Essential oil Composition and Antioxidant Activity of *Levisticum officinale* Koch. at Various Phenological Stages

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Abstract

Background: The leaves, roots and seeds of Lovage (*Levisticum officinale* Koch) being used as an herb, vegetable and spice.

Objective: The aim of this study is evaluation of the changes of phytochemical compound in aerial parts of the Lovage plants at different developmental stages as well as antioxidant activity.

Methods: The plants were harvested from Hezar Mountain. After drying, essential oils were extracted by steam distillation. To identify the essential components GC and GC-MS was used. Antioxidant activity of samples was examined by diphenylpicrylhydrazyl (DPPH) assay.

Results: The average yield of essential oil was respectively, 2.3% and 3.1% and 1.5% respectively, in vegetative, Flowering and seed stage. The oil analysis results showed that 21 compounds were identified in the vegetative stage. β-phellandrene (10.7%) and α-Terpinyl acetate (% 38.9) and Curzerene (% 10.6) were the major compound. in the reproductive stage, 22 compounds were identified including β-phellandrene (20.3%) and α-Terpinyl acetate (% 20.4) and γ-Cadinene (12.1%). in the seed stage β-phellandrene (21.1%) and α-Terpinyl acetate (% 25.3) and Sabinene (10.2%) were the highest. Analysis of variance showed that the effect of harvesting time has significant effect on plant height, stem branch number and yield of oil. The Higher antioxidant power was observed respectively in the flowering stage (83%), vegetative stage (68%) and seed stage (60%).

Conclusion: The phytochemical and antioxidant compounds in *Levisticum officinale* depend on the phenological stage.

Keywords: Lovage (*Levisticum officinale* Koch.), Essential oils, Antioxidant activity, vegetative stage, flowering stage, seed stage

Introduction

Lovage (*Levisticum officinale* Koch.) is known as ‘‘Karafse kuhi” in Persian. It is a perennial plant belongs to the family Apiaceae. The traditional usage of *Levisticum officinale* in several diseases has been documented in previous resources and in folk tradition. The lovage root is utilized as a diuretic drug [1]. A lot of phytochemical studies have been done to determine the chemical composition of the essential oil of lovage. Terpenes and phthalides, β-phellandrene, α-terpinyl acetate, and Z-ligustilide are the main components of lovage oil that present in different value in the various plant organs. The Essential oil compounds of the hairy roots of lovage were falcarinol, (Z)-ligustilide, (Z)-3-butylidenephthalide, trans-b-farnesene, b-phellandrene, n-octanal, g-elemene and n-heptanal [2]. In other reports, ligustilide, a natural phthalide, is the most abundant bioactive component in essential oils of *L. officinale* roots [3-6]. *L. officinale* exhibits diverse pharmacological activities, containing estrogenic [7], apoptotic [8], and antimycobacterial activities [9].

The chemical composition of different extracts of *Levisticum officinale* revealed more than 190 volatile compounds, frequently monoterpenes and phtalide[10] .Eskin and Tamir found that n-butylidene-4,5-dihydrophtalide is One of the major components at 67% concentration range [11]. Cichy *et al.* (1984) and Bradley (2006) found two phtalide dimers, levistolide A and levistolide B in lovage roots [12]. Up to 70% of the oil is composed of alkylphtalides which are responsible for the characteristic odour [13].

The essential oil content in lovage roots is determined by the harvesting time. Harvesting the leaves during the vegetation season has a negative effects on the gathering of essential oil in the roots [14]. Keeping shoot of *Levisticum* until autumn significantly increased the amount of essential oil from 0.52 to 0.85%. literature reviews present following composition of lovage root oil as % of oil yield - α-terpinyl acetate: 0.1-0.2, β-phellandrene: 1.7-15.5, α-phellandrene: 0.2-0.5, myrcene: 0.3-0.9, (Z)-ligustilide: 37.0-67.5 and pentylcyclohexadiene: 7.4-29.3 [15]. Acording to Samiee et al research, the main constituents in the oil of *Levisticum officinale,* were α-terpinyl acetate (40.5%) and β-phellandrene (16.7%) were the major components, but in the extract, β-phellandrene (23.0%), naphtalene (20.6%) and γ-terpinene (12.1%) [16]. The main components of the oil in the other report were ß-phellandrene (42.5%), cc-terpineol (27.9%), cis-ocimene (7.5%) [17].

