DETERMINATION OF VOLATILE COMPONENTS OF BUNIUM LURISTANICUM RECH.F USING MAHD AND HD EXTRACTION TWCHNIQUES AND ANTIOXIDATIVE ACTIVITY OF METHANOLIC EXTRACT- A GREEN CHEMISTRY APPROACH

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This paper is devoted to an investigation carried out onmicrowave assisted hydrodistillation (MAHD) and hydrodistillation (HD) techniquesto extract essential oils from aerial parts of aromatic herb *Bunium luristanicum Rech.f* from Iran which is a highly advanced and ogeneous family, largely used in food industries, perfumery and medicine. Chemical characterizations of volatile components were investigated by GC/MS. Fifty-seven components were characterized. The major components of the extracted oils using HD and MAHD were Anethole-E (60.9%), gamma -Terpinene(2.5 %), α –fenchylpinene(2.5%) .acetate(5.2%), and MAHD , α – pinene (16.2 %), β–Myrcene (3.4%), Camphene (8.2%). Terpinene (6.0%) **,** respectively The methanolic extract of *Bunium luristanicum Rech.f* was also examined for free radical scavenging activity. Antioxidant activity was examined using 2, 2-diphenyl-1-picrylhydrazyl radical scavenging method (DPPH). The results indicated the novel evidences of free radicalscavenging activity of MeOH extract (IC_{50}=89.2±.0.6 μ g/ml) and phenol content in samplem (Gallicacid equivalent=574.8±1.1 mg/)

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1.Introduction

Essential oils represent a small fraction of the composition of plants but confer the characteristics for which aromatic plants are used in the pharmaceutical, food and fragrance industriesEssential oils have a complex composition, containing from a few dozen to several hundred constituents, especially hydrocarbons (terpenes and sesquiterpenes). Both hydrocarbons and oxygenated compounds are responsible for the characteristic odours and flavours[1-2]. The essential oils of plants have usually been isolated by either hydro-distillation or solvent extraction. The distillation method has traditionally been applied for the recovery of essential oils from plant materials. One of the disadvantages of the distillation method is that essential oils undergo chemical alterations and the heat sensitive compounds can easily be destroyed [1].The other method applied for oil recovery from plant materials is using organic solvent extraction, which has limitations with regard to the loss of valuable volatiles during vacuum evaporation of solvent. A recent patent describes a new method for extracting natural products without added any solvent or water by using microwave energy [3].The solvent free microwave extraction apparatus is an original combination of microwave heating and dry distillation at atmospheric pressure. MAHD was conceived for laboratory scale applications in the extraction of essential oils from different kind of aromatic plants. Based on a relatively simple principle, this method involves placing plant material in a microwave reactor, without any added solvent or water.

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The internal heating of the in situ water within the plant material distends the plant cells and leads to rupture of the glands and floriferous receptacles. This process thus frees essential oil which is evaporated by the in situ water of the plant material. A cooling system outside the microwave oven condensed the distillate continuously. The excess of water was refluxed to the essential oil extraction vessel in order to restore the in situ water to the plant material. The extracted by MAHD for 3 min were quantitatively (yield) andqualitatively (aromatic profile) similar to those obtained by conventional hydro-distillation for 1.5 h. The MAHD method yields allows substantial savings of costs, in terms of time, energy and plant material. essential oil an MAHD is a green technology and appears to be as a good alternative for the extraction of from aromatic plants In this paper, the potential of the MAHD technique has been essential oils compared with a conventional method, hydro-distillation, as the current technique and commercial situation call for research into new extracts and new extraction techniques. We have applied MAHD and HD techniques to extract essential oils from aerial parts of aromatic herb *Bunium luristanicum Rech.f* from Iran which is a highly advanced and homogeneous family, largely used in food industries, perfumery and medicine. We make appropriate comparisons in term of extraction yields and rates, essential oil composition, and energy consumption.The genus *Bunium luristanicum Rech.f* (Black cumin) is an economically important umbellifer growing wild in the dry temperature regions of Jammu-Kashmir, Himachal Pradesh, Afghanistan, Baluchestan and Iran . Species have been used in the folk medicine as emulient, carminative [2] tonic, Anti flatulent, anathematic, antifungal andante bacterial agents[4]. It is herbaceous and perennial and grows up to 1 m high. The flowers are hermaphrodite (have both male and female organs) and are pollinated by Insects.The hydro-distillation of black cumin seeds has been reported previously [5]. According to the reports, black cumin seeds contain essential oils rich in monoterpene aldehydes; the main components are cuminaldehyde, *p*-mentha-1, 3-dien-7-al and *p*-mentha-1,4-dien-7-al; terpene hydrocarbons are *γ*-terpinene, *p*-cymene, *β*-pinene. The latter compounds are thought to reduce the quality of the spice. Herbs have been used for a large range of purpose including medicine, nutrition, flavoring, beverages, dyeing, repellents, fragrances, cosmetics, charms, smoking and industrial uses[2]. The importance of antioxidants in the maintenance of health and in protection from the damage induced by oxidative stress is coming to the forefront of dietary recommendations, the development of functional foods and the extraction of novel potentially therapeutic compounds from medicinal plants. Moreover antioxidants offer an effective way to prevent a variety of lifestyle-related diseases and aging that result from lipid per oxidation and active oxygen[6]. Usually, synthetic antioxidants such as butyl hydroxyl anisole (BHA) and butyl hydroxyl toluene (BHT) are used to decelerate these processes. Because of the possible toxicities of the synthetic antioxidants, increasing attention has been directed toward natural antioxidants[7]. It seems that antioxidant activity is mostly related to the presence of the phenolic compounds such as flavonoids and phenolic acids in the polar fraction. The key role of phenolic compounds as scavengers of free radicals is emphasized in several reports[8] . Moreover, radical-scavenging activity is one of various mechanisms to contribute overall activity, thereby creating a synergistic effect. In this paper, we report the chemical composition and antioxidant activity of the methanolic extract of *Bunium Luristanicum Rech.f*

