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Chemical Properties of Four Valuable Medicinal Plants on Oshtorankoh, Iran

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ABSTRACT

Iran has different environmental conditions due to specific geographical. Therefore, exploring essential oil and flavonoids components among different medicinal may be caused discoveries about valuable components. The present study aimed to study flavonoids profile and essential oils composition of four Lamiaceae species i.e. Stachys lavandulifolia, Teucrium polium, Thymus daenensis, and Ziziphora clinopodioides from Oshtorankoh located on Zagros Mountains, Iran. The plants were collected during 2014-2015 and were identified using available references. A chemical study using two-dimensional paper chromatography (2-DPC) and thin-layer chromatography (TLC) showed that the four studied species contained flavone C and C-/O glucosides and flavonoid sulfates. The result of the present study revealed that T. polium had high levels of many flavonoids such as apigenin, genistein, isorhamnetin, kaempferol, quercetin, rutin, andvitexin compared to the other studied plants. According to the results of GC/MS analysis, S. lavandulifolia was rich in α-pinene (21.16%) and 1,8-cineol (16.95%), T. polium in α-pinene (12.21%) and sabinene (9.55%), T. daenensis in γ-Terpinene (36.98%) and p-Cymene (26.87%), and Z.clinopodioides in piperitone (16.84%) and 1,8-cineol (16.11%) components.

Keywords: Flavonoids, essential oil, Lamiaceae, mint, Zagros

INTRODUCTION

Mint (Lamiaceae or Labiatae) is a family with about 236 genera and 6900 to 7200 species worldwide, among which about 124 species and subspecies (30%) are endemic to Iran [1] Many of these species are cultivated for their fragrant leaves and attractive flowers. Aerial parts and leaves are the most widely used plant parts. Nepeta (76 spp.), Salvia (56 spp.), Stachys (34 spp.), Scutellaria (19 spp.), Phlomis (17 spp.), Eremostachys (16 spp.), Thymus (16 spp.) and Teucrium (12 spp.) are the largest genera in Iran [2]. Mint plants are characteristically aromatic, and many of them are cultivated for their essential oils. They are widely used as spices and flavoring in food industries. Their oils are used in the perfumery, cosmetics, and pharmaceutical industries as bactericides, fungicides, virucides, antiparasitics, and pesticides [3]. More than 8000 flavonoid compounds have been identified in plants that afford protection against ultraviolet radiation, pathogens, and herbivores. They are considered health-promoting and disease-preventing dietary supplements. Epidemiological, clinical, and animal studies reveal that flavonoids may exert protective effects against various disease conditions including cardiovascular disease and cancer. Flavonoids also possess antibacterial, antiviral, and anti-inflammatory effects [4].

A high-level of variation in flavonoids profiles of four Stachys species [5]. Anti-inflammatory effects of Stachys tibetica polyphenols and flavonoid extracts [6]. The leaves and aerial parts of Thymus serpyllum had antidiabetic effects [7]. 55 compounds in Stachys lavandulifolia essential oils, of which the major components were α-pinene (20.1%), β-pinene (12.1%), and spathulenol (7.2%) [8]. Also, myrcene, sabinene, β-phellandrene, and β-caryophylene were reported as the main components in S. lavandulifolia essential oils [9]. Studies on different S. lavandulifolia populations indicated that the essential oil compounds can be varied genetically (ecotype), environmentally, and geographically [10]. Chemical compositions and antifungal activity of Thymus daenensis, T. caramanicus, and Ziziphora clinopodiaides essential oils [11]. Twenty-four components were distinguished in T. daenensis essential oil that thymol, α-terpinene, pcymene, methyl carvacrol and α-thujene were the major

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components [12]. The major essential oil compositions of Teucrium polium L. fruits were as follow α-pinene, β-pinene, limonene, elemol, and cubenol [13]. In addition, eighty-six compounds in T. polium essential oil that the main components were α-pinene, β-pinene, and p-cymene [14]. Antispasmodic effect of aerial part of T. polium essential oil on rat isolated ileum in vitro reported [15]. The main chemical compositions of Ziziphora clinopodiaides aerial part essential oils were reported as carvacrol, α- & β-pinene)ulegone, cymene, iso-menthone, iso-menthol, menthyl acetate)-menth-3-en-8-ol, neo iso-menthol, limonene, myrcene)iperitenone, 1,8-cineole)iperitone, spathulenol, β-pinene, borneol, germacrene D, ɣ-terpinene, and β-bourbonene [16]. The antinociceptive effect of Z. clinopodioides essential oil and opioidergic system was studied in male rats [17]. Due to the general tendency to use natural alternative medicines for the prevention and treatment of diseases, the consumption of plants containing flavonoids and essential oils as food and medicine has increased. Therefore, the present study aimed to identification of flavonoids and essential oils compositions of four Lamiaceae species including Stachys lavandulifolia, Teucrium polium, Thymus daenensis, and Ziziphora clinopodioides collected from "Oshtorankoh" protected area in Lorestan Province located on Zagros Mountains, Iran.