It seemed, different developmental stages of *L. officinale* would have different oil compositions therefore, in this study we report the variation of the essential oil composition from the aerial parts of the plant harvested at different developmental stages as well as antioxidant activity. These results can be used to investigate the optimal harvesting time of this plant for relevant industries.

Materials and Methods

Plant material and oil isolation

The aerial parts of *Levisticum officinale* were collected at different developmental stages (vegetative, full flowering and seed

stage) from Hezar mt. (29°30/ N, 57°20/ E), Kerman province at an altitude of 3440 m. Voucher specimen was deposited at the Herbarium of Islamic Azad University, Kerman branch. The essential oil of all air-dried samples was isolated by hydrodistillation for 3 h, using a Clevenger-type apparatus. The distillated oils were dried over anhydrous sodium sulfate and stored at 4 ºC until analysis.

GC and GC/MS analysis

GC-MS analysis was performed using a Hewlett-Packard 5973 mass spectrometer coupled to a Hewlett-Packard 6890 gas chromatograph equipped with a HP-5MS capillary column (5 % phenylmethylpolysiloxane, 30 m × 0.25 mm, film thickness 0.25 µm). The carrier gas was helium and the chromatographic conditions were as above. All mass spectra were acquired in electron-impact (EI) mode with an ionization voltage of 70 eV.

Identification of compounds

N-alkanes (C6–C24) were applied as reference points in the computation of relative retention indexes. The essential oils components were identified by comparing of the retention times of their mass spectra with the mass spectra library data search [18]. To determine the value of relative area percentages obtained by FID were utilized without the usage of correction factors.

Antioxidant activity

Antioxidant activity was calculated by a spectrophotometric method based on the reduction of a methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) [19]. Samples were replicated 3 times. 50 µl of sample was added to 5 ml of methanolic solution of DPPH (0.02 mM). The absorbance was measured after 30 min incubation period at room temperature at 517 nm. Methanol was used as blank. Butylated hydroxytoluene (BHT) and the solvent were used as positive and negative controls respectively. The percentage of inhibition was calculated according to formula: $I\%=(A_b-A_s/A_b) \times 100$.

Where A_b is absorbance of the control mixture (containing all reagents except the test Sample), and A_s is the absorbance of the test Sample.

Results

Soil characteristics of the study area

Experimental results show that soil samples of the study area was sandy loam that is not suitable for agriculture. Soil phosphorus was 6.9 mg per kg while the optimal value is between 7-10 mg per kg (Table 1).

Chemical composition of the essential oil

The essential oil yields (w/w %) based on the dry weight of the plant, in different harvesting times of developmental growth stages were in the order of: flowering (3.1%)> and vegetative (2.3%) > seed stage (1.9 %). In total, 21, 22 and 19 constituents were identified and quantified in the subsequent stages, respectively (Table 2). At vegetative stage the highest amount of compound belonged to α-Terpinyl acetate (38.9), β-phellandrene (10.7) and Curzerene (10.6) while α-Terpinyl acetate (20.4), β-

phellandrene (20.3) and γ-Cadinene (12.1) were highest amount in full flowering stage. At seed stage, α-Terpinyl acetate (21.1), β-phellandrene (25.3) and Sabinene (10.2) were highest value.

