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COMPARISON OF CHEMICAL COMPOSITION OF *TEUCRIUM POLIUM* L. ESSENTIAL OIL AFFECTED BY PHENOLOGICAL STAGES

MOHAMMAD R ASGHARIPOUR* AND HOSSEIN GORGINI SHABANKARE

College of Agriculture, Department of Agronomy, University of Zabol, Zabol, Iran

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Abstract

This study was carried out to examine the essential oil composition of *Teucrium polium* L. during the phenological stage in a population growing wild in Zibashahr, Iran. Chemical analysis of extracted essential oil was performed using combination of capillary GC and GC-MS after fractionation on column chromatography. Gas chromatography coupled with mass spectrometer (GC-MS) detected 24 components in essential oil of *T. polium* in full bloom constitutes 98.8% of total oil composition. The main components of oil were α-pinene, β-pinene, linalool, carvacrol, β-caryophyllene, germacrene D, farnesene-cis-b and valenene. The amount and composition of the essential oil were affected by phonological stages. The most important change in essential oil composition was an increase in α-pinene, β-pinene, β-caryophyllene and cyperene at full bloom. Camphene was not detected in oil samples at full bloom stage, while at vegetative growth and budding 3-octanol, myrcene, bornyl acetate and β-bisabolene were not detected. These results suggested that the best time for collecting of the plant to achieve the highest yield and quality of essential oil is full blooming stage.

Introduction

Iranian natural habitat would be considered as valuable inheritance resources for endangered medicinal plant (Ebrahimi *et al*. 2008, Alizadeh *et al.* 2013, Asgharipour *et al.* 2016). Phenological stage is an important factor which can affect plant growth and the composition of essential oil.

Teucrium is a genus of perennial plants, the largest of the Labiatae (Lamiaceae) family in the Mediterranean area, which constitutes more than 300 species widespread all around the world and comprises about 12 species in Iran (Tutin *et al*. 1972, Sadeghia *et al.* 2014). Plants belonging to the genus *Teucrium* have evolved through natural hybridization and selection, showing substantial variation in terms of their natural habitats, growth characteristics, and aromas (Lakušić *et al.* 2006, Tutin *et al.* 1972).

Teucrium polium L. locally named "Kalpooreh" is one of 300 species of the genus *Teucrium* and found mainly in the hills and deserts of Mediterranean and Western Irano-Turanian sphere. The leaves, 1 - 3 cm long, are sessile, oblong or linear, the stems are ending in shortly paniculate or corymbose inflorescences, corolla is white or pale cream colored (Lakušić *et al.* 2010). Phytochemical investigations have shown that *T. polium* contains various compounds, such as iridoids, flavonoids and diterpenoids (Piozzi *et al.* 2005). Several researchers have evaluated the composition of the essential oil of *T. polium* growing in different geographic areas (Moghtader 2009, Djabou *et al.* 2012a). These studies revealed some chemical differences in the oil compositions, probably related to the different subspecies and/or the geographical origin of the plants. The aim of the present study was to determine if the composition of the essential oils varies during the phenological cycle of this species growing wild.

∗Author for correspondence: <m_asgharipour@yahoo.com>.

Materials and Methods

The aerial parts of *Teucrium polium* was collected from naturally growing plants in June, July and August 2013, at Zibashahr (29.3912° N, 52.3323° E, 1600 m above sea level), Fars Province, corresponding to the vegetative growth, budding and full bloom, respectively (Fig. 1). The majority of population were mixed with species such as *Thymus vulgaris* L., *Origanum majorana* L., *Achillea millefolium* L., *Borago officinalis* L., *Hyssopus officinalis* L. and *Hypericum perforatum* L. Site characteristics of identified population are presented in Table 1. Bioclimatic zones were defined according to Emberger's (1966) Q2 pluviothermic coefficient.