MATERIAL AND METHODS

Plant Sampling and Preparation

The aerial parts of four Lamiaceae species including Stachys lavandulifolia, Teucrium polium, Thymus daenensis, and Ziziphora clinopodioides were collected from "Oshtorankoh" protectedarea, Lorestan, Iran during 2014-2015 (Table 1).

Table 1 Collection information and aerial parts two-dimensional paper chromatographical data of four studied Lamiaceae.

Voucher samples	Taxon	Altitude (m)	Total flavonoids no.	Flavone C-& C-/O glucosides no.	Flavonoid sulphates no.
CEF02	Stachys lavandulifolia*vahl*	2400	6	3	3
CEF55	Teucrium polium *L.*	1951	6	2	4
CEF70	Thymus daenensis*Celak*	2235	9	5	4
CEF73	Ziziphoraclinopodioides *Lam.*	2223	3	2	1

Lorestan with a 28,294 Km^2 area is located in western Iran in the Zagros Mountains (33°58´N, 48°39´E) (Figure 1).

Fig. 1 The sampling site, Oshtorankoh in Lorestan Province located on Zagros Mountains, Iran.

Herbarium voucher samples of each species were prepared for reference and deposited at the Arak University Herbarium (Figure 2).

Stachys lavandulifolia vahl

Thymus daenensis Celak

Teucrium polium L.

Ziziphora clinopodioides Lam

Fig. 2 The four Lamiaceae species from Oshtorankoh in Lorestan Province located on Zagros Mountains, Iran

The samples were air-dried for isolation and determination of their flavonoids and essential oils.

Flavonoids

Flavonoids of plants aerial parts were extracted using 70% ethanol and the extracts were vacuum concentrated to dryness in a rotary evaporator. The flavonoids in each extract were isolated and detected using two-dimensional paper and thin-layer chromatography according to Markham (1982) method [18]. Flavonoids extracts were prepared using the acid hydrolysis method adding Chloride acid 2 M hydrochloric acid and placing them in a water bath at 100 °C for 30 min. After cooling, ethyl acetate was added to separate the flavonoid extract from the non-flavonoids extract. The flavonoid extract, soluble in ethyl acetate at 40 °C and 40 m/sec, was distilled off in a vacuum. Co-chromatography with standards was also performed where possible. Flavonoid standards available for comparison during the study were apigenin, chrysin, genistein, hesperidin, isorhamnetin, kaempferol, luteolin, morin, myricetin, naringenin, quercetin, rhamnetin, rutin, tricine and vitexin (all obtained commercially, Rutin from Merck, apigenin and luteolin from sigma and the rest from Fluka). Cellulose sheet TLC plates were used and after running in three solvents separately, then were viewed in UV245 nm each spot Rf -values and color comparing to standards helped flavonoids identification. Rf values were calculated and in comparison with used flavonoid standards identification and concentration of each spot were determined using Camag UV 254 and 366 nm, chromatographic map, and UV spectroscopy. Also, Perkin-Elmer Lambda 15 UV/Viz Spectrophotometer was used for spots having similar Rf.

Essential Oil Compositions

A total of 50 g of dried aerial parts were hydro-distilled in a Clevenger type apparatus for 2.5 h in three replications. The EOs were kept at 4 °C in the dark before analysis. The essential oils were injected into a gas chromatograph and the most appropriate temperature planning of the column was determined to complete the separation of essential oils. Then, essential oils were injected into the Gas Chromatograph/Mass Spectrophotometer Aglient 7890 and compound mass spectra were obtained. Essential oils components of each sample were identified using Retention Index (RIs), studying mass spectra comparing to Reference Spectra Library [19]. In this study, GC including a mass detector Aglient 5975 C, an Electron Ionization source coupled with an Aglient 7890 GC apparatus was used. A 30-meter-long HP-5MS column, 0.25 mm internal diameter, and 0.25 µm internal layer thickness (film) were used. The GC injection site temperature (Inlet) were regulated 280 °C, mass detector ionization source temperature 150 °C, analyzer (Quadrupole) temperature on 230 °C, and the GC/MS intermediate section temperature 280 °C were set. Each extract was inserted into the device through the injection site and the carrier gas (N) was entered into the device column. Then bypassing the sample and gas, extract components are separated and detected by the detector. Results, and graphs were represented and each essential oil constituents' percentages were recounted according to the FID chromatographic peaks areas assuming that all the essential oil compounds comprise 100%.

