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Seasonal variation in yield and chemical composition of Moroccan Thymbra capitata (L.) Cav. essential oil and its corresponding hydrolat extracted essential oil

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Abstract

The aim of the present work was to study the variation in yield and chemical composition of the pure essential oil (EO) and essential oil dissolved in its corresponding hydrolat (HEO) of *Thymbra capitata* (L.) Cav. of Morocco according to its stage of growth. The results obtained revealed that the yield of essential oil increases with the growth of the plant to reach its maximum at post-flowering stage (2.90%). On the other hand, the yield of dissolved essential oil in the corresponding hydrolats peaked at the flowering stage (0.53%) and then dropped. The analysis of these oils was performed by the GC-MS and showed that carvacrol was the major compound of this plant during its growth stage. The highest carvacrol content was noted at the flowering stage for EO (84.26%) and at the pre-flowering stage for HEO (98.54%). However, the evolution of concentration of *p*-cymene (3.84-6.01%) and *γ*–terpinene (3.45-5.68%) in EO seems to be the opposite of carvacrol. Moreover, these two monoterpene hydrocarbons are absent or almost absent in HEO.

Keywords: *chemical composition; essential oil; carvacrol; growth stage; hydrolat ; Thymbra capitata (L.)Cav.*

1. Introduction

For thousands of years, various plants have been used to treat and cure all kinds of diseases. These plants contain numbers of compounds with different chemical structure and a wide range of biological activities. However, the assessment of these activities remains a very interesting task that can be the target of numerous studies.Within the Labiate family, with about 220 genera, the genus Thymus is one of the eight most important genera with regard to the number of species included, although this number varies depending on the taxonomical point of view. Thyme is an aromatic plant utilized for medicinal purposes and as flavor ingredients in food almost everywhere in the world. This type of plant is very common in Mediterranean regions where some species display a special type of bushy vegetation; around 50 cm tall at most, well adapted to the dry and hot summer climate [1]. Recent studies have shown that thyme has strong antifungal, antimicrobial and antibacterial activities [2-4]. Due to its geographical position, Morocco offers a wide range of Mediterranean bioclimates allowing the growth of rich flora, which is constituted by more than 4200 species. Aromatic and medicinal plants are estimated by 500 to 600 species, and most of them are endemic [5]. In Morocco, the rate of endemism in genus thymus is 57%, representing 13 species [6] which makes a 46.6% endemism rate [7]. It is widely distributed in several regions in Morocco; it is found in plains, mountains, rockeries, scrublands, lawns or undergrowth [8]. It is used fresh or dry as culinary plants. This plant has been used as a part of Moroccan traditional medicine for the treatment of several medicinal disorders such as diarrhea, fever, coughing, infected areas and wounds. It has also been used as a tonic and stimulant [9]. It is known that genetic constitution [10] and environmental conditions [11;12] influence the yield and chemical composition of essential oil produced by plants. Correlations between chemotype polymorphism, sexual polymorphism and the environment have been detected [13]. Essential oils from medicinal plants are no exception; Cabo et al. (1987) [14] and Ozguven and Tansi (1998) [15] reported that their yield and composition are also influenced by management practices such as harvest time as well as ecological and climatic conditions. These are some of the reasons why it is very difficult to determine the best harvesting period of plants in order to obtain an essential oil with better quality and quantity without any kind of preselection. In the present work, hymbra capitata (L.) Cav. samples were used to monitor changes during vegetative cycle by studying yield and chemical composition of essential oils (EO) and those dissolved in there corresponding hydrolats (HEO). This plant grows in the Mediterranean basin; in Morocco, it is found only in south and west of Tetouan city (northern Morocco) at temperate bioclimate [6].

2. Materials and methods

2.1. Plant material and essential oil extraction

The studied samples of *Thymbra capitata* (L.) Cav. were harvested in 2015 from the Tetouan area in northern Morocco (Latitude: 35°34'42" N, Longitude: 5°22'06" W; at 121m above sea level). The aerial part of the plant was harvested during the growth stage of the plant, every two weeks in the morning in the same field and at the same atmospheric conditions, during a period from the beginning of April to the end of June (from the pre-flowering to the post-flowering stage). This region is known to be sunny all day with significant rainfall season every year. The identification of this plant was confirmed by Professor Mohamed Kadiri (botanist in Biology Department, Faculty of Sciences, Tetouan, Morocco). The essential oil of all air-dried samples (500 g) was isolated by simple hydrodistillation for 3 h . The distillated oils were dehydrated by anhydrous sodium sulfate and stored in tightly closed dark vials at 4 °C until analysis.