2. Experimental

2.1. Plant material

The fresh plantof *Bunium Luristanicum Rech*.f was collected during ful-flowering stage from Zagros Mountain of altitude 1600 m in the Lorestan province, west of Iran, on May 2009. The plant was identified and authenticated by Dr. N.Akbari at the faculty of agriculture Lorestan University. Voucher specimens were deposited in the Herbarium of Research Institute of Forest and Rangeland Tehran.

2.2. MAHD apparatus and procedure

Microwave extraction has been made in a Samsung microwave laboratory oven. This is a multimode microwave reactor 2455 MHz with a maximum delivered power of 1000 W variable in 10 W increments. The dimensions of the PTFE-coated cavity are 35 cm × 35 cm × 35 cm. During the experiment, time, temperature, pressure, and power could be controlled with the "easy-WAVE" software package. Temperature was monitored by a shielded thermocouple (ATC-300) inserted directly into the sample container and by an external infrared (IR) sensor. Temperature was controlled by a feedback to the microwave power regulator. The experimental SFME variables have been optimized in order to maximize the yield of essential oil. In a typical MAHD procedure performed at atmospheric pressure, 50 g of fresh plant material was heated using a fixed power of 600 W for 3 min without adding of any solvent or water. A cooling system outside the microwave cavity condensed the distillate continuously. Condensed water was refluxed to the extraction vessel in order to provide uniform conditions of temperature and humidity for extraction. The extraction was continued at 100 °C until no more essential oil was obtained. The essential oil was collected, dried over anhydrous Sodium Sulphate

2.3. Hydro-distillation apparatus and procedure

Hundred grams of aromatic herb were chosen for hydro-distillation with a Clevenger-type apparatus according to the European Pharmacopoeia [9]and extracted with 600 ml of water for 1.5 h (until no more essential oil was obtained). The essential oil was collected, dried over anhydrous sodium sulphate and stored at 0 °C until used.

2.4. Gas chromatography–mass spectrometry identification

GC analyses were carried out on a Shimutzu 17A gas chromatograph and a BP-5 (non-polar and 95 % dimethyl polysiloxane) capillary column (30 m × 0.25 mm; 0.25 µm film thickness). The oven temperature was held at 60 °C for 3 min then programmed at 5°C /min to 300 °C. Other operating conditions were as follows: carrier gas He, with a flow rate of 5 ml/min; injector temperature 230°C; detector temperature 300 °C; split ratio, 1:8. GC/MS analyses were performed on a Shimutsu 17A GC coupled with Shimutsu QGD5050 Mass system. The operating conditions were the same conditions as described above but the carrier gas was He. Mass spectra were taken at 70 eV. Mass range was from m/z 50–450 amu. The constituents of the oil were identified by calculation of their retention indices under temperature-programmed conditions for identification of individual n-alkanes (C_6–C_{24}) and the oil on DB-5 compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley 5.0) or with authentic compounds or with those of reported in the literature[10]. Quantitative data was obtained from FID area percentages without the use of correction factors.

