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STUDY OF CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *FERULAGO ANGULATA* (SCHELCHT) BOISS. FROM IRAN USING SUPERCRITICAL FLUID EXTRACTION AND NANO SCALE INJECTION

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The essence of aerial parts of *Ferulago Angulata* was extracted through supercritical fluids technology (CO2). The essential oil was analyzed by GC and GC/MS via nano scale injection method. The total extract yield was 0.8 percent. The 37 components of the essential oil have been identified. The 14 main components are included 83.3% of the total essence. This is the first report representing the extract of *Ferulago Angulata*. Essence extracted through supercritical fluid method by carbon dioxide and the ultimate amount of its yield in this way is more than the amount extracted through hydrodistillation and solvent extraction. Major components were Suberosin (12.36%), Spathulenol (10.9%), Trans β-Caryophyllene (7.32%), Arcurcumene (7.07%), and Bicyclogermacrene (6.96%).

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1. Introduction

In botany the umbllifererae (apiaceae) family is widespread and includes 300 genus and 3000 aromatic herbaceous species. Although the species related to this family are spreaded throughout the word, its centrazation is in alpine and temperate zones of ancient word. Petroselinum, anethugraveal, foeniculum vulgare, conrum carui, pimpinella anism and etc. are some species of this large family [1] Ferulago is a significant species of apiaceae family. There are 35 to 40 species of this plant in the word and in Iran 7 to 8 species grow naturally, originally and wild. *Ferulago angulata* is a natural and native plant in western parts of Iran [2,3]. Except for Iran, it grows in other countries including: Turkey, Greece, Serbia and Macedonia. Some studies have been also performed in those countries [4,5]. In different parts of Turkey, different species of this plant have been used as sedutive, tonic, remedy of digestive pains, aphrodisiac properties, hemorrhoids and etc [6-10]. Since long time ago any way, Ferulago species are good for ulcers, snake bits, spleen and headache [11]. In those regions of Iran where *FerulagoAngulata* grows, it is traditionally common to add this plant to diary and oil ghee in order to give a pleasant taste to them as well as preventing from their decay. There have been some special reports proving its properties as being anti oxidation and having anti diabetic effect. Besides extracted essence is useful for cosmetic industries and perfumery, furthermore, this plant contains a strong preservative substance for diary and it is effective for digestive system diseases [12]. Major chemical compounds of some species of Ferulago have been reported in table (1).

Table 1: Major constituents of ferulago Essential oil

Ferulago species	Major components	no.
f. aucheri &mughlae&sandrasica&phialo carpa	α-pinene	1
f. humillis&trachycarpa	(z)-β-ocimene	2
f. trachycarpa	gama-terpinene	3
f. maeroseiadia	Methyl carvacrol	4
f. sylvatica&idaea	p-cymene	5
f. confusa	2,5dimethoxy-p-cymene	6
f .thirkeana	ferulagone	7
f. thyrsiflora	ent-3β-hydroxy-13-epi-manoyl oxide	8
f. asparagifolia	2,3,6-trimethyl benzaldehyde	9
f. silaifolia&galbani fera	trans-chrysanthenyl acetate	10

The extraction of *Ferulago Angulata* essence has already been done through hydro distillation and extraction by solvent, its major chemical compounds have been identified and their percentages are different [13-17]. Essence extraction of Ferulago Angulata has been done by supercritical method for the first time and the total yield of the process has been more than the old ones. The old methods of essence extraction such as distillation have been based on temperature increase which is not suitable for volatile components and sensitive to temperature. On the other hand, considering solvent removal after extraction and essence contamination, cost, sometimes poisonous solvents, the solvent extraction method is not comparable with supercritical method in which quality and even quantity of the essence is higher. The aim of this research is extraction of *Ferulago Angulata* by supercritical extraction method through carbon dioxide that has been performed by the first times and then comparison of the results with the other previous methods.

2. Materials and methods

The samples of components *Ferulago Angulat*, as its aerial parts, were collected in high 2500 to 3000 meter altitude of Dena mountain, located in the south Zagross, before flowering time in may, 15th, 2009. The samples were dried under the shadow in a cool room with low humidity and were crushed in appropriate size. The drying period took nearly 2 weeks with more than 85% humidity. The eatable ready samples were stored in suitable containers still the time of supercritical extraction.

