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Full Length Research Paper

Variation of thymol, carvacrol and thymoquinone production from wild and cultivated *Origanum syriacum* of South Lebanon

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Essential oils from wild and cultivated *Origanum syriacum* were prepared monthly by hydrodistillation. Their composition in thymol, carvacrol and thymoquinone were studied by Gas chromatography–mass spectrometry (GC-MS). Essential oils extraction ranged from 3.82 and 3.44% in February to 8.4 and 6.8% in may, from cultivated and wild samples respectively. The essential oil composition for all samples was dominated by carvacrol. The cultivated populations harvested in April just before flowering had a higher peak content in thymol (31.5%) and carvacrol (49.8%). Thymoquinone was detected in traces only in February, and was not found in any other population studied from *O. syriacum* of South Lebanon.

Key words: *Origanum syriacum*, essential oil, carvacrol, thymol, thymoquinone.

INTRODUCTION

Origanum syriacum belongs to the mint family, Labiates, *Origanum* genus and Majorana section group B (Skoula and Harborne, 2002). It inhabits large area in the eastern Mediterranean, and grows from nearly sea level up to at least 2000 m in rocky soils, often on limestone. In South Lebanon, *O. syriacum* grows naturally in mountains and regions that surround the Litanie River. It is an important dietary source, collected from wild, as well as from cultivated fields. The production of cultivated *Origanum* has increased in recent years in response to increasing local demands. Owing to the antioxidant, antimicrobial, insecticidal and antifungal activities of their essential oils, *Origanum* species have recently been of great interest as potential therapeutic substances and as natural additives to replace synthetic products in food industry (Aslim and Yucel, 2008; De Souza et al., 2010; Ocana-Fuentus et al., 2010). The biological activity of essential oils is related to the chemical composition, functional groups and their synergistic effects.

The *O. syriacum* essential oils, like many species of the genus, *Origanum* and *Thymus*, are dominated by two phenolic compounds, thymol and carvacrol. Essential oils composition can vary according to climate, geographical location and vegetative stage (Abu Lafi et al., 2007, 2008). Thymoquinone, a bioactive compound, was detected in the populations of *O. syriacum*. It is a phytochemical compound found essentially in the plant *Nigella sativa*. It has antioxidant, analgesic, anti-convulsant effects, it is an angiogenesis inhibitor that has been shown to protect against heart, liver and kidney damage in animal studies, as well as having possible anti-cancer effects (Gali-Muhtasib et al., 2006; Koka et al., 2010; Torres et al., 2010). In order to obtain favorable contents of bioactive components, we study the variability of essential oils production and composition in different vegetation conditions. So, in this work we analyze the monthly production of essential oils and their composition in thymol and carvacrol in wild and cultivated *O. syriacum* from South Lebanon. The presence of thymoquinone in Lebanese *O. syriacum* was never been documented. So, in order to diversify the use of this plant, the detection of thymoquinone in the samples studied, was planed.

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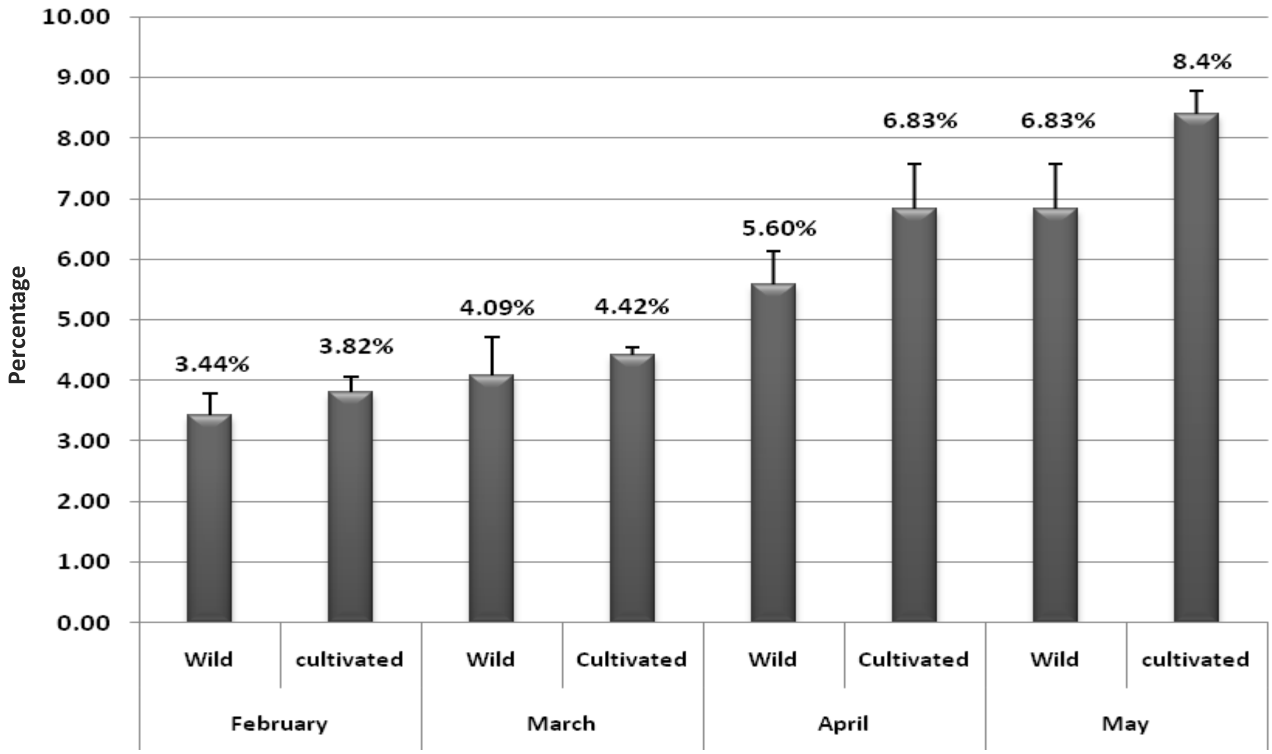