$Q^2 = 2000P/M^2-m^2$

where Q^2 is pluviothermic coefficient, P is the average of annual rainfall (mm), M is the mean of maximal temperature (K: Kelvin) for the warmest month (July) and m is the average of minimal temperature (K) for the coldest month (February).

Twenty individuals were sampled at random with a minimum distances exceeding 50 m from each other to avoid collecting multiple plants from the same parent. The limited number of samples analyzed was due to the small size of the existing populations. From each individual, branches with young leaves were taken by hand. Samples were placed on ice in plastic bags and transported to the laboratory for morphological and chemical analyses. Voucher specimens are kept in herbarium of the University of Zabol (Voucher specimens were TFF-2677-2684).

To obtain essential oils, the fresh aerial parts of plants (400 - 700 g) were subjected to hydro-distillation for 2.5 hrs. The distillate was dried over anhydrous sodium sulphate and stored at 4 ± 6 °C.

GC analyses were carried out using a Konic gas chromatograph (model 2000 C) equipped with a flame ionization detector (FID) and a Spectra Physic (model 4290) electronic integrator. An OV-17 (60 m × 0.22 mm, film thickness 0.40 μm) capillary column was employed. The oven temperature was programmed to: 60°C for 6 min, then increased by 5°C/min to 150°C and held isothermally for 10 min; injector and detector temperatures were 225 and 250°C, respectively. The carrier gas was hydrogen and the samples were injected using the splitless technique. The percentages of the components were calculated from the GC peak areas, using the normalization method.

The GC/MS analyses were done on a Thermo mass spectrometer (model Trio 1000), combined with a Thermo gas chromatograph (model 8000) using an OV-17 column (25 m × 0.25 mm film thickness 0.40 μm). Other conditions of GC±MS were the same as set out above. Oil injected volume: 0.1 l L, fraction injected volume: 0.2 l L. Identification of the components was based on the comparison of their GC retention indices (RI) on non-polar and polar columns, determined to the retention time of a series of n-alkanes with linear interpolation, with those of authentic compounds or literatures data (Djabou *et al.* 2012a). To estimate the concentration of the constituents of the oils, we calculated response factors for all chemical groups relative to tridecane used as internal standard.

Each variable was tested using the ANOVA test in SAS 9.1 program. Pairwise comparisons of the percentage of each compound at each stage were performed using DMRT.

Results and Discussion

The yields of essential oils were low (Table 1) and ranged from 0.21% for budding, 0.57% for vegetative growth and 0.74% for full bloom. Since the protrusions of capitate secretory cells have only a small storing space, there is a continuous evaporation of essential oils (Werker *et al.* 1985) and thus reducing essential oil of leaves. It is well known that all species of the genus *Teucrium*

are distinguished by only a low quantity of essential oils (Kovačević *et al.* 2001, Djabou *et al.* 2011, Djabou *et al.* 2012a,b). According to Voirin *et al.* (1990), the yield of oil is favored with higher temperatures, water deficit and higher summer sunshine, which was the case in the Zibashahr. The essential oil yield, in the 10 wild populations of 2 Mediterranean subspecies of *T. scorodonia*, collected at full flowering ranged from 0.4 to 1.4% (essential oil weight per plant dry weight, w/d.w.), averaging 1.1% (w/d.w.) (Djabou *et al.* 2012b). These values are in accordance with the reported oil yield studied by Djabou *et al.* (2011) and with some reported oil yields at full flowering for 2 Mediterranean subspecies of *T. polium* (Djabou *et al.* 2012a).

Table 1. Main ecological and soil physic-chemical characteristics for the Iranian *Teucrium polium* population analysed.

