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Identification of terpens in methanol extracts of *Heracleum Persicum*

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ABSTRACT

Today, due to the increasing importance of plants as a natural resource containing different active ingredients, many people use herbal medicines and its products. Because, it can reduce the adverse effects of the drug as an alternative to chemical drugs. The aim of this study was to compare the levels of Terpenoids in the fractions of hydromethanolic , petroleum ether and chloroform in Heracleum persicum fruits. For Qualitative detection The fractions used to method of the thin layer chromatography (TLC) when a solvent phase were Benzene and Ethyl acetate (1:1). Fractions were also analyzed to measure the amount of terpenoides. The major constituents of the Terpenoids in study showed Decane 100%, Cyclodecane 61.23%, Dodecane 56.78%, Butanoicacid 50.37% , Cyclohexasiloxan 40.14%, Phenonemethoxynaphthoquinone 34.39%, 1,4-Naphthoquinone 19.87%, 2-methylbutanoate 17.6% , N-Aethylpiperidin 17.59%.

Key words: Heracleum Persicum, Terpenoids ,Thin Layer Chromatography (TLC), Gas Chromatography - Mass Spectrometer (GC-Mass).

INTRODUCTION

Today more than million species of herbs are used as an alternative to chemical drugs. Medicinal plants in the world can provid a revolution in treatment of medical activities, Because it can reduce the side effects of the drug as an alternative to chemical drugs considered [1].

Medicinal plants are resources that developed countries have to attention to them. This plants are used as raw materials for drug production through the extraction of active ingredients. In this sense Iran is one of the richest sources of plant diversity of its habitats [2, 3].

Phytochemical Products (phyto means plant in the Greek language) are active biological materials. The natural chemical compounds found in plants due to macronutrients trace elements are very useful for human health benefits. These compounds representing the flavor and color of the plants against environmental hazards such as air pollution, stress, drought and exposure to disease-causing attacks UV protection. To date, more than 4,000 kinds of phytochemicals and recognition on 150 especially it was carried out [4].

These compounds, depending on their role in plant divided into two categories: primary and secondary. Primary compounds, including sugars, amino acids, proteins, nucleic acids, purine and pyrimidine of chlorophyll and, as a stream of defense for plant secondary compounds that are considered include chemicals like alkaloids, terpenes, flavonoids and saponins and phenols [4, 5, 6].

Heracleum Persicum or Iranian Angelica belong to Apiaceae and one of the ten genus of this family in Iran. There are two types of Heracleum persicum [7].

Heracleum persicum has a warm nature and chemical composition of including acetate Hecilic, astilic acetate, ethyl butyrate and ethyl butyrate and some other acids, fats, minerals and vitamins. Other components found in the essential oil of Angelica include aliphatic esters such as butyl ethylhexyl, octyl acetate, and monoterpenes including limonene and Anatole [8, 9, 10].

Other members of the Apiaceae family, including plants, carrots, parsley, celery, coriander, fennel and caraway are. Which are rich in essential oils [11].

Heracleum persicum fruit is commonly used in the country as a seasoning for preparation of pickles. In traditional medicine, Heracleum persicum as increasing stomach secretions, appetite, milk and sweat, carminative, strong germicidal, anti-toxins and to improve the sores around the lips and used epilepsy. also should not have a positive effect on the circulatory system can not be overlooked [2, 8, 9, 12].

The terpenoids are natural products that have been extracted from five-carbon isoprene units. Most of the terpenoids have multi cyclic structures that differ from each other by basic carbon skeletons. These types of natural lipids can be found in every class of living beings, and resulting in regarded as the biggest group of natural products [13, 14].
Terpenes are Spread in nature. Their main building is isoprene (CH2=C(CH3)-CH=CH2). Terpene hydrocarbons thus have molecular formula (C5H8) n and they are categorized conforming by the number of isoprene units [15].

Most of terpenoids are commercially interesting due to of their use as savor and odor in foods and cosmetics examples menthol and sclareol or because they are important for the quality of the Crops, such as the flavour of fruitage and the potpourri like linalool [16].