2.5. GC–MS analysis condition

The oil was analyzed by GC/MS using a Gas Chromatography Analysis GC analysis of the oil was conducted using a Varian CP-3800 instrument equipped with a DB-1 fused silica column (60 m × 0.25 mm id., film thickness 0.25 µm).Nitrogen was used as the carrier gas at the constant flow of 1.1 mL/min. The oven temperature was kept at 60°C for 1 min, then programmed to 250°C at a rate of 4°C/min, and kept constant at 250°C for 10 min. The injector and detector (FID) temperatures were kept at 250°C and 280°C, respectively. GC-MS analysis was carried out on a Thermoquest-FinniganTrace and DB-Wax columns under the same conditions.GC-MS instrument equipped with a DB-1 fused silica column (60 m× 0.25 mm i.d., film thickness 0.25 µm).The oven temperature was raised from 60°C to 250°C at a rate of 5°C/min, and then held at 250°C for 10 min.; transfer line temperature was 250°C . In this case, the oven temperature was

raised from 40°C to 250°C at a rate of 4°C/min, then held at 250°C for 10 min. with the transfer line temperature adjusted at 250°C The flow rate of helium as carrier gas was 1.1 mL/min. split ratio was, 1/50.The quadrupole mass spectrometer was scanned over the 45-465 amu with an ionizing voltage of 70 eV and an ionization current of 150 µA. The constituents of the oil were identified by calculation of their retention indices under temperature-programmed conditions for Identification of individual n-alkanes (C_6–C_{24}) and the oil on DB-1 compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley 7.0) or with authentic compounds or with those of reported in the literature [1] . Quantitative data was obtained from FID area percentages without the use of correction factors.The list of compounds identified in the oil of Bunium Luristanicum Rech.f can be seen in Table 1.

Table 1. The composition of essential oils of Bunium luristanicum Rech.f using HD and MAHD

NO	Compound	RI_{cal}	Relative content (%)	
			HD	MAHD
1	Tricylene	927	....	0.5
2	alpha-thajene	930	0.1	0.2
3	α -pinene	939	2.5	16.2
4	Camphene	945	....	6.8
5	Sabinene	975	0.3	0.9
6	β -pinene	979	2.4	2.8
7	1-octen-3-ol	979	....	0.2
8	3-octanone	984		3.2
9	β -Myrcene	991	0.5	3.4
10	3-octanol	991	....	0.2
11	1-phellandrene	1003	....	0.3
12	Cymol	1016	1.7	0.2
13	α -Terpinene	1017	0.1	0.4
14	p- cymene	1025	....	0.5
15	dl-limonene	1029	9.2	1.5
16	1,8 – cineole	1031	....	13.7
17	gamma-ter pinene	1060	2.5	0.2
18	Cis-sabinene hydrate	1070	....	0.3
19	L- Fenchone	1087	1.5	0.4
20	α - Terpinolene	1089	1.1	0.3
21	Linalool	1097	....	4.0
22	D-Fenchylalcohol	1109	0.1	
23	chrysanthenone	1128	....	1.2
24	Cis- verbenol	1141	....	0.4
25	Camphor	1148	0.1	8.2
26	Pinocamphone	1152	....	0.7
27	4-Terpineol	1166	....	0.9
28	Borneol	1669	....	5.5
29	1,3cyclohexadiene	1175	0.2	....
30	p-Allylanisole	1179	4.5	....
31	p-Menth-1-en-80L	1189	....	1.6
32	alpha-thajene	1196	....	0.3
33	Fenchyl acetate	1220	0.4	....
34	Anethole-E	1225	60.9	....

35	α -fenchyl acetate	1227	5.2	....
36	Cuminaldehyde	1242	1.5	....
37	Anethole-Z	1253	1.2	....
38	Sabinene	1253	....	0.9
39	Carvone oxide- trans	1276	....	0.9
40	p-Allylanisole	1279	0.1	
41	β -Myrcene	1285	....	12.7
42	α -Terpinene	1289	....	6.0
43	Cymol	1355	....	0.3
44	Tetradecane	1400	0.1	....
45	trans β Caryophyllene-	1419	0.2	....
46	β -Farnesene (E)	1457	0.1	....
47	α -Humulene	1455	0.1	....
48	Fenchyin-valepate	1465	0.1	....
49	Fenchyin-valepate	1472	0.1	....
50	Germacrene D	1485	0.2	....
51	Nerolidol-E	1563	0.1	....
52	(-) – spathalenol	1578	0.1	....
53	Hexadecane	1600	0.1	....
54	Benzyl benzoate	1760	0.3	....
55	Octadecane	1800	0.1	....
56	neophytaDine	1839	0.1	....
57	phytol	1943	0.8	....

2.6. Preparation of the methanolic extract

The air-dried and finely ground samples were extracted by using the method described previously [12]. Briefly, the sample, weighing about 150 g was extracted in a Soxhlet apparatus with methanol (MeOH) at about 60 °C for 12 h. The extract was then filtered and concentrated in vacuo at 45 °C, yielding a waxy material .3.87%, w/w. Finally, the extracts were then lyophilized and kept in the dark at -4 °C until tested.