Rainfall (mmyr-1)	Mean tem (°C)	Mean RH	Q2 coeffi-cient a	Biocli-mates b	N (%)	P (ppm)	K (ppm)	OM (%)	Soil pH	Soil texture
324.2	18	41	70.91	semi-arid	0.265	38.80	29.12	0.75	7.3	Loamy

GC and GC/MS analysis of essential oils allowed the identification of 20 compounds for vegetative growth, 23 compounds for budding and 24 compounds for full bloom, ranging from 89.17 to 98.8% of the total oil composition. The concentrations (%) of all the identified oil components are listed in Table 2 in order of their elution on column. Fourteen of the identified compounds were common at all the essential oils corresponding to 82.43, 83.27 and 85.98% of the identified compounds for vegetative growth, budding and full bloom, respectively. The main components of *T. polium* oil collected in vegetative growth were linalool (15.65%), *β*-caryophyllene (15.3%), germacrene D (13.4%), carvacrol (7.1%), farnesene-cis-b (5.6%), valenene (5.4%) and spathulenol (4.2%). The main components of *T. polium* oil collected in budding were farnesene-*cis*-*b* (18.4%), β-caryophyllene (12.8%), bicyclogermacrene (12.0%), germacrene D (11.8%), linalool (7.8%) and α-camphene (6.1%). The main components of *T. polium* oil collected in full bloom were β-caryophyllene (28.4%), β-pinene (10.9%), farnesene-cis-b (10.7%), carvacrol (8.6%), α-pinene (6.4%) and bicyclogermacrene (6.2%). It is noticeable that the chemical composition of *T. polium* population studied here was in accordance with those previously reported (Tomi and Casanova 2006, Djabou *et al.* 2012a).

Some quantitative differences were also observed between compounds that were present at each stage. α-pinene, β-pinene, β-caryophyllene and cyperene were in significantly higher percentages during the growth stage. Sabinen, linalool, germacrene D, valenene, spathulenol and caryophyllen oxide were higher during the vegetative stage. α-Thujene, limonene and p-cymene were not observed at vegetative growth, while 3-octanol, myrcene, bornyl acetate and β-bisabolene only occurred at full bloom.

Among the essential oils, 8 to 10 sesquiterpenes, 5 to 8 monoterpenes, 1 to 3 aromatics, 1 to 2 alcohols and 2 esters components were identified. Hydrocarbon sesquiterpenes and monoterpence were the predominant classes of compounds in the essential oil (Table 3): 75.4, 61.8 and 74.4% in vegetative growth, budding and full bloom, respectively. Sesquiterpenes and monoterpenes had a high contribution in the composition of the essential oils. The aromatic, alcohols and esters fraction was present in oil samples at low levels. Monoterpence and alcohols were the only group of compounds that seemed to vary depending on the phenological stage with the least level at the full bloom; However the plant collected in full bloom revealed higher amounts of sesquiterpenes than those of vegetative growth and budding, while the latter displayed higher amounts of

monoterpenes than the first. Thus, the qualitative chemical composition of leaf essential oil was relatively constant during the phenological cycle of this species.

Table 2. Chemical compositions of *T. polium* essential oils collected from different location.

Retention index[a]	Composition of essential compounds	R.t.[b]	Vegetative growth	Budding	Full bloom
1	α-thujene	936	-	0.80	0.10
2	α-pinene	942	3.40	2.70	6.40
3	α-camphene	954	2.22	6.10	2.60
4	Sabinen	972	4.10	1.80	2.40
5	β-pinene	977	2.40	0.87	10.90
6	3-octanol	982	-	-	0.84
7	Myrcene	986	-	-	1.10
8	Limonene	1023	-	0.10	1.40
9	p-cymene	1025	-	1.90	2.90
10	1,8-cineole	1032	0.80	1.60	0.78
11	Linalool	1127	15.65	7.80	1.80
12	Bornyl acetate	1267	-	-	0.80
13	Carvacrol	1272	7.10	1.70	8.60
14	Camphene	1385	0.30	0.80	-
15	β-caryophyllene	1417	15.30	12.80	28.40
16	Farnesene-cis-b	1445	5.60	18.40	10.70
17	α-humulene	1450	2.32	-	2.30
18	Germacrene D	1475	13.40	11.80	2.10
19	Cyperene	1484	0.76	0.90	2.40
20	Bicyclogermacrene	1491	3.40	12.00	6.20
21	β-bisabolene	1500	-	-	1.60
22	Valenene	1503	5.40	0.80	2.40
23	γ -cadinen	1506	2.60	3.20	0.30
24	Spathulenol	1564	4.20	2.70	0.98
25	Caryophyllen oxide	1570	1.80	0.40	0.80

[a]Order of elution is given on column. [b]Retention time on the column (Second).