Activity of Terpenes: Among plant secondary metabolites terpenoids are a structurally most differing group; they function as indirect defense responses their Invaders the nature [17].
Order of terpenes to attract insects for pollination. terpenes are fugacious and bitter. toxic terpenes also defence some plants from Frittered by animals [18].

terpenes Are important as signal compounds and growth regulators (phytohormones) of plants. Also, terpenoids can have Pharmacological properties such as anti-carcinogenic (e.g. perilla alcohol), anti-ulcer, antimalarial (e.g. artemisinin), hepaticidal antimicrobial or diuretic (e.g. sesquiterpenoid) antimalarial drug artimisinin and the diterpenoid anticancer drug taxol. [15, 19, 20].

MATERIALS AND METHODS

2.1. Preparation of plant samples:

Heracleum Persicum fruit be prepared with registered number of 091,073,001 herbarium.

2.2. Method :

2.2.1. method of extraction of methanol:

For the extraction of methanol 99.99%, weight 50 grams of the fruit, then 250 ml of methanol was added to the powder plant, then in the dark and shaking was continued for 48 hours on a shaker.

2.2.2. method of preparation of water-methanol and petroleum ether fractions:

After removal of solvent solved in 100 ml of water and methanol at a ratio of (1 : 3) to total extract. Then add 200 ml of petroleum ether into the funnel. To remove the solvent, both phase Were transferred to rotary device. Petroleum ether phase was yellow and hydro-methanol was dark green.

2.2.3. Method of preparation of hydro-methanol and chloroform fractions:

After removal of solvent solved in 100 ml of water and methanol at a ratio of (1 : 3) to total extract. Then add 200 ml of Chloroform into the funnel. To remove the solvent, both phase Were transferred to rotary device. Chloroform phase was dark green and hydro-methanol was Brown.

2.2.4. Qualitative detection terpenoids thin layer chromatography (TLC):

The paper TLC (thin layer of 250 micrometers, 2.25 micrometers average particle size, pore diameter 60 Angstrom silica gel coating on polyester 254 nm) with dimensions of 6.5 by 3.2 cm was cut. The dropper to a ratio of 1: 1 ethyl acetate and benzene were added to the chromatography tank. Each of the fractions were loaded with a distance of 4

mm from the end of the paper. Then blow-dry with a paper and placed inside the tank. After a period when the samples reached their peak TLC paper out and 10% sulfuric acid was sprayed on paper, the resulting color change as a result of using a ruler and reported in cm [21].

2.2.5. Gas Chromatography-Mass Spectametry (GC-MS) Analysi:
The chemical composition of the fruit extract was analyzed using GC and GC-MS. The GC/MS analysis was carried out with an 20 Agilent 5975 GC-MSD system in research laboratory of Islamic Azad University, Khorasgan Branch, Isfahan, Iran. HP-5MS column (30m × 0.25mm. 0.25mm film thickness) 20 used by helium as carrier gas (1.2mL/min). GC oven temperature was kept 20 at 50 C2 B0C for 3 min and programmed to 280 C2 B0C at a rate of 5 C2 B0C/min, and kept 20 constant at 290 C2 B0C for 3 min, at spilitless mode. The injector temperature was at 20 280 C2 B0C. Transfer 20 line temperature 280 C2 B0C. MS were taken at 70 20 eV. Mass ranger was from m/z 35 to 450. Head space GC-MS was used in this study. This method can use plant dry matter for chemical analysis (Figure 2 and 3).

RESULTS AND DISCUSSION

Results of TLC for Terpenoids
Quality test results of fractions of petroleum ether and hydro-methanol of the first stage and second stage hydro-methanol -chloroform fractions by thin layer chromatography (TLC). According to the results of tests the following formula is as follows:

$$Rf=\frac{\text{Distance travel by solute}}{\text{Distance travel by solvent}}$$

TLC of the fruit extract of Heracleum Persicum revealed the presence compounds terpenoid when a solvent phase of Benzene: Ethyl acetate (1:1) was used The first stage hydro-methanol fraction Rf value has 36/0 (Figure1, Column 1), hydro-methanol fraction of a second stage Rf value has zero (Figure1, Column 2), fractions of petroleum ether Rf value has 40/0 (Figure1, Column 3), and chloroform fractions Rf value has zero (Figure1, Column 4). If Rf presence should 36/0 and 40/0 is the terpenoids [21].