2-1 Apparatus

In order to obtain experimental data, there has been used the pilot laboratory of supercritical extraction which its specifications has been presented in the reference [18], containing multipurpose system with different supercritical extraction applications. The machine had a special extractor container for herbal essence with dimensions of (r=8.8mm), (h=56cm) and volume is 35 mL. Carbon dioxide with 99.9% purity was used in the mobile stick tank with pressure of at most 80 bar as supercritical fluid. The machine is capable of enduring pressure to 400bar. Besides since there are cooling and heating systems, extraction containers and essence receiver, supercritical essence extraction tests can be done in different and controlled pressure and temperature. The capacity of the

extractor is about 15 to 20 g of crushed (powder) feed.

2-2. Experimental procedure

The temperature control system of the pilot SFE extraction container is set with a low temperature (of about 35oC) 1 hour before starting. By circulation water in the wall of extraction container (shell) and controlling its temperature, the temperature of the test can be adjusted. The leakage test is performed before anything else. The powdered material is placed in extraction container after being weighed and the extraction pressure is adjusted on 190 bar and maintained in fixed temperature and pressure for one hour as static time.

Static time for maximum solubility of essence in supercritical fluid and is determinable incomplementary tests. Then the extractor output and production of essence is commenced after gradually opening of needle valve. The supercritical fluid along with the solved essence goes through the pressure reducing valve in the receiver container, the essence is sediment and carbon dioxide is released as gas. In order to measurement of the flow and fluid on carbon dioxide rate, we put wet test meter and bubble flow meter respectively after receiver and CO2 is then released. Since plant essences are sensitive to temperature, the temperature of the extractor container is set low and for determining the appropriate range of extraction pressure, through several error and trial test and repeating them. Range of 190 to 200 bar has been determined. To collect the extracted essence, due to its little amount, there has been used about 1mL of appropriate solvent such as *n*-pentane or *n*-hexane and ethanol or pure acetone in the receiver container for the sediment essence to be solved. Then it is stored in special bottle (dark and without leakage) in nitrogen atmosphere and kept there till the time of analysis in a cool place and far from sunlight, some where like refrigerator, hereby it should be reminded that in supercritical extraction test using acetone as solvent, the pressure was100 bar but with the same temperature as the former test. Using two solvent is to identify more components of the essence.

2-3. SFE total yield

SFE total yield of essence is expressed as percentage of fraction. At the end of the test, through dividing the extracted essences weight by the consumed powder, the total yield is measured. In this research, the total yield is about 0.8% which is considerable and more than the extraction yield gained in other methods as hydrodistillation and solvent extraction.

2-4. Analysis method

To analysis the components of extracted essence of *Ferulago Angulata*, a gas chromatography with the model of GC3600 (FID) and coupled gas chromatograph by mass spectrometry GC/MS (HP-5973) equipped with a column, 30m length, internal diameter of 0.25 mm and thin layer of 0.25 micro meter thick, were used.

Temperature Profile was as follow: at first the temperature of the oven was fixed on 42oC for 1 min. and then increased to 180oC with the temperature rate of 10oC/min, again 1 minute fixed. It totally took 14 minutes from the time of sample injection to the last peak appearing and completing the analysis graph. The temperature of the container of injection the detector was 250 oC and the volume of the sample was 1μL diluted by 1lit. of *n*- pentane (nano scale injection). Ionization energy was 70 ev., and carrier helium speed was 1mL/min. The adjustment of the GC/MS has been take in a way that the first related peak to the first component of the essence appeared after the end of retention time of all compound of solvent. In this case the given percentages mentioned in the analysis are without solvent.

2-5. Identification of essential oil components

Retention time indices are calculated using retention time of normal alkanes injected after the essence in the same temperature and under the same chromatography conditions. Retention indices of all composition are calculated based on Kovats Index method, when the normal alkanes are used as a standard. The chemical components of essence are identified by comparing its retention index with what is published in the reports or in the data bank of data base, Wiley library, stored in the computer memory of GC/MS. [19,20].