Table 1- Soil characteristics of the study area

Clay%	Silt%	Sand%	P (mg/kg)	K (mg/kg)	OC%	PH	EC (ds/m)
10	17	73	460	48	1.86	6.9	1.8

Table 2- Composition of essential oil of *Levisticum officinale* Koch at different developmental stages

No	Compound	R.I	Vegetative stage	Flowering stage	Seed stage
1	α-Thujene	930	-	0.2	0.2
2	α-Pinene	939	1.2	0.6	0.7
3	Camphene	954	0.2	0.4	-
4	Sabinene	975	2.2	4.3	10.2
5	β-Pinene	979	1.3	1.1	1.1
6	Myrcene	991	0.7	1.3	0.9
7	α-Phellandrene	1003	0.5	0.6	1.2
8	α-Terpinene	1017	0.2	0.4	0.3
9	*p*-Cymene	1025	1.3	2.4	1.5
10	β-phellandrene	1030	10.7	20.3	21.1
11	z-β-Ocimene	1037	7.0	6.4	-
12	γ-Terpinene	1060	7.4	8.9	8.0
13	α-Terpinolene	1089	0.4	1.4	0.4
14	allo-Ocimene	1132	0.1	0.5	-
15	Terpinene-4-ol	1177	0.6	1.6	0.5
16	α-Terpineol	1189	1.7	2.2	0.5
17	Pulegone	1237	0.2	0.5	0.7
18	α-Terpinyl acetate	1349	38.9	20.4	25.3
19	Methyl Eugenol	1359	0.2	0.4	0.4
20	N-Buthyl Phthalide	1400	4.3	0.8	-
21	Curzerene	1499	10.6	8.2	9.9
22	γ-Cadinene	1514	4.3	12.1	5.8
	Total		**94**	**95**	**88.7**