2.7. Antioxidant activity: Free Radical Scavenging Capacity (RSC)

RSC was evaluated by measuring the scavenging activity of examined extract on the

$$RSC(\%) = 100\times (A_{blank} - A_{sample}) / A_{blank}$$

2,2- diphenyl-1-picrylhydrazyl radical scavenging method (DPPH) . The DPPH assay was carried out as described elsewhere previously. Various concentrations of the sample were mixed with 1 ml of 90 µM DPPH. Solution were filled up with 95% methanol to a final volume of 4 ml .After 60-min incubation period at room temperature, absorbance of solutions and blank (with same chemicals, except for the sample) were recorded against tert-butylated hydroxy toluene (BHT) as positive control. Three replicates of sample were recorded. The disappearance of DPPH was measured spectrophotometrically at 517 nm on a Shimadzu 2501UV spectrophotometer.The percentage of RSC was calculated in following way.

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound .The IC_{50} value, which represented the concentrations of the essential oil and extracts that caused 50% inhibition, was

determined by linear regression analysis from the obtained RSC values

2.8. Assay Phenolic content

The amount of phenolic content in the herb extract was determined with the Folin-Ciocalteu reagent according to the method of slinkard and slingleton using gallic acid as standard [12] . Twenty micro-liters of extract solution was taken in cuvette , then 1.58 ml of distilled water and 100 µl of Folin-Ciocalteu reagent were added , and was shaken thoroughly. After 3 min, 300 µl of the sodium carbonate solution (7% w/v) was added, and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm

3. Results and discussion

3.1. Chemical composition of the essential oil

We compared our results with those of the Indian [12]and Tajikistan[13]black cumin samples. In all oils, *γ*-terpinene and cuminaldehyde were the major constituents, but our oil was higher in Isopulegol acetate(24.64%), Camphor(10.43%), Anethole (20.36%). On the other hand, in Indian and Tajikistan oil, *p*-mentha-1,4-dien-7-al was the major constituent, whereas, this component was not found in our oil. It is worthy to note that Indian and Tajikistan oils were obtained by hydro-distillation method. These differences might have been derived both from harvest time and local, climatic and seasonal factors or we may hypothesize.The volatile oil of the *Bunium luristanicum Rech.f* at flowering was obtained by a conventional hydro-distillation method using a Clevenger-type apparatus and the yield of the oil was found to be in 3.1% (w/w) components. Identification of the constituents was accomplished by comparing their mass spectra and retention indices with those given in the literature and those authentic samples [1].Relative percentage amount were calculated from TIC by computer. This study showed that sp growing in this region contained a maximum amount of 1, 3-Decadiene (19.93, 18.07 %). The results of the GC/MS analysis of the oil of aerial parts of (at flowering stage) are listed in Table I. fifty-nine compounds were identified representing (HD, 98.3 %)and (MAHD,95.8%) of total oil. Comparing these results with previous studies on *Bunium* species, it is worth mentioning here that there is variation in the chemical composition of. *Bunium luristanicum Rech* f of this region with other species[7-14-16].

3.2. Antioxidant activity

The antioxidative capacity of *Bunium luristanicum Rech.f* methanolic extract was determined by comparing with the activity of BHT as antioxidant. Free radical scavenging capacity of the extract, measured by DPPH assay. The results indicated the free radical scavenging activity of MeOH extract (IC_{50}=89.2±.1.6µg/ml) . Effect of *Bunium luristanicum Rech.f* methanol extract and positive control (BHT) on the in vitro free radical (DPPH) are given in Table 2

Table 2. Effects of Bunium luristanicum Rech.f methanol extract and positive control on the in vitro free radical (dpph) assay, IC_{50} (µg/ml)

Sample	Gallic acid Equivalent(mg/l)[a]	DPPH IC_{50} (µg/ml)
Methanolic Extract	574.8 ±4.2	89.2 ± 1.6
BHT (positive control)	198±0.3	26.5 ±1.0

a) Result is given as mean ± S.D. of three different experiments.

3.3. Amount of Phenolic content

Typical Phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids[11] . Phenolic acids have been repeatedly implicated as natural antioxidants in fruits, vegetables, and other plants. Amount of Phenolic content based on the absorbance value the extract solution reacting with Folin –Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents, as described above. The phenolic content of methanol extract was high (574.8±4.2 mg/l) as measured by gallic acid test Table2.

In conclusion**,** the results reported here can be considered as the first information on essential oil analysis and antioxidant property and microwave assisted hydrodistillation (MAHD) and hydrodistillation (HD) techniques to extract essential oils from aerial parts of *Bunium luristanicum Rech.f* from Iran. The molecular mechanism of radical scavenging activity of methanol extract of *Bunium luristanicum Rech.f* could be attributed to the Presence of polyphenolic compounds. It has already been shown that polyphenolic compounds were responsible for radical scavenging activity in Lamiacea family due to ease of their hydrogen atom donation to active free radical.

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