RESULTS

Flavonoids

According to the results, all of the studies samples contained flavonoid sulphates and flavone C- &C-/O-glucosides (Table 2). T. daenensis had the most total flavonoids number and Z. clinopodioides had the lowest one. Aglycones were not found in any of them. Flavone C- &C-/O-glucosides and flavonoid sulphates were identified flavonoids series in 2-DPC and TLC shows kind of flavonoids. As Table 2 shows rhamnetin, tricin, and morin were not found in the four studied species. However, all of them had isorhamnetin, kaempferol, luteolin, orientin, and quercetin. In addition, the samples had apigenin and vitexin (exception for T. daenensis). High levels of many flavonoids such as apigenin, genistein, isorhamnetin, kaempferol, quercetin, rutin, andvitexin were observed in T. polium.

Table 2 Thin Layer Chromatographical data of four studied Lamiaceae taxa aerial parts flavonoids from Iran.

Species	Flavonoids identification														
	Apigenin	Chrysin	Genistein	Isorhamnetin	Kaempferol	Luteolin	Morin	Myricetin	Naringenin	Orientin	Quercetin	Rhamnetin	Rutin	Tricin	Vitexin
S. lavandulifolia	+	-	-	+	+	±	-	++	-	++	+	-	++	-	++
T. polium	++	+	++	++	++	+	-	-	-	+	++	-	++	-	++
T. daenensis	-	-	-	+	+	+	-	+++	+	++	+	-	+	-	-
Z. clinopodioides	++	+++	++	+	+	+	-	+	-	+	+	-	-	-	+

Concentration of flavonoids:(nonflavonoid), ± (non or a few flavonoid), + (few flavonoid), ++ (middle concentration of flavonoid), +++ (high concentration of flavonoid).

Table 3 Comparison of essential oils analysis results of four studied Lamiaceae from Iran using GC/MS.

Component	RI[a]	*S. lavandulifolia*	*T. polium*	*T. daenensis*	*Z. clinopodioides*
α-Pinene	938	21.16[b]	12.21	2.03	3.8
(E)-2-Heptenal	965	4.73	5.85	0	0
Sabinene	979	3.58	9.55	0	4.52
β-Pinene	985	3.03	7.87	0.45	3.93
p-Cymene	1037	0	0.13	26.87	0
Limonene	1039	2.44	2.27	0.29	17.4
1,8-Cineol	1041	16.95	0.21	2.66	16.11
γ-Terpinene	1069	0	0	36.98	0.93
n-Nonanal	1110	4.88	6.73	0	0
Piperitone	1257	0	0	0	16.84
(E)-2-Decenal	1271	5.46	7.07	1.76	1.5
Thymol	1329	0	0.46	3.63	0
Carvacrol	1339	0	0	4.83	0
(E)-2-Undecenal	1374	5.32	6.87	1.68	1.04
Total (%)	-	67.55	59.22	81.18	66.07

[a] RI: Retention indices .

[b] Values are the relative area percent (peak area relative to total peak area in total ion chromatogram).

Essential Oils

Results of four Lamiaceae taxa aerial parts essential oils studies from Iran using GC/MS showed, S. lavandulifolia was rich in α-Pinene (21.16%) and 1,8-Cineol (16.95%), T. polium in α-Pinene (12.21%) and Sabinene (9.55%), T. daenensis in p-Cymene (26.87%) and γ-Terpinene (36.98%), and Z. clinopodioides in 1,8-Cineol (16.11%) and piperitone (16.84%). Carvacrol and piperitone components were just found in T. daenensis and Z. clinopodioides, respectively. Based on the results, the maximum and minimum percentages of essential oil compositions detected by GC/MS belonged to T. daenensis (81.18%) and T. polium (59.22), respectively. 1,8-cineol, (E)-2-decenal, limonene, α-pinene, β-pinene, and (E)-2-undecenal compositions were found in the four studied species. According to the results, 14 components were detected in the essential oils of the four studied species, among which 9, 11, 10, and 9 components were observed in S. lavandulifolia, T. polium, T. daenensis and Z. clinopodioides, respectively. All of the studied species had monoterpene, alcohol, aldehyde, and sesquiterpene while aromatic and fatty acid ester were just found in S. lavandulifolia and T. polium, respectively (Table 4).