Figure 1. Yield variation of essential oil in wild and cultivated *O. syriacum* from February until May 2010.

MATERIALS AND METHODS

Reagents

The essential oil standards (thymol, carvacrol and thymoquinone), were purchased from Sigma-Aldrich Inc. (USA), and GC grade n-hexane 95% from Merck.

Plant material

Leaves of wild and cultivated *Origanum* samples were collected from the same environment site in Tyre region of South Lebanon, on February, March, April and May 2010. The identification of plant material was done according to botanic description as indicated by Mouterde (1978). Voucher specimens are kept at the laboratory of Biotechnology of Natural Substances and Health products, Faculty of Pharmacy. All plant samples were air dried in the absence of light at room temperature for one week.

Hydrodistillation

The determination of essential oils was carried out by steam distillation as prescribed by European Pharmacopeia (2008).

GC-MS analysis

The quantitative and qualitative analyses were performed on an Agilent Technologies GC-6890N coupled with an Agilent Technologies 5975B inert Mass Selective Detector (MSD) (mass range m/Z 50 to 550). The GC was equipped with a silica capillary column HP-5MS (30 m x 0.25 mm i.d., 0.25 µm film thickness). The GC-MS operating conditions were as follows: Helium was used as carrier gas. Injector and detector temperatures were 260 and 280°C, respectively. Split ratio was 1:50. The column temperature was held at 60°C for 4 min, then raised from 60 to 100°C at 5°C/min, and from 100 to 280°C at 9°C/min. Injection volume was 1µL. Duration program was 36 min. The identification of the compounds was based mainly on their retention times (RT) and mass spectra in comparison with those from authentic standards (RT: 15.518, 16.312, 16.505 and m/Z= 164 to 149,150 to 135,150 to 135 respectively for thymoquinone, thymol and carvacrol). The quantitative composition was obtained by peak area normalization. Each sample was analyzed in triplicate.

RESULTS AND DISCUSSION

The aim of this study is to compare, first, a monthly variation of essential oils quantities yielded from leaves samples of both wild and cultivated *O. syriacum* harvested at the same region (Tyre) of South Lebanon. A comparison between essential oils yield in these 2 populations on each month, from February to May 2010, is represented in Figure 1. Secondly we compare the composition of these oils in thymol, carvacrol and thymoquinone. The ratios in thymol and carvacrol are represented in Table 1. Variation in phenol content is illustrated in Figure 2, while the chromatogram obtained from wild *O. syriacum* in February is represented in Figure 3. As mentioned earlier, the composition of

Table 1. Ratios of thymol and carvacrol from essential oils of wild (W) and cultivated (C) samples in February until May 2010*.

Month (samples)	RT**	Thymol (%)	RT**	Carvacrol (%)
February (W)	-	-	16.60	34.58±2.58
February (C)	-	-	16.62	37.42±2.31
March (W)	16.39	7.15±0.26	16.79	38.81±2.64
March (C)	16.47	12.46±1.53	16.77	43.75±2.89
April (W)	16.49	10.55±0.68	16.77	39.00±0.48
April (C)	16.40	31.47±3.59	16.89	49.77±1.63
May (W)	-	-	16.67	36.82±0.15
May (C)	16.47	10.64±0.15	16.84	47.54±3.08

*Values are arithmetic mean peak area percentages± standard deviations. **RT=retention time.