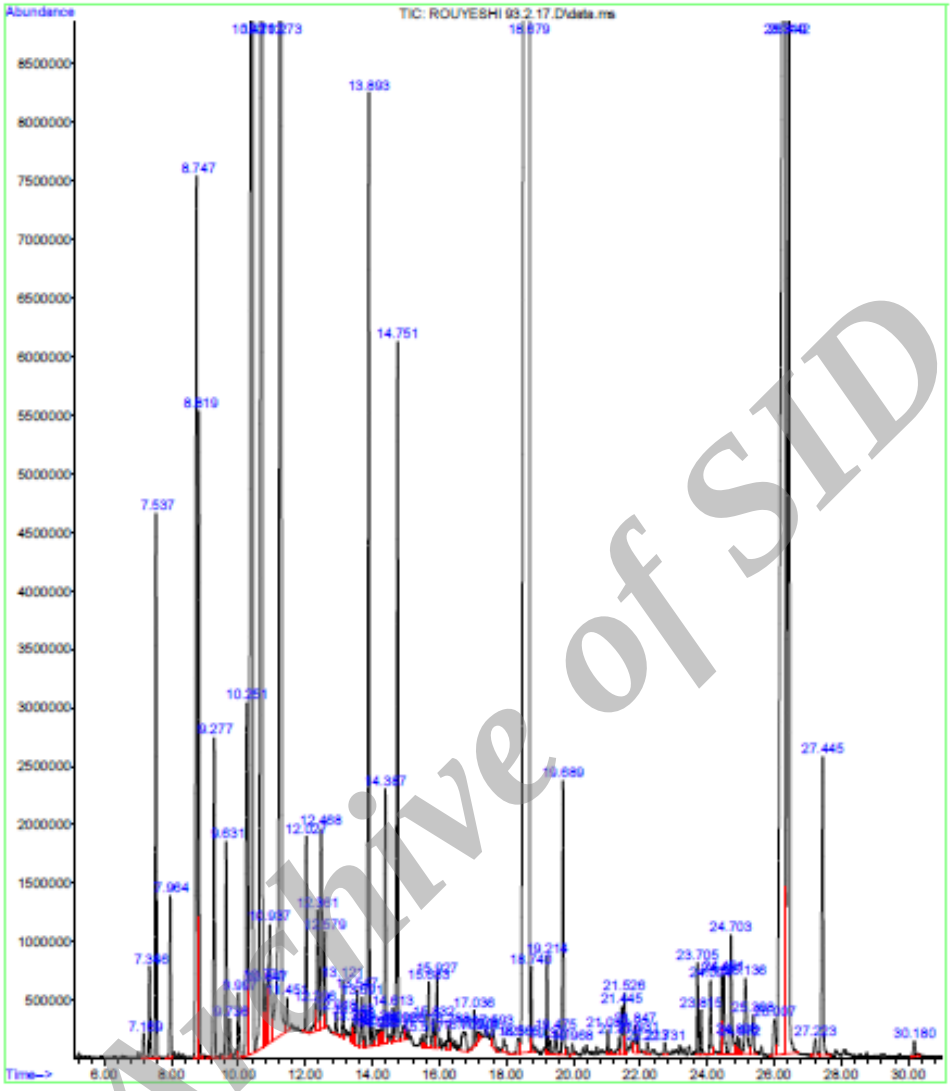

Fig. 1- GC-MS Chromatogram of *Levisticum officinale* Koch essential oil in the vegetative stage

