Analysis of the essential oil components from different *Carum copticum* L. samples from Iran

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Submitted: 21-02-2013 Revised: 03-05-2013 Published: 12-12-2013

ABSTRACT

Background: The family Apiaceae is defined with the diversity of essential oil. Fruits of Ajwain (*Carum copticum*), a famous herb of Apiaceae, accumulate up to 5% essential oil which is remarked as important natural product for food and flavoring industry, as well as pharmacological approaches. It is believed that differences in essential oil profile in a certain plant are resulted from various cultivation situations and locations, time of cultivation and also different extracting method. **Objective:** Present study aimed to evaluate major components of ten different collected Ajwain samples from random cultivation locations of Iran. **Materials and Methods:** Samples were individually subjected to hydrodistillation using a Clevenger-type apparatus for the extraction of essential oil. GC/MS analysis for samples was carried out using Agilent technologies model 7890A gas chromatograph with a mass detector. **Results:** The yield of extracted essential oil was calculated as 2.2 to 4.8% (v/w) for ten samples. Major oil components were thymol, para-cymene and gamma-terpinene. Five of ten samples have thymol as the main component with amount of 35.04 to 63.31%. On the other hand, for four samples, para-cymene was major with amount of 40.20 to 57.31% and one sample had gamma-terpinene as main constituent containing 37.43% of total oil. Accordingly, three different chemotypes, thymol, para-cymene and gamma-terpinene can be speculated from collected samples. **Conclusion:** While these components possess pharmacological effect, screening of different chemotypes not only represent the effect of cultivation situations and locations but also can be beneficial in further investigation.

Key words: Ajwain, *Carum copticum* L, GC analysis, essential oil

Access this article online
Website: www.phcogres.com
DOI: 10.4103/0974-8490.122920
Quick Response Code:

INTRODUCTION

Essential oil is one of the most active and effective components of medicinal plants which can be extracted from different parts. These compounds that are generally oil soluble are derived from main parts such as leaves, seeds and bark.[1] Usual methods for the separation of essential oils are water and steam distillation.[2]

The family Apiaceae is remarked as a family with the diversity of essential oils.[3] One of the famous herbs of this family is *Carum copticum* L. which is commonly known as Ajowan.[4] Ajowan is an annual herbaceous plant and is widely distributed and cultivated in various regions such as Iran, Egypt, Pakistan, Afghanistan, India and Europe.[5] The herb fruits accumulate up to 5% essential oil in its compartments and are defined as important commercial products for food and flavoring industry.[6]

Beside these industrial purposes, Ajowan has a number of pharmacological properties and biological actions which can be considered for clinical approaches. Seeds of the Ajowan revealed to have anti-inflammatory,[7] analgesic,[8] antibacterial, antihelmintic, antihypertensive, antiflatulent, antispasmodic, bronchodilator, hepatoprotective,[8,9] antiplatelet,[10] insecticidal and spermicidal activities.[11] Moreover, it has been remarked in Traditional Persian Medicine that *C. copticum* has therapeutic effects on diarrhea, dyspepsia, colic and digestion[1,12] and also can relief rheumatic and neuralgic pain.[9] Furthermore, the herb exhibited the antilithiatic activity in animal models.[13]

The total amount of extracted essential oil of the herb has been reported differently by the investigators. Studies have

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remarked the percentage of volatile oil from 0.5% (v/w) up to 9% (v/w).[14,15] Besides the amount, the total constituents of *C. copticum* are also reported different. Although the maximum constituents in an investigation are remarked as 40 components, most reports have mentioned the total of 9-17 chemical compositions in different collected samples.[16,17]

On the other hand the major constituents of Ajowan and their percentage in evaluated samples are reported different. While thymol, gamma terpinene and para cymene are reported as the main compositions, other investigation remarked para cymene and carvacrol or even the four mentioned components are reported by the investigators.[14,17,18] It is believed that these differences in essential oil profiles are resulted from different cultivation situations and locations, different time of cultivation and also different extracting method.[14,16,19,20]

In this regard, present study was done to evaluate the major components of ten different samples of *C. copticum* using GC/MS method of analysis.

