plant is related to alkaloids such as alpha-pyrrolidine, which need to be tested and confirmed in the laboratory. This family plants have been used to treat dandruff and hair regrowth (Safaei, 2010). According to the presence of vitamin B7 in this plant, we can say this plant is a good candidate for the treatment of hair loss and alopecia.

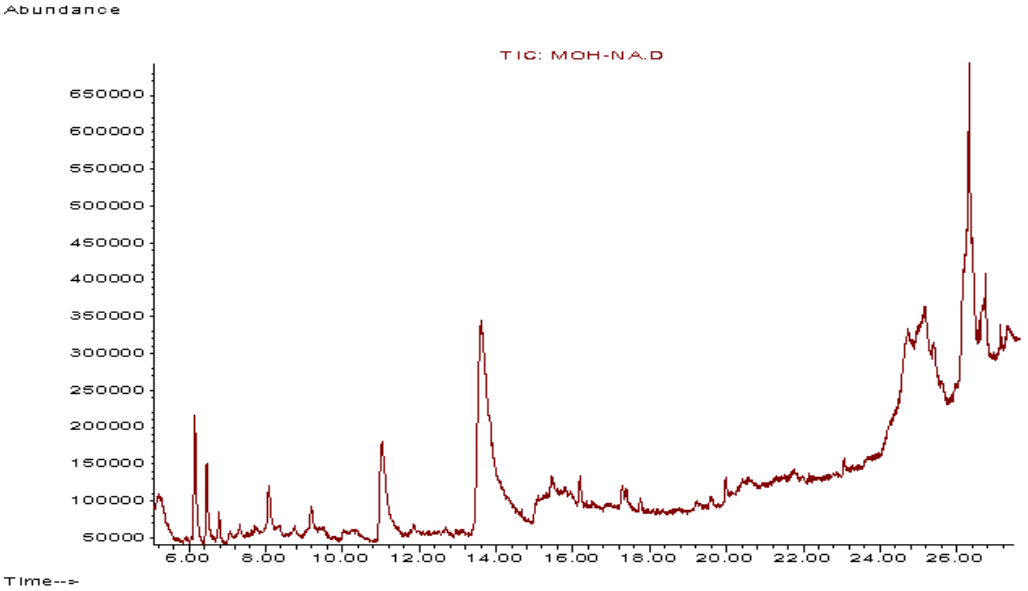

Figure 1. Chromatogram of *M.anisodan*.

Table 2. The compound present in *M.anisodan* (more than 1%).

No.	Name of the compounds	RT[a]	MF[b]	MW[c] g/mol	Peak area%
1	Furancarboxaldehyde	13.63	$C_5H_4O_2$	96.09	20.43
2	Cyclopentane	26.41	C_5H_{10}	70.1	13.26
3	Lactose	26.64	$C_{12}H_{22}O_{11}$	342.3	9.53
4	Neo-inisitole	24.86	$C_6H_{12}O_6$	180.16	8.55
5	4H-pyran-4-one	11.08	$C_5H_4O_2$	96.085	5.42
6	12-methyl-E,E-2,13-octadecadien-1-ol	20.09	$C_{19}H_{36}O$	280.496	3.22
7	Cyclotetradecan	16.17	C14H28	196.37	2.32
8	Alpha-pyrrolidone	9.21	C_4H_7NO	85.106	2.21
9	Propanoic acid	8.05	$C_3H_6O_2$	74.07854	1.88
10	Pentadecanoic acid	25.11	$C_{15}H_{30}O_2$	242.3975	1.55
11	Dodecanoic acid	24.47	$C_{12}H_{24}O_2$	200.32	1.03

a: Retention Time, b: Molecular Formula, c: Molecular Weight.