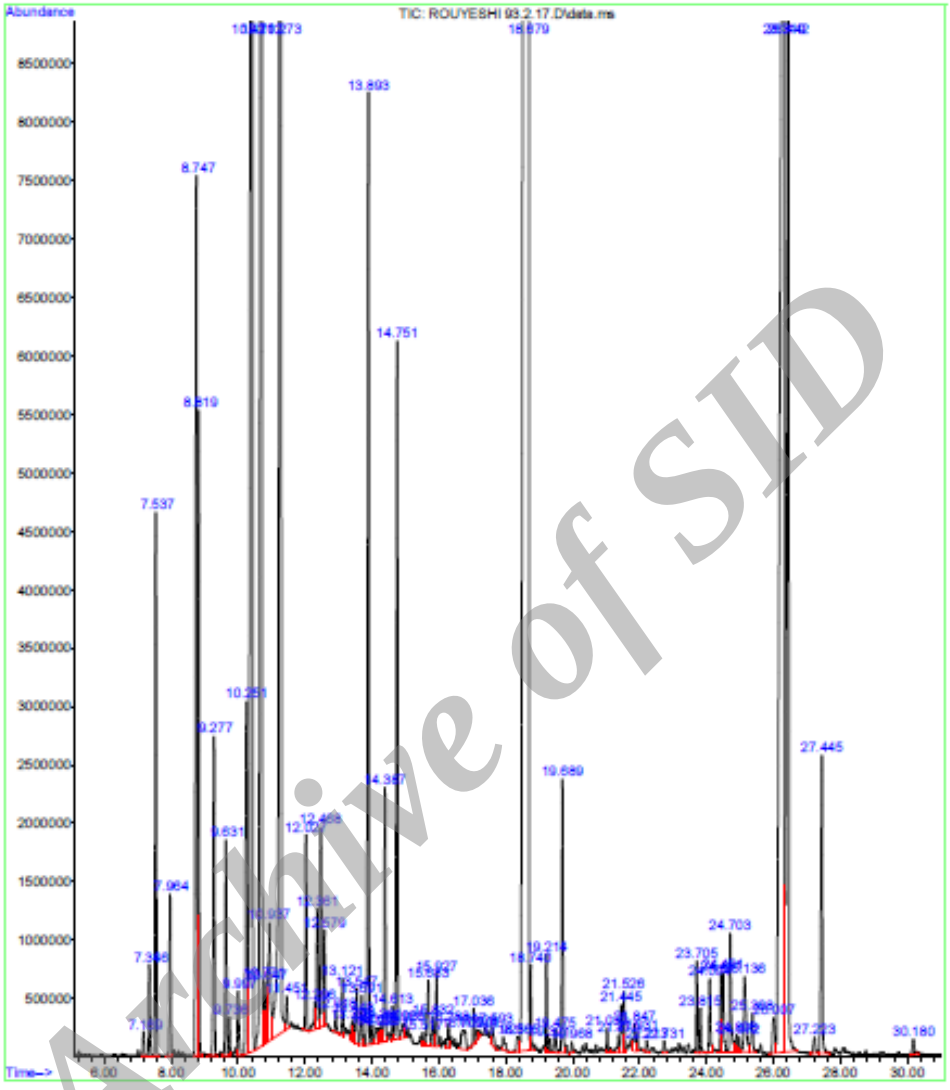

Fig. 2- GC-MS Chromatogram of *Levisticum officinale* Koch essential oil in the flowering stage

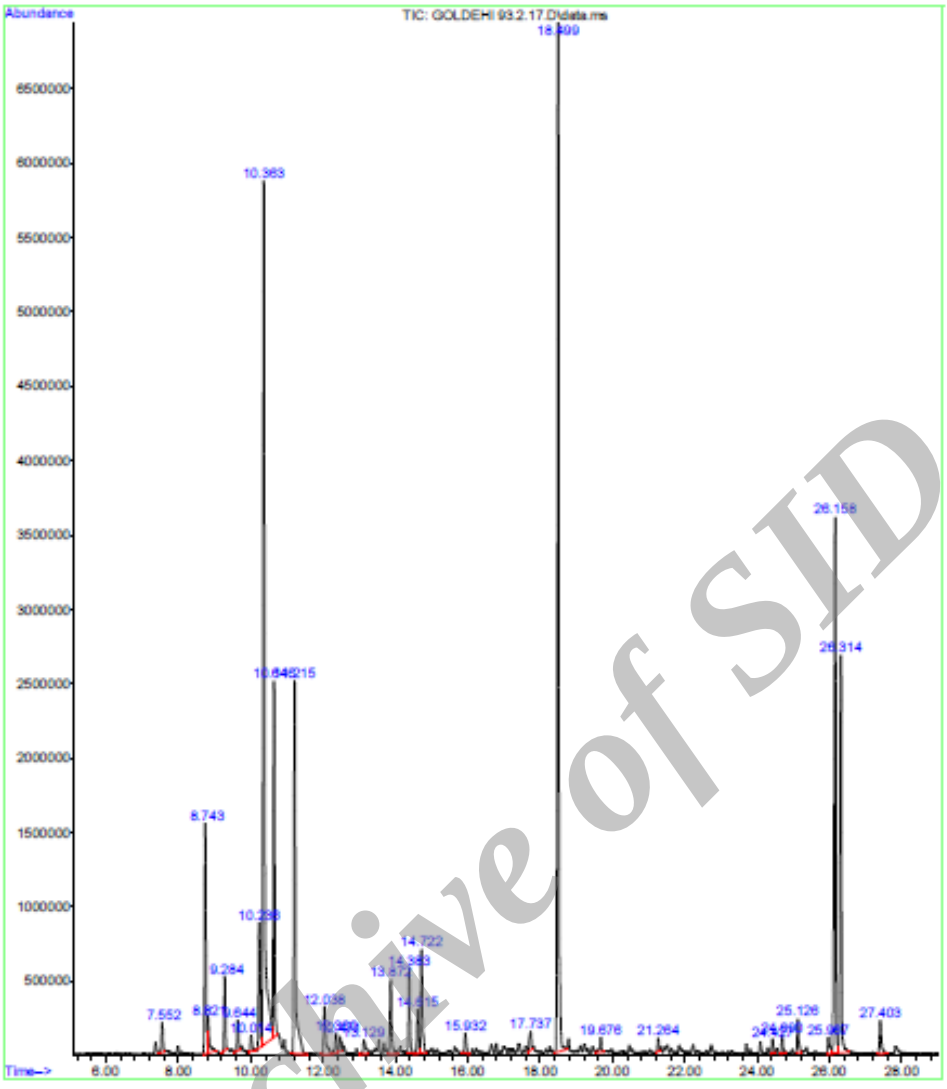

Fig. 3- GC-MS Chromatogram of *Levisticum officinale* Koch essential oil in the seed stage

Antioxidant activity

The results of DPPH inhibition assay of different phenological stages samples of *L. officinale* have shown in Table 3. The Higher antioxidant power was observed respectively in the flowering stage (83%), vegetative stage (68%) and seed stage (60%).