3. Results and discussion

Results of *Ferulago Angulata* analysis are given in tables 2 and 3. When absolute ethanol was used, 30 components were identified and for acetone only 10 components (3 common components and total components are 37) were identified. Tables 2 and 3 show the percentages of composition of major components. Among 30 identified components, the 20 first main ones form 94.14% of the essence and the first 14 ones, 83.3%. The major components are: Suberosin (12.36%), (+) Spathulenol (10.90%), Trans-β-caryophyllene (7.32%), Curcumene (7.07%) and Bicyclogermacrene (6.96%). When acetone was used as solvent, 10 components were identified from which the first 5 ones formed 88.15% of the whole essence. The main components have been identified and the percentage of their composition is different with the former research (hydrodistillation and solvent extraction). In this new method, the yield has been up to 0.8%.The first 3 components had been identified in hydro-distillation method and 62 components including 89.7% of the essence had been also discovered including: (z)–β–Ocimene (35.5%), Terpinolene (5.7%) and α–Phellandrene (5.4%) and by the same method in the former research, 33 other ones also were identified whose major components with their percentage are: α-Pinene (17.3%), Bornyl acetate (14.45%) and Cis-ocimene (14.41%). In another research (solvent extraction) with absolute ethanol as the solvent, 63 components including 97.6% total essence were identified [15,16]. In this research the first 14 components included 90.45% of the extracted essence and 7 ones were the same as our extraction method (SCE). When acetone was used, 3 components were common. Anyway, as seen in table 4, the percentage composition of the common components in solvent method, when ethanol was used, Cis-ocimene was the most important component with the highest percentage. Another important point is that, when acetone or ethanol were used at the end of SCE method for collecting the essence in the receiver, the first component with the highest percentage was common in both but different in quantity [13].

Table 2. Important components of the essential oil of Ferulago Angulata with absolute ethanol extraction (S.F.E. method)

TOTAL PERCENT OF 20 COMP = 94.14 %	TOTAL PERCENT OF 14 COMP = 83.3 %	%	R.T(min)	IR	Chemical components	No.
		0.90	8.65	1189	α-terpineol	20
		1.05	11.24	-	α-humulene	19
		1.45	8.18	-	1-(Methylen cyclopropyl)-cyclopentane	18
		2.09	11.16	1509	Trans-β-farnesene	17
		2.41	6.92	1050	Trans- β- Ocimene	16
		2.94	12.33	-	Calcalol methyl ether	15
		3.43	8.99	1245	Citronellol	14
		3.66	9.58	-	Bornyl acetate	13
		3.81	10.47	1368	Piperitenone oxide	12
		3.82	9.20	736	2-cyclohexane-1-one, 2-methyl	11
		3.95	9.65	1075	Benzene ,1-ethyl-2,4-dimethyl	10
		4.60	10.76	1396	Cis-jasmone	9
		5.06	5.75	940	α-pipene	8
		5.08	7.64	1104	Linalool	7
		5.28	11.87	-	δ-cadinene	6
		6.96	11.64	1518	Bicyclogermacrene	5
		7.07	11.46	1483	Curcumene	4
		7.32	10.92	1444	Trans- β-Caryophyllene	3
		10.90	12.59	1622	spathulenol	2
		12.36	*4.17-14.47	*	Suberosin 2H-1-benzopyran	1

Table 3: Important components of the essential oil of Ferulago Angulata with acetone extraction (S.F.E. method)

TOTAL PERCENT OF 10 COMP. = 100 %		2.06	8.45	-	Calcalol methyl ether	10
		2.30	5.68	-	α–Pinene	9
		2.36	10.58	1385	β-Bourbonene	8
		2.43	7.63	1104	Linalool	7
		2.71	6.92	1050	Trans – β - Ocimene	6
		3.99	10.91	1417	Trans – caryophyllene	5
		8.09	11.47	1480	Germacrene - D	4
		16.42	9.19	1210	L – Carvone	3
		21.64	4.54	849	2- pentanone , 4 –hydroxy -4 – methyl	2
		38.01	*1.77-1.59	*	Suberosin 2H-1-benzopyran	1
		%	**R.T(min)**	**IR**	**Chemical components**	**No.**

Table 4: Important components of the essential oil of Ferulago

No.	Components	Solvent ext. with EtOH.
1	Cis - ocimene	30.17
2	α - pinene	15.41
3	β - germacrene	6.64
4	γ - terpinene	5.77
5	Trans- β-ocimene	5.70
6	Germacrene - D	5.03
7	limonene	4.88
8	Bornyl acetate	4.58
9	myrcene	3.62
10	camphene	2.41
11	Neo-allo-ocimene	1.87
12	β- pinene	1.84
13	Bicyclo germacrene	1.29
14	subirosin	1.24

4. Conclusion

In this research, supercritical fluid CO_2 has been used for extraction of *Ferulago Angulata* plant essence for the first time. The quality of extracted essence is more than the quality of essence extracted by other methods like hydro distillation and solvent extraction.

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