GC-MS analysis:
Iran has specific conditions of geography, climate and vegetation varied very interesting is the critical [9].
Heracleum genus has many species that different researchers have been working on a variety of its forms. The composition of the index family umbrella that is abundant in a variety of Heracleum genus furanocoumarin be found [22].

Heracleum persicum has a hot nature and chemical substances such as acetate Hecilic, acetate astilic, ethyl butyrate, ethyl butyrate, and some other acids, fats, minerals and vitamins are. Heracleum persicum essential oil also contains elements such as ethylhexyl butyrate (56.5%), octyl acetate (16.5%), ethylHexylisobutyrate (3.4%) and ethylHexyl-2-methylbutanoate (5.2%) is. Heracleum persicum can extract hydro plant on Pentylenetetrazole induced seizures in Suri mice is useful [8, 23].

Heracleum persicum alcoholic extract contains several furanocoumarin is like sphondin inhibitor of IL-1 and Beta cyclooxygenase 2. The compounds in this plant alkaloids, terpenoids, including cineol and linalool, Triterpenoids aliphatic esters (95%), aliphatic alcohol (4%), monoterpenes (1%), 37 of polyester and 17 have been identified monoterpenes [6].

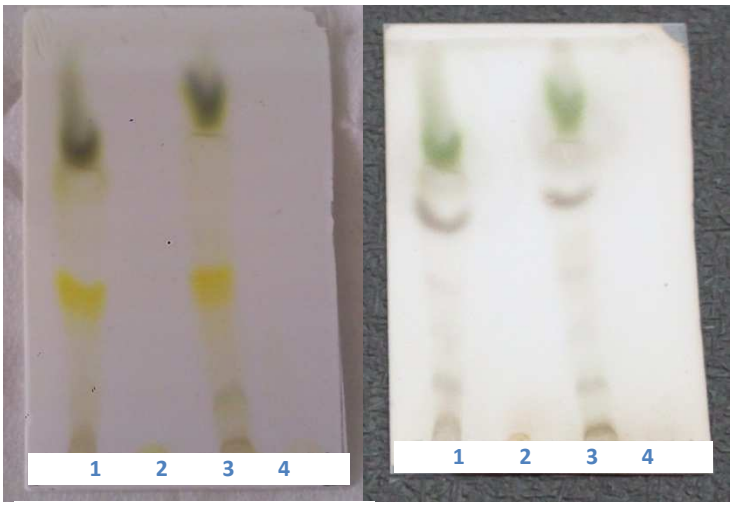
The results of GC-Mass Heracleum persicum thymol (63.4%), Carveol (61%) and trans-anethole (39%) have been extracted [24].

There are several chemical compounds in the herb Heracleum persicum, including Pimpinellin, isopimpinellin, bergapten, isobergapten and sphondin are furanocoumarins that the roots of this plant have been found. There are 6 types furanocoumarin research and a variety of flavonoids in the fruit Heracleum persicum show. The research on essential oils of fruit, leaves and roots of this plant compounds such as ester aliphatic (95%), alcohol Aliphatic (4%) and monoterpenes (1%) is proof. Compound leaves and roots are the main trans-anethole [25].

About 75% of the chemical compounds in Heracleum persicum seeds of which have been identified, 12 of which remain unknown. The main components of these compounds include ethylhexyl butanoate (37.7%), ethylhexyl butanoate (36.7%), octyl acetate (16.3%), ethylHexyl-2-methylbutanoate (5.7%), ethylhexyl Iso Butyrate (4.7%), Hptyl-2-methyl butyrate (2.3%), n- Butylbutanoate (2.25%), ethylhexylvalerate (1.9%), Octylbutanoate (1.7%) and Linalol [8].

In addition to the basic compounds mentioned above specifically on terpene compounds work, which confirms results from other researchers. In this study, more than a hundred combinations of hydro-methanol 1 and petroleum ether fractions were identified. According to the results of GC-Mass spectrum of the methanol fraction can be used in this isolated fractions of terpenes of high molecular weight and Light, but terpene fractions are not found.