MATERIALS AND METHODS

Dried seeds of *Carum copticum* were procured from medicinal plants markets of Iran having the information of the cultivation location [Figure 1]. In order to rule out any bias concerning the variability, we had collected three samples from each location. The collected seeds were rendered free from soil and impurities manually. Plant seeds were then authenticated by S. Khademian, the botanist of the Department of Traditional Pharmacy of Shiraz Faculty of Pharmacy. While the identification completed, each sample was deposited in the Shiraz School of Pharmacy herbarium with individual voucher number.

Figure 1: Cultivation locations of collected Ajwain samples in Iran

The seeds were grounded into coarse powder (30 g) by electrically grinding mill and were then soaked in fresh distillated water (300 ml). The mixture was then subjected to hydrodistillation for 4 h using a Clevenger-type apparatus for the extraction of the essential oil.[21] This procedure was done in an appropriate time of day for each sample. The resulting oil was dried over anhydrous sodium sulphate and kept at 4°C.

Initially, GC/FID analysis of the oils was performed in order to achieve to a desirable analytical condition. Therefore, GC/FID analysis was carried out on a gas chromatograph Agilent technologies model 7890A apparatus attached to HP-5 column (25 m, 0.32 mm, 0.52 μm film thickness) and connected to flame ionization detector (FID). Nitrogen was selected as the carrier gas with a flow rate of 1 ml/min, split ratio was 1:30. The injector temperature was 250°C, and detector temperature was 280°C, while column temperature was linearly programmed from 60 to 250°C (at rate of 5°/min) and then held for 10 min at 250°C. Solutions of essential oil samples in dichloromethane (~1%) were consecutively injected.

The same analytical method and condition as those mentioned for GC/FID were applied for GC/MS analysis. The process was carried out by using Agilent technologies model 7890A gas chromatograph connected to a mass detector (Agilent technologies model 5975C). The gas chromatograph was equipped with a HP-5MS capillary column (phenyl methyl siloxane, 30 m × 0.25 mm i.d., Agilent technologies). Helium was selected as the carrier gas with the same flow rate as for GC/FID. The mass spectrometer was acquired in EI mode (70 eV) in a mass range of 30-600 m/z. The interface temperature was 280°C. Identification of components was based on a comparison of their mass spectra with Willey (nl7) and Adams libraries spectra, as well as with those reported in literatures.[22]

RESULTS

We have carried out an investigation on the essential oil chemical composition of ten samples of dried seeds of Ajwain. As each location had three collected seeds, results for each sample is the mean of three collections of each location. So for all ten samples of the herb seeds, mean yield of extracted essential oil for each triplicate samples was obtained from 2.2 to 4.8% (v/w). The oil content for each sample is the mean of three times of extraction. Table 1 represents places of purchasing and cultivation locations of Ajwain seeds along with the yield of the essential oil obtained for each sample. On the other hand, the constituents of essential oil are reported in Table 2.

Table 1: Average values of essential oil content in *Carum copticum* samples

Sample no	Medicinal plant store	Cultivation location	Coordinates	Oil content (%)
1(S_1)	*Torbat-e heydaryeh*	*Torbat-e heydaryeh*	35°16′N; 59°13′E	2.3
2(S_2)	*Tehran*	*Tehran*	35°41′N; 51°25′E	2.2
3(S_3)	*Darab*	*Darab*	28°45′N; 54°32′E	2.8
4(S_4)	*Mashhad*	*Mashhad*	36°18′N; 59°36′E	2.2
5(S_5)	*Kerman*	*Kerman*	30°17′N; 57°50′E	3.3
6(S_6)	*Shiraz1*	*Shiraz* (Northwest)	29°37′N; 52°32′E	4.5
7(S_7)	*Zahedan*	*Zahedan*	29°29′N; 60°51′E	3.2
8(S_8)	*Meymand*	*Meymand*	28°52′N; 52°45′E	4.0
9(S_9)	*Bandar-abbas*	*Bandar-abbas*	27°11′N; 56°16′E	3.1
10(S_{10})	*Shiraz 2*	*Shiraz* (South)	30°17′N; 57°50′E	4.8