Table 3. Concentration (%) of identified oil components arranged according to the five types of chemical groups.

Different chemical groups	Vegetative growth	Budding	Full bloom
Monoterpence hydrocarbons	16.4	15.0	21.0
Sesquiterpene hydrocarbons	54.0	46.8	53.4
Aromatic	4.7	5.4	6.5
Alcohols	7.8	15.6	5.0
Esters	6.3	7.9	7.0
Total	89.2	90.7	92.9

Physiological variations (i.e. organ and leaf position), environmental conditions (i.e. soil condition, moisture availability and temperature), geographic variations, genetic factors and evolution are known to affect the biosynthesis of the essential oils (Figueiredo *et al.* 2008). Thus, these type of variations, that where already seen in *T. polium* may be due to the influence of the developmental stage on the regulation of the essential oil biosynthesis.

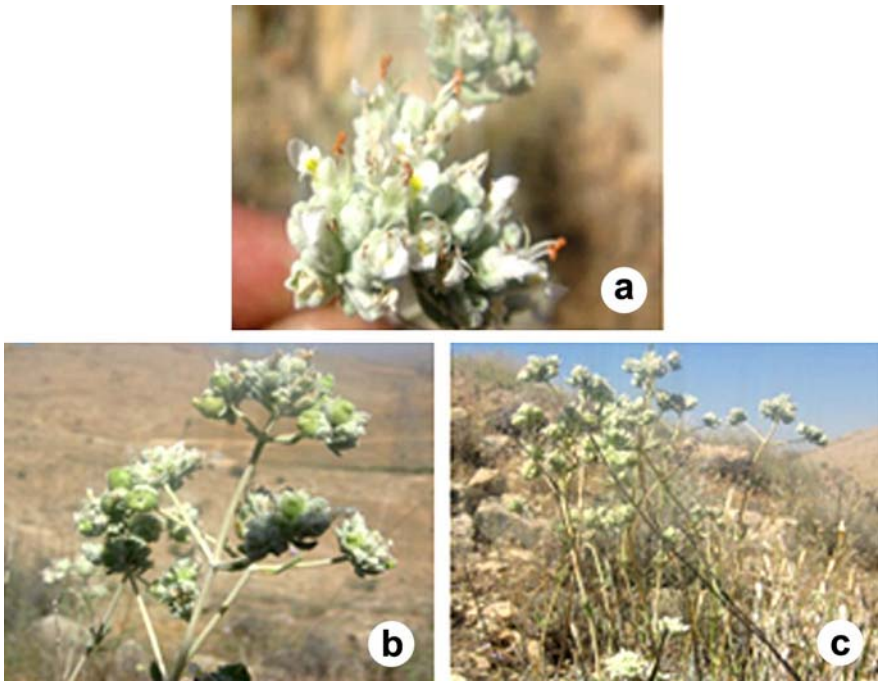

Fig. 1. Vegetative growth (a), budding (b) and full bloom (c) of *T. polium.*

These results extend our knowledge about *T. polium* essential oils; We previously established that, at the same phenological stage, essential oils of different habitats were different (Gorgini *et al.* 2015); therefore, variation in *T. polium* essential oil composition is more habitats-dependent than phenology-dependent. As species of *T. polium* were little studied for their essential oils, it is difficult to compare our results with others. Further studies with the essential oil of *T. polium* are needed.

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