Table 4 Essential oils compounds data of four Lamiaceae taxa aerial parts from Iran using GC/MS.

Compounds	*S. lavandulifolia*	*T. polium*	*T. daenensis*	*Z. clinopodioides*
EO Compounds N.	9	8	6	4
EO Compounds %	54.76	41.64	91.55	90.43
Total EO N.	56	46	27	30
Total EO %	100	99.98	100	99.99
Monoterpene N.	14	10	14	21
Monoterpene %	53.90	37.92	90.95	89.45
Alcohol N.	5	4	2	1
Alcohol %	2.26	2.18	1.95	0.93
Aldehyde N.	23	21	4	7
Aldehyde %	35.61	47.18	9.38	8.63
Sesquiterpene N.	1	1	1	1
Sesquiterpene %	0.86	3.72	0.6	0.98
n-Alkane N.	3	1	0	0
n-Alkane %	1.43	0.47	0	0
Alkane N.	3	0	2	0
Alkane %	1.43	0	0.71	0
Alkene N.	3	7	0	0
Alkene %	5.09	7.19	0	0
Aromatic N.	1	0	0	0
Aromatic %	0.38	0	0	0
Fatty acid N.	1	1	0	0
Fatty acid %	0.47	0.74	0	0
Fatty acid ester N.	0	1	0	0
Fatty acid ester %	0	0.58	0	0
Ketone N.	0	0	1	0
Ketone %	0	0	0.31	0