Table 2: Chemical composition of volatile oils obtained from Ajwain samples

Component	%S1	%S2	%S3	%S4	%S5	%S6	%S7	%S8	%S9	%S10	KI (DB5)	Identification
Thujene	1.51	-	0.96	1.29	0.89	0.51	0.88	0.56	0.27	0.57	928	MS, KI
α-pinene	0.82	0.10	1.31	0.61	0.90	0.47	-	-	0.26	0.27	936	MS, KI
β-pinene	3.70	3.04	3.64	3.46	3.40	1.50	2.58	0.99	1.23	1.80	980	MS, KI
Myrcene	1.31	0.80	0.96	1.32	0.92	0.66	0.85	0.94	0.40	0.63	992	MS, KI
α-terpinene	0.70	-	0.88	0.69	-	-	0.80	0.64	0.33	0.55	1018	MS, KI
ρ-cymene	31.80	57.31	43.30	37.30	40.20	22.38	20.73	34.64	19.15	19.67	1029	MS, KI
Limonene	-	-	-	-	-	-	1.32	-	-	-	1033	MS, KI
β-phellandrene	-	-	-	0.49		-	-	0.65	0.48	0.62	1035	MS, KI
γ-terpinene	23.11	35.12	28.04	32.54	21.31	16.98	21.71	37.43	12.93	19.21	1056	MS, KI
Terpinene-4-ol	0.86	0.37	-	-	-	-	-	0.53	-	-	1180	MS, KI
Carvone	-	0.23	-	-	-	-	0.91	-	-	-	1242	MS, KI
Thymol	35.04	1.50	19.58	21.29	30.40	53.66	49.16	20.62	63.31	55.21	1286	MS, KI
Carvacrol	-	-	-	-	-	0.65	-	1.01	-	0.43	1302	MS, KI
Identification (%)	98.85	99.87	98.67	98.99	98.02	96.81	98.94	98.01	98.38	98.96		
MH1 (%)	62.95	97.37	79.09	77.70	67.62	42.50	48.87	75.85	35.07	43.32		
OCM2 (%)	35.90	1.50	19.58	21.29	30.40	54.31	50.07	22.16	63.31	55.64		

The compounds have been sorted according to retention indices on HP-5 MS capillary column. MH1: Monoterpene hydrocarbons; OCM2: Oxygen containing monoterpene

For all ten samples, main components of total essential oil were determined as thymol, para-cymene and gamma-terpinene. These components were different in ranking amount. According to Table 2, five of ten samples represented thymol as the main component with the amount of 35.04 to 63.31%. On the other hand, in four other samples, para-cymene was the main component with the amount of 40.20 to 57.31% and only one sample contained gamma-terpinene as the main ingredient (37.43%). The variation of thymol, para-cymene and gamma-terpinene contents in the investigated oil samples are shown in Figure 2. In this regard, three different chemotypes namely thymol, para-cymene as well as gamma-terpinene can be speculated from collected samples. Previous studied revealed that *C. copticum* has two chemotypes as thymol and carvacrol[12] while none of these collected samples in the current study had carvacrol even as the fourth major component. In contrast, Beta-pinene represents as fourth major component at least in nine of total sample.