Table 3- Inhibition percentage of *Levisticum officinale* Koch essential oil at different developmental stages

Phenological stages	Inhibition persentage (%) (Mean±SE)
vegetative stage	68± 3.2[b]
flowering stage	83±5.1[a]
seed stage	60±2.8[c]

have different composition depending on the different factors [37].

Morphological study

The highest stem height (103 cm) was observed in the seed stage. The highest number of branches and the highest essential oil was obtained from the flowering stage. The number of branches from 12 at the vegetative stage reached to 18 at the flowering stage and at the seed stage remains the same number.

Discussion

From an agricultural perspective, carbon and organic matter is very important that the analyzed samples in this area have been largely poor. Further, the supporting results were also observed earlier in other members of the family Apiaceae. The essential oil content of *Foeniculum vulgare* (fruit) was evaluated at four different growth stages (immature, premature mature and full mature). Results showed that essential oil content declined with fruit maturity [25]. Similarly, in *Coriandrum sativum* L., the green fruit stage produced more essential oil than the brown fruit stage [26]. The results of Karimi et al study showed that the different phonological stages did not influence on chemical composition but has effects on the essential oil content [27]. Results showed that monoterpenes are the major portion of all samples. In Bylaite et al report, β-phellandrene was the most abundant component in all parts of lovage except the roots [11]. They showed seeds and flowers possessed the highest yield of oil. α-Terpinyl acetate was the highest compound in leaves and stems, β-phellandrene in seeds and flowers, Z-ligustilide was found as a major lovage phthalide in the stem's essential oils depending on the harvesting time [28]. The results of Nejad Ebrahimi et al are consistent with the present research. They reported quality and quantity of essential oil components were different in various developmental stages including vegetative, floral budding, full flowering and seed stage. Flowering and floral budding stages have the highest percentage of essential oil and vegetative stage has the lowest one [29]. Marotti et al found different ontogenetic stages (early, late waxy and ripe seed) influence on the chemical composition and biological activity of *Foeniculum vulgare* [30]. The variation in the quantity and quality of *Origanum majorana* L. essential oil in different phonological stages has been demonstrated by Sellami et al. They found bioactive compounds were in maximum value in later vegetative stage [31]. Effect of developmental stage on yield and components of essential oil has been confirmed in other plants such as *Salvia officinalis* [32], *Ribes nigrum* [33], *Mentha piperita* [34] and *Coriandrum sativum* [35]. Plant metabolism is different in various stage of growth; therefore the production of bioactive components of essential oils could be affected [36]. The antioxidant activity depends on different factors. Then the chemical composition of the essential oil potentially influence on the biological activities of it. Shafaghat indicated that extracts from different parts of *L. persicum* possess considerable antioxidant activity. The highest radical scavenging

activity was detected in seed [37]. The changes in chemical composition of plant essential oil depends on different factors such as genotype, Climate, plant organs, geographical locations, season of sampling, developmental stages. Thus, the essential oils of the same species probably have different composition depending on the different factors [38].

Conclusion

Present study clearly demonstrated that the optimal time for harvesting of *Levisticum* is at flowering stage, because of the highest content of bioactive compounds in this period. The essential oil of lavage show strong antioxidant activity special in flowering stage, so it can be used as a natural preservative in food industries. GC-MS analysis displayed that the oil of lovage is rich in case of bioactive compounds such as β-phellandrene and α-Terpinyl acetate and Curzerene , γ-Cadinene, Sabinene.

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