DISCUSSION

Species of the Teucrium genus have been used for centuries, the plants are used for different purposes such as diabetes, gastrointestinal disorders, rheumatism, inflammations, tuberculosis, diuretic, antipyretic, tonic, diaphoretic, analgesic, and antihyperlipidemic [20] In the present study, high levels of many flavonoids such as apigenin, genistein, isorhamnetin, kaempferol, quercetin, rutin, andvitexin were observed in T. polium. Therefore, different applications of these plants are maybe related to these components. The plant also had a low level of luteolin, this component was reported in Teucrium species [21]. Different components such as luteolin-7-O-rutinoside and luteolin-7-O-glucoside, which having antioxidant activities, were identified in Teucrium orientale [22]. Luteolin 3',4',5,7-tetrahydroxyflavone, is a common flavonoid that has been used in Chinese traditional medicine for treating various diseases such as hypertension and inflammatory, it also has a high level of antioxidant activity (Lin et al., 2008). Our results showed from the isolated compounds of Teucrium polium, 47.18% were aldehydes. Monoterpenes with 37.92% consist of α-pinene, β-pinene, and sabinene are a part of species essential oil [23]. Also, α-Muurolene, α-Cadinol, and δ-Cadinene were isolated from T. Polium [24]. T. polium extract enhances the anti-angiogenesis effect of tranilast on human umbilical vein endothelial cells [25]. Based on the results, luteolin, isorhamnetin, kempfrol, myricetin, orientin, quercetin, and rutin were found in Thymus daenensis. Luteolin, luteolin 7-O-glucoside, and rosmarinic acid were the most evident of phenolic compounds in the species [26]. The results of GC/MS analysis revealed that the essential oil compounds of the plant containing monoterpenes (90.95%), γ-Terpinene (36.98%), and p-Cymene (26.87%). Previous studies reported the following essential oil components in this species, γ-terpinene, α-pinene, camphene, myricetin, sabinene hydrate, terpenine, isovaleric acid, δ-3-carene, thymol, linalool, thymoquinone, and carvacrol [26]. The carvacrol component has therapeutic potential for the prevention and treatment of colon cancer [27]. In addition, sesquiterpenes (60%) consisting β-caryolhyllene, α-humulene and allo-aromadendrene, alkeholes (1.95%), aldehydes (47.18%), alkenes (9.38%), alkanes (0.71%), and ketones (0.31%) were reported in T. daenensis (Tables 3 & 4). Growth inhibition of Gram-positive bacteria, i.e. Staphylococcus aureus, Micrococcus luteus, Entrococcus faecalis, Streptococcus pyogenes was observed using T. daenensis methanolic extract [28]. This growth inhibition of the species on different Gram-positive bacteria could be due present of different components in the essential oil of aerial parts of the plant. The plants in Stachys species are consumed as herbal tea in the major parts of the world, and due to their antibacterial, antifungal, anti-inflammatory, and antioxidant capacity, they are widely used as an herbal remedy in alternative medicine [29]. Flavonoids components are known for their antioxidant properties and one of the most important sources for humans is the diet. Flavonoids make up one of the most pervasive groups of plant phenolics. In this study, high levels of some flavonoid components such as luteolin, myricetin, orientin, rutin, and vitexin were observed in aerial parts of S. lavandulifolia. There is a strong positive correlation between total phenolic content and antioxidant activity in this species [29]. Due to different flavonoids contents the species can be used potentially as a readily accessible source of natural antioxidants [30]. The flavonoids were also identified in S. lavandulifolia methanolic extract [31]. The essential oil extracted from S. lavandulifolia subsp. lavandulifolia had antimicrobial and antioxidant activities [32]. The GC/MS analysis showed that the essential oil containing monoterpenes (53.9%) and sesquiterpenes (0.86%). In addition, sabinene, β-pinene, β-myrcene, 3-δ-carene, limonene, terpinolene, allo-Ocimene, verbenone, trans-carveol, nerol, geraniol, caryophyllene, and caryophyllene oxide components were detected in the essential oil of this plant. Two sesquiterpenes components including caryophyllene and caryophyllene oxide in S. benthamiana and S. officinalis, respectively [33, 34]. Arial parts including leaves, flowers, and stem of Z. clinopodioides are valuable parts of the plant, are used mostly for traditional food and medicine purposes. It is also used in the preparation of an aromatic tea for gastrointestinal disorders and as an aperitive, carminative, and antiseptic [35]. Our results revealed that the plants were rich in apigenin, chrysin, and genistein. The high level of chrysin component was the previous report in this plant, however, some other components such as kaempferol, myricetin, orientin, and quercetin were also observed [36]. The chrysin flavonoid by different properties such as anti-oxidative and anti-inflammatory effects is a potential prophylactic agent in immunopathological and physicochemical injuries [37]. Therefore, it seems that the plant may have health-promoting and disease-preventing in modern lifestyles. A variation in antioxidant and antimicrobial properties of essential oils of Z. clinopodioides accessions which were collected from different regions in Iran [38]. The antioxidant activity of the plant could be due to the high level of 1,8-cineol in its essential oil [39]. The component also has anti-inflammatory properties used for co-medication in inflammatory airway diseases [40]. Based on the results, the plant was rich in piperitone component, which was not observed in the three other studied plants.

Piperitone component (1 µl/ml) enhanced the antimicrobial activity of furazolidone and nitrofurantoin againstSalmonella spp., E. coli strains)roteus spp., Enterobacter spp., Citrobacter spp., Klebsiella spp., and Serratia spp[41].In the present study such valuable components including carvacrol, 1,8-cineol)-cymene, (E)-2-decenal, (E)-2-heptenal, limonene, n-nonanal, α-pinene, β-pinene)iperitone, sabinene, γ-terpinene, thymol, and (E)-2-undecenal were detected in the essential oils of S. lavandulifolia, T. polium, T. daenensis, and Z. clinopodioides. The essential oil components can be affected by different factors including plant species, plant genetics, soil conditions, altitude, growth phase) lant part used, and environmental factors. Therefore, exploring phytochemical variations in different medicinal plants species could lead to discoveries about valuable components.

CONCLUSION

Iran has different environmental and climatic due to specific geographical and climatic conditions. Therefore, exploring essential oil components among different medicinal may be caused to discoveries about valuable components. There is little information about the essential oil components in medicinal plants from Oshtorankoh region. The result of the present study revealed that T. polium had high levels of many flavonoids such as apigenin, genistein, isorhamnetin, kaempferol, quercetin, rutin, andvitexin compared to the other studied plants. According to the results of GC/MS analysis, S. lavandulifolia was rich in α-pinene and 1,8-cineol, T. polium in α-pinene, and sabinene, T. daenensis in p-Cymene and γ-Terpinene, and Z. clinopodioides in 1,8-cineol and piperitone components.

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