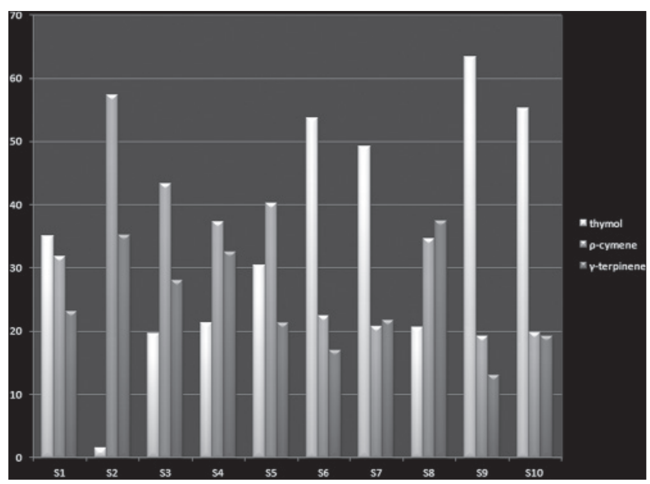

Figure 2: Variation of thymol, para-cymene and gamma-terpinene contents in the investigated oil samples

DISCUSSION/CONCLUSION

The essential oil analysis of selected samples revealed that thymol, para-cymene as well as gamma-terpinene are main essential oil constituents in all samples. The maximum calculated amount of thymol in current study was up to 63%. While it was reported as 73% in a sample with collection area near to our first sample, *Torbat-e-heydaryeh*.[23]

It is believed that many parameters involving intrinsic or genetic as well as extrinsic or environmental may affect on the yield of oil content and oil constituents. Picking or harvesting time,[24,25] distillation method or procedure time,[24] as well as cultivation location and harvesting conditions are often spoken as effective parameters. These conditions affects on the amount of main constituents of herbal essential oils. While for these main components, as the formation of thymol and para-cymene are done by the bioconversion of gamma-terpinene,[26] the low amount of thymol in sample 2 may be in that of early harvesting. In a study, different times of harvesting and subsequent drying period have had significant influences on type and amount of major components.[27] This may result in different chemotypes. It is also remarked that essential oil content of aromatic plants may decreases according to the storage time.[28] According to Table 1, samples 1, 2 and 4 contain lowest content of essential oil. This may be due to poor storage conditions. Furthermore, mentioned samples are collected from herbs which were cultivated in nearly north parts of Iran. Others with higher amount of essential oil were collected from southern areas with higher temperature. This may be due to the cultivation location.

It should be noted that these main components, thymol, para-cymene and gamma-terpinene, exhibit different pharmacological and nutritional properties, individually. Among these major components, thymol is well known in commercial and pharmacological approaches. Many investigations have explored the antimicrobial and antioxidant properties of this component.[29,30] Thymol also possesses anti-inflammatory,[31] analgesic and local anesthetic[32] effects. Moreover, it is reported that thymol can be applied for the inhibition of food borne pathogens.[33] In addition to thymol, other major constituents of Ajwain, para-cymene and gamma-terpinene, have shown beneficial effects in clinical and nutritional approaches similar to those of thymol. Although, less certain evaluation has been done on these components, properties such as anti-inflammatory and analgesic,[34] antioxidant,[35] antimicrobial[36,37] and inhibition of LDL oxidation[38] are considerable.

Many factors, as chemical and physical are responsible for the alteration on essential oils chemical components and change the types and amounts of major constituents. Regarding to the clinical properties of Ajwain major compositions as well as cultivation location, harvesting time and storage condition, desirable chemotypes can be screened among various samples. Having a specific chemotype with the high amount of major component may be beneficial for investigational and functional approaches in food and pharmaceutical industries.

ACKNOWLEDGEMENT

This paper is derived from Mohammad M. Zarshenas thesis for obtaining PhD in Traditional Pharmacy. Authors wish to express their thanks from Shiraz University of Medical Sciences for providing the grant of this project (Project Number: 5802 and 5494).

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Cite this article as: Zarshenas MM, Samani SM, Petramfar P, Moein M, Analysis of the essential oil components from different *Carum copticum* L. samples from Iran. Phcog Res 2014;6:62-66.

Source of Support: Shiraz University of Medical Sciences, **Conflict of Interest:** None declared.