2.2. Extraction of essential oils from hydrolats

To extract the essential oils dissolved in hydrolats, we used a liquid-liquid extraction using a polar organic solvent capable of extracting the maximum of remaining essential oil dissolved in the hydrolat (Dichloromethane CH_2Cl_2)

[16]. The recovered organic phase was dehydrated by anhydrous sodium sulfate, and then the oily residue was obtained after removal of solvent under reduced pressure by rotary evaporator.

2.3. Chromatographic analysis

The analysis of the EOs was performed on a GC–MS (Agilent Technologies, J&W Scientific Products, Palo Alto, CA, USA), equipped with an Agilent Technologies capillary DB-5MS column (30 m length; 0.25 mm i.d.; 0.25 mm film thickness), and coupled to a mass selective detector (MSD5975B, ionization voltage 70 eV; all Agilent, Santa Clara, CA). The carrier gas was He and was used at 1 ml min^{-1} flow rate. The oven temperature program was as follows: 1 min at 100 ◦C ramped from 100 to 260 ◦C at 4 ◦C min−1 and 10 min at 260 ◦C. The chromatograph was equipped with a split/splitless injector used in the split mode. The split ratio was 1:100. Identification of components was assigned by matching their mass spectra with Wiley and NIST library data, standards of the main components and comparing their Kovats retention indices with reference libraries [17] and from the literature. The component concentration was obtained by semi-quantification by peak area integration from GC peaks and by applying the correction factors.

3. Results and Discussions

3.1. Yields of essential oils and those extracted from corresponding hydrolats

As shown in Figure 1, the yields (w/v) of EO obtained from Thymbra capitata samples increase with the growth of the plant to reach the maximum at the post-flowering stage. It ranged from 0.29% to 2.91% (sample harvested at the end of June) (figure 1). This result is consistent with a study conducted by Moldão-Martins et al. (1999) [18] for EOs of species of Thymus zygis. It also coincides with that of Bounatirou et al. (2007) [19] who showed that the yield of Thymbra capitata EO from Tunisia harvested in various localities during different growth phases varied between 1% and 6%. Tonçer and Kızıl (2005) [20] reported also that the highest EO content of Thymbra spicata (L.) (2.33 %) was at the full-flowering stages. This is due to high yields of fresh and dry biomass and content of oil at this stage. Similarly, the yield of HEO kept the same sense of variability up to the flowering stage (0.54%) by increasing from 0.11% (pre-flowering stage). And then, it began to decrease to reach 0.31% (sample harvested by the end of June at post-flowering stage) (figure 1).

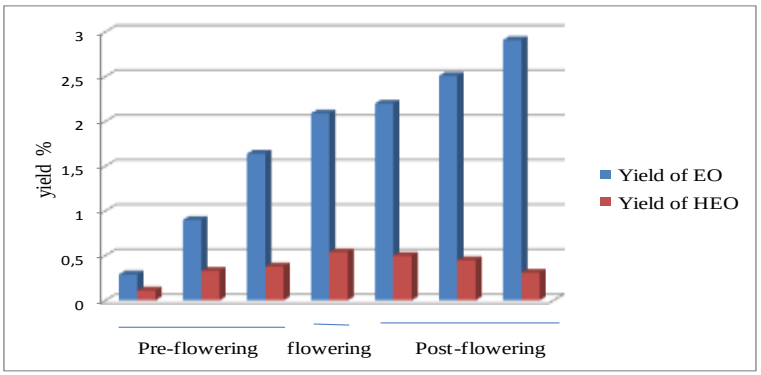

Figure 1. yield variation of *Thymbra capitata* essential oils and those extracted from corresponding hydrolats through its growth stage

This variation of yield was explained by Müller- Riebau et al. (1997) [21] who proposed that a close relationship exists between the growth stages and production of essential oil in the plants. Also, according to Sellami et al. (2009) [22]**,** the accumulation of essential oils during the full-flowering stage could be related to ecological roles such as intensifying antifungal defenses and attracting pollinators.