Chemical Composition of *Echium Khuzestanium (Flowers)*

According to GC/MS results, 46 compounds were found in methanol extract of *E.khuzistanicum* flower. Each of these compounds made a peak on chromatogram (Figure2). The plant compounds with more than 1% are shown in Table 3 including the mucilage, fatty acids, flavonoids and diterpenes Mucilage, fatty acids, flavonoid and diterpenes. According to the results, glucose is the highest compound in the flower extract

of this plant (22.32%). Mucilage in *Borago officinalis* is hydrolyzed to glucose, galactose, arabinose and allantoin (Khan.andAbourashed, 2009). As a result, the glucose present in the extract of this plant can be obtained by hydrolysis of mucilage (Javadzadeh, 1995). Mucilages are carbohydrates with very complex chemical structures and high molecular weights. One of the most important medicinal properties of mucilages is their anti-inflammatory property. Mucilage is used to treat gastrointestinal ulcers (stomach and intestines) and infections of the throat mucous (Bone andMills, 2013). In flower extract of this plant, 11.23% of 9, 12, 15-Octadecatrien-1-ol was found. This compound is also present in the spartium junceam extract. Fatty acids such as capric acid (12.6%), octadecanoic acid (3.75%) and botanoic acid (1.62%), alkaloids such as indole (2.57%), phenolic compounds such as 4-vinyl-2-methoxy-phenol (4.4%), diterpenes such as phytol (5.7%) and flavonoids such as 4H-Pyran-4-one (1.91%) and 3-Hepten-2-one (1.54%) are found in the extract. Capric acid is a 10-carbon fatty acid found in palm and coconut oil and less in animal fats and milk. This oil reduces insulin resistance and balances insulin level in humans. 36.7% of furfural and 1.99% of sulfur compound such as dimethylsulphon compound are also found in this plant. It is used as a food additive to maintain the quality and taste of food and treat parasitic infections and carriers of drugs (Jacob et al., 1999). Researchers try to increase the level of this fatty acid in transgenic plants (Dehesh, 1996). Diterpene available in this plant is an alcoholic non-cyclic diterpene with antibacterial, anticancer, anti-inflammatory and diuretic effects (Furumoto, 2002). Delorme et al. (1977) reported that E.amoenum have anthocyanins (13%), flavonoids (0.15%) and a small amount of alkaloids (Delorme *et al.*, 1977). Javadzade (1995) reported that *Borago officinalis* have mucilage, tannins, Na, Ca and K. Due to presence of different materials such as mucilage, flavonoids, phenolic compounds, diterpenes and useful fatty acids in this plant, it could be a good candidate for the treatment of many diseases and it is necessary to examine the effects of secondary metabolites of this plant.