The chemical composition of the fraction of hydro-methanol1 from the fruit of Heracleum Persicum, analyzed by GC-MS are shown in Table 1 The relative percentage of the individual components was calculated based on GC peak area. The major constituents of the Terpenoids in our study showed compounds of the fraction of hydro-methanol1 were Decane 100%, Cyclodecane 61.23%, Dodecane 56.78%, Butanoicacid 50.37% (Table 1) and Cyclohexasiloxan 40.14%, Phenonemethoxynaphthoquinone 34.39%, 1,4-Naphthoquinone 19.87%, 2-methylbutanoate 17.6% , N-Aethylpiperidin 17.59% were the most of compounds on petroleum ether fraction (Table 2).



A **B**

Figure1: A: Terpenoids detected by thin layer chromatography (TLC), hydro-methanol fractions of petroleum ether chloroform fractions of the first and second phase before the spray reagent hydro-methanol, B: terpenoids detected by thin layer chromatography (TLC), the first phase of hydro-methanol fractions of petroleum ether and chloroform fractions of a second stage after hydro-methanol spray reagent

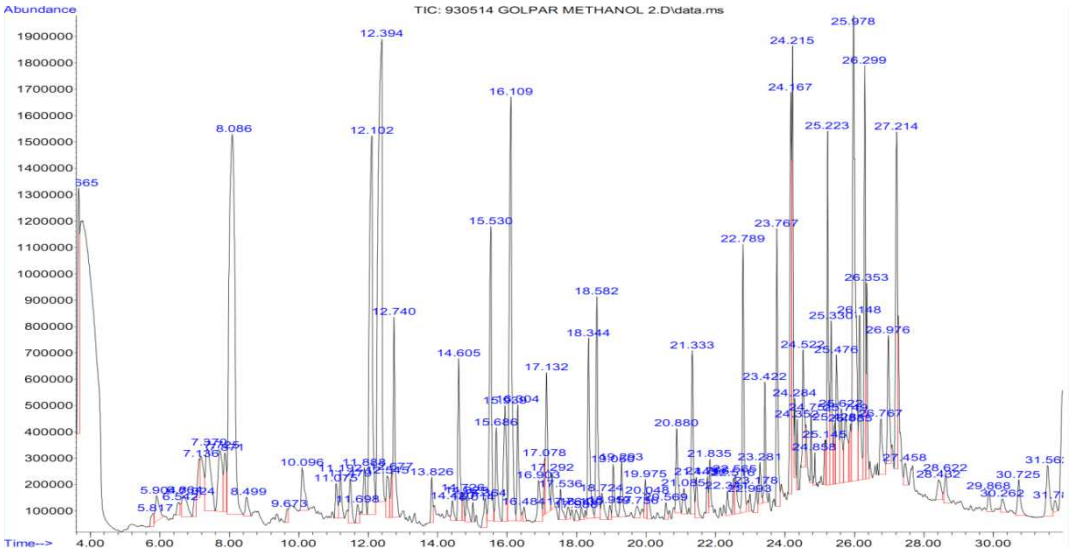


Figure 2: spectrum terpenoids to gas chromatography - mass spectrometry of The fraction of hydro-methanol1