3.2. ***Chemical composition of essential oils and those extracted from corresponding hydrolats.***

The chemical constituents identified by GC–MS are presented in the Table. Fifteen constituents were identified in oils. Their sum constituted the bulk of the oils and ranged from 95.64% up to 97.82% of EOs. The phenolic compound carvacrol was the predominant constituent of Thymbra capitata EO at all growth stages, confirming that Thymbra capitata is the carvacrol chemotype. This result is in line with other studies carried out on this species worldwide [19, 23-32]. The concentration of carvacrol ranged from 77.97% to 84.26% with the higher concentration noted at the flowering stage in a sample harvested on May 16th (84.26%), which is consistent with the results obtained by Bel Hadj Salah et al. (2006) [33] and Bounatirou et al. (2007) [19]. p-cymene and γ-terpinene concentrations ranged between 3.84 -6.01% and 3.45-5.68% respectively. Furthermore, the only sesquiterpene present was β-caryophyllene with concentrations between 2.33% and 3.84%. In other hand, Nejad Ebrahimi et al. (2008) [34] reported also that Thymus caramanicus EO is rich in carvacrol at flowering stage (68.9%). In HEO, GC/MS analysis resulted in the identification of 09 compounds representing from 98.31 to 98.98% of the oils through the growth stage of the plant (table). Carvacrol concentrations varied between 96.39 and 98.54% with the maximum concentration noted at the pre-flowering stage. The presence of carvacrol in hydrolats with this important rate is due to its hydrophilic character. Moreover, contrarily to EO, HEO was characterized by the absence of *p*-cymene, *γ*–terpinene and *β*-caryophyllene at the pre-flowering stage and very low concentrations at the other stages; from 0.22 to 0.54% for *p*-cymene; from 0.16 to 0.49% for *γ*–terpinene and from 0.21 to 0.32% for *β*-caryophyllene. Other monoterpene hydrocarbons such as *β*-thujene, *α*-pinene, *β*-ocimene, *3*-carene and terpinolene were completely absent in HEO. Generally, the hydrophilic oxygenated constituents are found in large quantities in hydrolats, whereas the terpene lipophilic compounds are almost absent [35-37], which is in concordance with our study. It is noteworthy that our results are completely in concordance with those of Amarti et al. (2011) [38] who conducted a study on certain species of Thymus genus and concluded that *Thymbra capitata* EO of Morocco is mainly composed of carvacrol (70.92%) along with other compounds at relatively much lower levels: *p*-cymene (6.34%), *γ*-terpinene (4.92%), linalool (3.86%), *E*-caryophyllene (3.57%) and *β*- pinene (2.48%). This dominance of carvacrol in *Thymbra capitata* EO is also reported by Karpouhtsis et al. (1998) [39] and by Goren et al. (2003) [40]. However, Cosentino et al. (1999) [41] reported that *Thymbra capitata* EO of Sardinia (Italy) is dominated by thymol (29.3%) while carvacrol accounts 10.8% of this oil. The evolution of carvacrol concentration in EO showed in the table seems to be the opposite of that of *γ*–terpinene and *p*-cymene, which might be explained by the simultaneous bioconversion of *p*-cymene and *γ*–terpinene to carvacrol as biogenetic precursors of carvacrol and thymol [21].

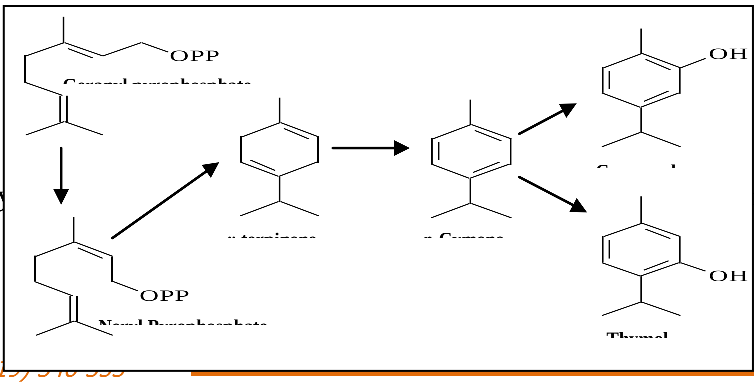

Figure 2: General biosy … rol

Table 1. Evolution of the chemical composition of pure essential oils and those dissolved in corresponding hydrolats of *Thymbra capitata* during the growth stage