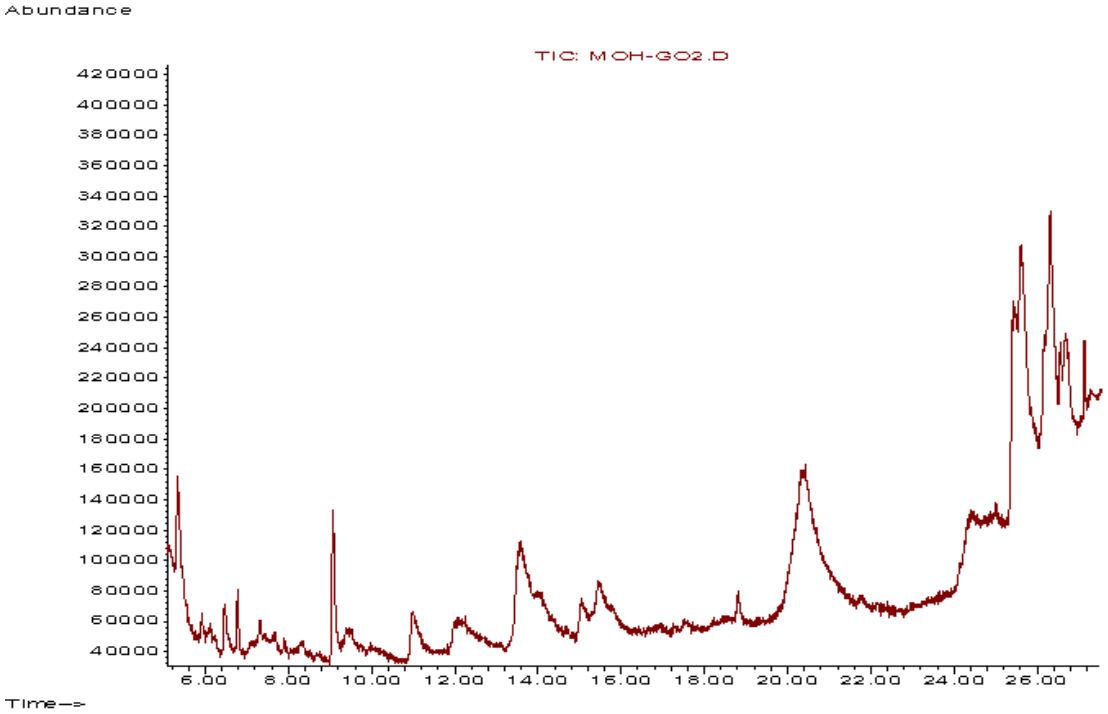

Figure 2. Chromatogram of *E.khuzestanicum*.

Table 3. The compound present in *E.khuzestanicum* (more than 1%).

No.	Name of the compounds	RT[a]	MF[b]	MW[c] g/mol	Peak area%
1	alpha-D-Glucopyranoside	20.43	$C_7H_{14}O_6$	194.1825	22.32
2	Decanoic acid	25.57	$C_{10}H_{20}O_2$	172.268	12.60
3	9,12,15-Octadecatrien-1-ol	25.81	$C_{18}H_{32}O$	264.453	11.23
4	2-Furancarboxaldehyde	13.83	$C_5H_4O_2$	96.09	7.36
5	Phytol	18.57	$C_{20}H_{40}O$	296.539	5.7
6	4-vinyl-2-methoxy-phenol	11.26	$C_9H_{10}O_2$	150.18	4.40
7	d-Talonic acid lactone	12.31	$C_6H_{10}O_6$	178.14	4
8	Octadecanoic acid	26.28	$C_{18}H_{36}O_2$	284.48	3.75
9	Indole	9.07	C_8H_7N	117.15	2.57
10	Dimethyl sulfone	13.62	$C_2H_6O_2S$	94.13	1.99
11	4H-Pyran-4-one	13.79	$C_5H_4O_2$	96.085	1.91
12	Butanoic acid	11.06	$C_4H_8O_2$	88.11	1.62
13	3-Hepten-2-one	21.61	$C_7H_{12}O$	112.172	1.54

a: Retention Time, b: Molecular Formula, c: Molecular Weight

Chemical composition of *Echinops cephalotes*

According to the results, 76 kinds of chemical compounds found in methanol extract of *E.cephalotes*. Figure 3 shows the chromatogram obtained from the methanol extract of *E.cephalotes*. The compounds present in this plant (with more than one percent), their retention time (RT), molecular formula, molecular weight (MW), and concentration (peak area %) are presented in Table 4. Aldehydes (7.9%), coniferol (4.8%), fatty acids (5.8%) and furfural (5.4%) are found in *E.cephalotes*. According to the results, Tridecanedinal is the highest compound in this plant (7.9%). In methanol extract of this plant, there are fatty acids, carboxylic acids, aldehydes, furfurals and phenolic compounds such as coniferol. The presence of alkaloids, saponins, plant sterols, polyphenols, and carotenoids has been detected in different parts of the genus *Echinops* (Hymete et al., 2005). Diisodecyl ether compound derived from Streptomyces has anti-bacterial effect and was found in this plant (Nandhini andSelvam, 2013). Benzenemethanol is a type of benzyl alcohol present in many plants and an important aglycone with antioxidant effect (Milos *et al.*, 2000). There are toxic compounds such as DDMP and benzyl alcohol in this plant and is necessary to examine its toxicity.