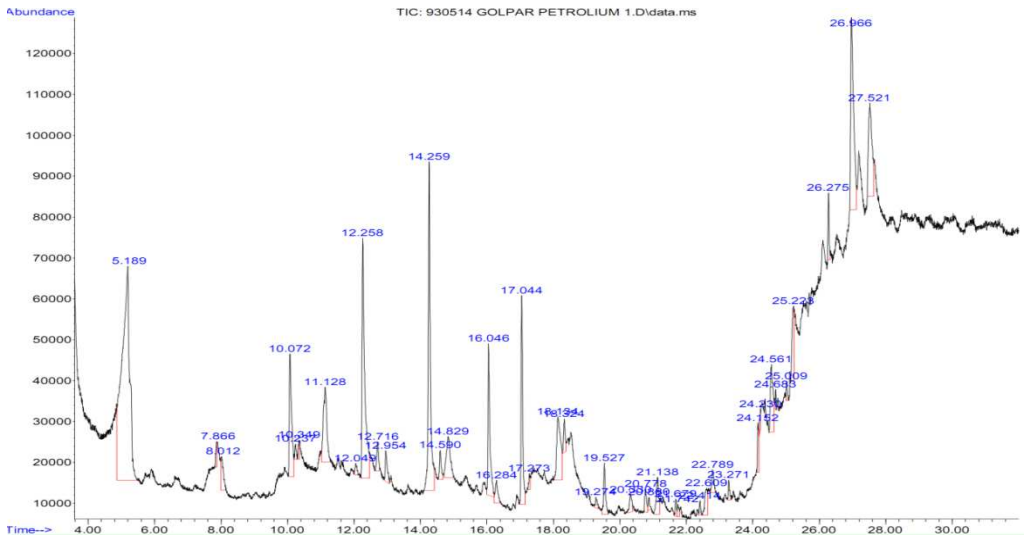


Figure 3: spectrum terpenoids to gas chromatography - mass spectrometry of The fraction of petroleum ether fraction

Table 1: The chemical composition of the fraction of hydro-methanol1 from the fruit of Heracleum Persicum

	chemical composition	Retention time R.T(min)	Content %
1	Octane	3.665	22.21%
2	Nonane	5.817	1.67%
3	Decane	8.086	100.00%
4	Dodecane	12.102	56.78%
5	Acetic acid	12.394	86.78%
6	hexyl 2-methylbutanoate	12.740	17.26%
7	Propanoic acid	14.605	14.73%
8	Tetradecane	15.540	29.42%
9	Butanoic acid	16.109	50.37%
10	Phenylacetylene	17.132	13.71%
11	Butyric acid	18.130	17.17%
12	Hexadecane	18.344	19.83%
13	1-Heptadecene	20.569	8.08%
14	Octadecane	21.085	13.52%
15	1-Octadecene	21.333	4.07%
16	Hexadecanoic acid	22.565	20.24%
17	1,2,4-Metheno-1H-cyclobuta[b]cyclopenta[d]furan	23.422	19.69%
18	10,13-Octadecadienoic acid	23.767	29.96%
19	9-Octadecadienoic acid	24.167	21.81%
20	Pimpinellin	24.352	9.46%
21	Hexadecanoic acid	25.145	21.98%
22	Eicosanoic acid	25.223	12.85%
23	Cyclododecyne	25.885	61.23%
24	1,2-Benzenedicarboxylic acid	26.148	26.02%
25	9,12-Octadecadienal	27.214	29.67%

Table (2): The chemical composition of The fraction of petroleum ether fraction from the fruit of Heracleum Persicum

	chemical composition	Retention time R.T(min)	Content %
1	2-Methyl-2-pentyl methylphosphonof	7.866	1.63%
2	N-Aethylpiperidin	10.072	17.59%
3	Cyclopentimine	11.128	15.75%
4	Acetic acid	12.258	37.87%
5	Cyclohexasiloxan	14.259	40.14%
6	2-methylbutanoate	16.46	17.60%
7	Pentasiloxan	17.044	20.20%
8	Cyclododecasiloxane	19.527	5.85%
9	Cypentil	22.609	4.11%
10	Pimpinellin	24.564	10.39%
11	7-cyclohexyl-2,3-dihydro-2-methyi-cis-8-(N-pyrrolidyl) -(2,2,5,5-tetradeutero)bicyclo[4.3.0]nona-3	24.683	1.02%
12	Cyclobutanone	25.009	1.19%
13	Isopimpinellin	25.223	8.42%
14	Octasiloxane	26.275	4.16%
15	phenonemethoxynaphthoquinone	26.966	34.39%
16	1,4-Naphthoquinone	27.521	19.87%

CONCLUSION

The highest detection rate of terpene compounds were fraction of hydro-methanol 1. In this experiment, methanol is a protic polar, chloroform is a nonprotic polar and petroleum ether is non-polar solvent. Polar solvents can to organize hydrogen bonds. In this way it could extracted compounds better than other solvents. The presence of water in reaction environment increases probability and to formation of these bonds. In fact, herbal drugs exert, their effects through phenolic agents, flavonoids which act as the best anti-inflammatory substances. The anti-inflammatory and antioxidant efficacy of flavonoids and other herbal compounds have been extensively investigated and their ameliorative properties have been shown in various animal models and human studies [26-37].

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