RI	Compounds	% peak area													
		Essential oil							Essential oil dissolved in hydrolat						
		Pre-flowering			Flowering	Post –flowering			Pre-flowering			floweri ng	Post –flowering		
		April			May	June			April			May	June		
		1th		16th		1th		16th	1th		16th		1th		16th
		30th			16th	30th			30th			16th	30th		
924	α–thujene	0.52	0.42	-	-	-	-	-	-	-	-	-	-	-	-
933	α–Pinene	0.21	0.17	0.29	0.19	0.36	-	-	-	-	-	-	-	-	-
1005	δ3-Carene	0.46	0.37	0.19	0.36	0.11	1.04	1.00	-	-	-	-	-	-	-
1014	*p*-Cymene	4.64	4.60	4.66	3.84	6.01	4.62	4.52	-	-	-	0.54	0.25	0.22	0.23
1021	β–Phellandrene	0.11	-	0.69	0.46	0.90	0.87	0.93	-	-	-	-	-	-	-
1028	b-Ocimene, (Z)-	-	-	0.52	-	-	0.34	0.62	-	-	-	-	-	-	-
1050	γ–Terpinene	5.68	3.99	5.66	3.66	5.12	4.09	3.45	-	-	-	0.49	0.16	0.18	0.23
1056	Sabinene hydrate, *cis*	0.39	0.28	0.30	-	-	0.38	0.44	0.15	0.12	-	0.15	0.13	0.12	0.16
1079	Terpinolene	1.03	0.77	1.12	0.76	1.21	-	-	-	-	-	-	-	-	-
1086	Linalool	-	-	-	-	0.95	0.99	0.95	-	-	-	-	0.18	0.19	0.11
1152	Borneol	0.18	0.21	0.15	0.19	0.21	0.14	0.15	0.07	0,07	0.07	0.09	-	-	-
1163	4-Terpineol	-	-	-	0.40	0.43	0.12	0.12	-	0,11	0.10	-	-	-	-
1270	Thymol	0.18	0.16	0.17	0.28	0.45	0.24	0.24	0.19	0,18	0.18	0.29	0.37	0.33	0.24
1278	Carvacrol	79.01	80.83	79.66	84.26	77.97	80.54	82.23	98.54	98.50	98.42	96.39	97.33	97.18	97.69
1419	β–Caryophyllene	3.80	3.84	3.30	2.33	3.16	3.58	3.17	-	-	-	0.32	0.27	0.27	0.21
	Total rate %	96.21	95.64	96.71	96.73	96.88	96.95	97.82	98.95	98.98	98.77	98.27	98.69	98.31	98.87
	Yield	0.29	0.90	1.64	2.09	2.2	2.51	2.91	0.11	0.33	0.38	0.54	0.5	0.45	0.31

In other hand, *p*-cymene recorded its maximum when carvacrol scored its minimum, which is confirmed by Hedhili et al., (2002) [27] and Bounatirou et al.(2007) [19]. The metabolic pathways of carvacrol and thymol formation begin with aromatization of γ-terpinene to *p*-cymene followed by hydroxylation of *p*-cymene to thymol. Carvacrol results from the desaturation of γ-terpinene to *p*-cymene followed by hydroxylation at C-2 of ring. Hence, this shows the key role played by the γ-terpinene in the flavoring process and by *p*-cymene as a precursor for oxygenates compounds [42,43]. The γ-terpinene results from the biosynthetic chain from acetyl-CoA, this later leads to the synthesis of terpenoids through the formation of geranyl-pyrophosphate (Trans), that turns into Neryl Pyrophosphate (Cis) then by

a simple cyclization of the latter [44] (Figures 2). The results represented in the Table, showed a remarkable concentration of carvacrol in the plant (in EO and HEO) throughout its growth stage, while the amounts of *p*-cymene and γ-terpinene remained low. It is so probable that almost all the *p*-cymene and γ-terpinene compounds are converted into carvacrol according to the general biosynthetic pathways of these aromatic monoterpenes represented in figure 2. In a major review of the composition of thymus oils, Stahl-Biskup (1991) [45] listed a number of contradictory studies of seasonal effects on oil yield. Most oils were produced from flowering plants, because the oil content was generally found to peak at that time. For phenol-containing oils, it seems that the phenol content peaked at the onset of flowering or when the plants were in full flower, which is in line with our results. However, a report on *Thymus vulgaris* grown in northern Italy indicated that phenol content at full flowering varied from year to year [46]. The main component was *p*-cymene (25.3%) in 1986, and thymol (38.2%) in 1987. Few of the previous studies used replicated sampling and statistical analysis techniques.

4. Conclusion

The result acquired by this work reveals the period when we can have a better yield in EO and HEO extracted from *Thymbra capitata* by hydrodistillation. It also shows the period in which the phenolic compound carvacrol reaches its maximum concentration in these oils. Moreover, this study confirmed that the monoterpene hydrocarbons, namely *p*-cymene and γ–terpinene, have a biosynthetic relationship with the phenolic compound. Hydrolats of this plant contain very high concentrations of carvacrol (98.54% in sample harvested in the beginning of April) which makes these oils as quasi-pure product. From an economic point of view, it is better to harvest this plant at its post-flowering stage to reach maximum yield, while its harvest at the flowering stage provides us with a maximum concentration of carvacrol as the active compound of this plant.

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