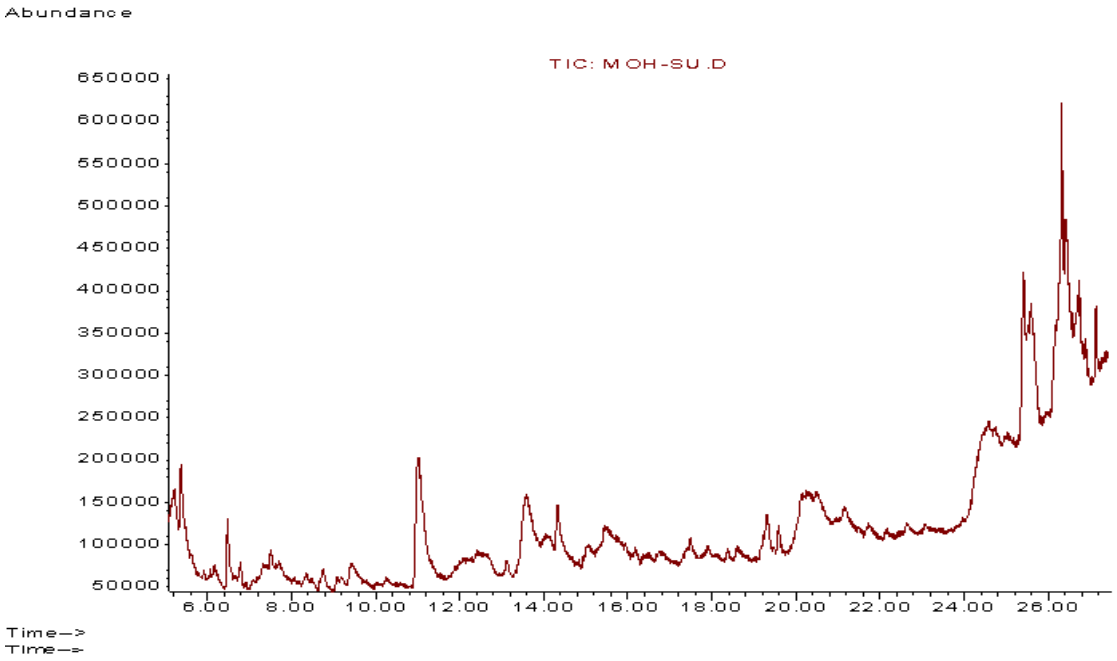

Figure3. Chromatogram of *E.cephalotes.*

Table 4. The compounds present in *E.cephalotes* (more than 1%).

No.	Name of the compounds	RT[a]	MF[b]	MW[c] g/mol	Peak area%
1	Tridecanedial	26.47	$C_{13}H_{24}O_2$	212.333	7.9
2	Capronic acid	25.14	$C_6H_{12}O_2$	116.16	5.82
3	11,13-Dimethyl-12-tetradecen-1-ol acetate	27.13	$C_{18}H_{34}O_2$	282.4614	5.76
4	2-Furancarboxaldehyde	13.83	$C_5H_4O_2$	96.09	5.46
5	Octadecanoic acid	26.47	$C_{18}H_{36}O_2$	284.48	4.91
6	Coniferol	25.61	$C_{10}H_{12}$	180.201	4.87
7	Phytol	18.57	$C_{20}H_{40}O$	296.539	4.15
8	2,2,3-Trimethyloxirane	11.21	$C_5H_{10}O$	86.132	4.08
9	2-Ethyl-2-hexen-1-ol	25.43	$C_8H_{16}O$	128.212	3.47
10	Benzenemethanol	24.38	$C_7H_{10}O$	110.156	2.94
11	2-Methyl-2-pentenal	13.90	$C_6H_{10}O$	98.145	2.59
12	Hexanoic acid	24.52	$C_6H_{12}O_2$	116.16	2.22
13	1-Methoxy-3-hydroxy methyl octane	24.81	$C_{10}H_{22}O_2$	174.281	2.13

a: Retention Time, b: Molecular Formula, c: Molecular Weight

CONCLUSION

According to the results, the previous reports on the medicinal properties of the examined plants were confirmed by identification of compounds in the extract of these plants. The synthetic pathways of many secondary metabolites and associated genes in medicinal plants have not yet been completely identified. The amount of a particular secondary metabolite can be increased or decreased by identifying these synthetic pathways and genetic engineering of them. In these medicinal plants, there are valuable secondary metabolites such as alkaloids, flavonoids, diterpenes, unsaturated fatty acids, vitamin B and phenolic compounds which can be used in Pharmaceutical and Cosmetics industries.

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