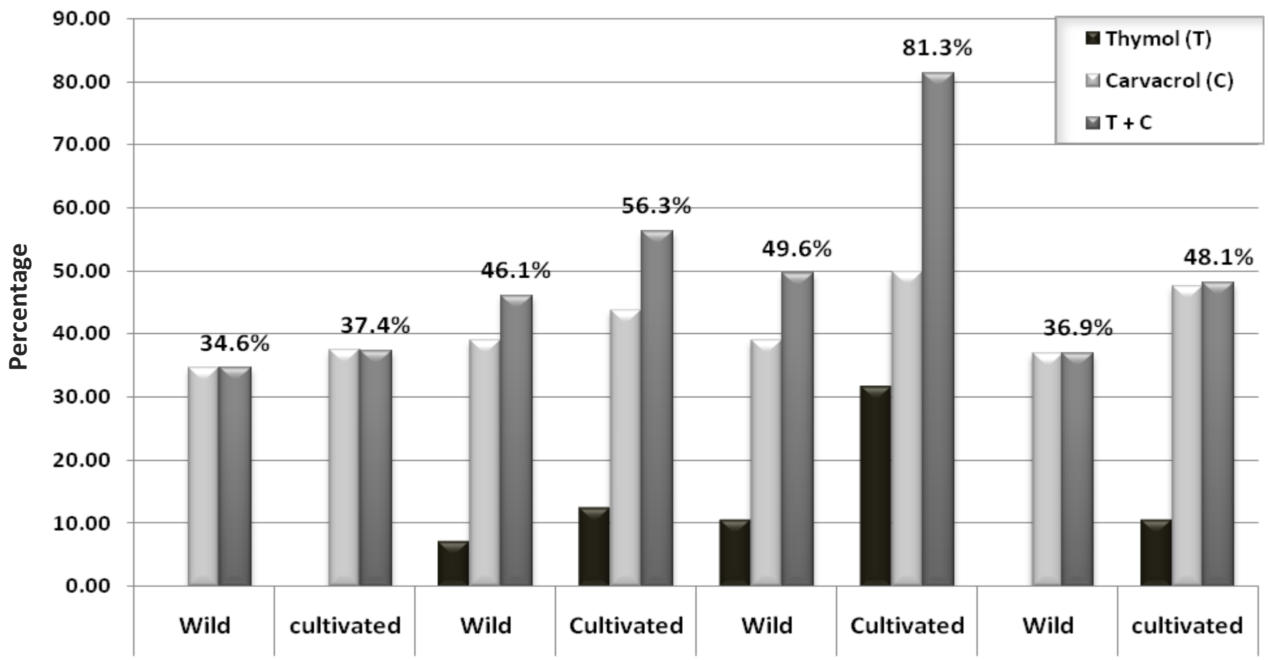

Figure 2. Yield variation in thymol and carvacrol in essential oil of wild and cultivated *O. syriacum* from February until May 2010.

essential oils can vary according to climate (temperature, light, and humidity), soil composition, geographical location and vegetation stage. But our focus was to study the variation of composition according to vegetation stage; therefore all samples were harvested from same region and in same climate factors in order to eliminate the impact of any other factors.

The quantity of essential oils obtained by hydrodistillation changed according to vegetation stage, and it was in increasing rate from February to May. It ranges from 3.44% in February to 6.83% in May in wild populations, and from 3.82% in February to 8.4% in May in cultivated populations. Comparable results were found in previous studies of *Origanum* sampled in Palestine (Abu-Lafi et al., 2007, 2008), in Lebanon (Zein et al., 2011) and in Turkey (Toncer et al., 2010). Cultivated populations yielded more essential oils than wild populations, harvested at the same vegetation stage (8.4% in May from cultivated versus 6.8% from wild *Origanum*). The maximum coefficient of variation (cv) was 15% which is an acceptable value according to the small number of samples (n=3). Thus, enhancement of cultivated *Origanum* and harvesting samples in May allowed more yield of essential oils. Extracting large quantities of essential oils is not suitable by hydrodistillation, because it is very time-consuming (Richter and Schellenberg, 2007). *O. syriacum,* with regard to its main compounds carvacrol and thymol, is highly polymorphic. In this study, all samples were of carvacrol type, and their average values range between

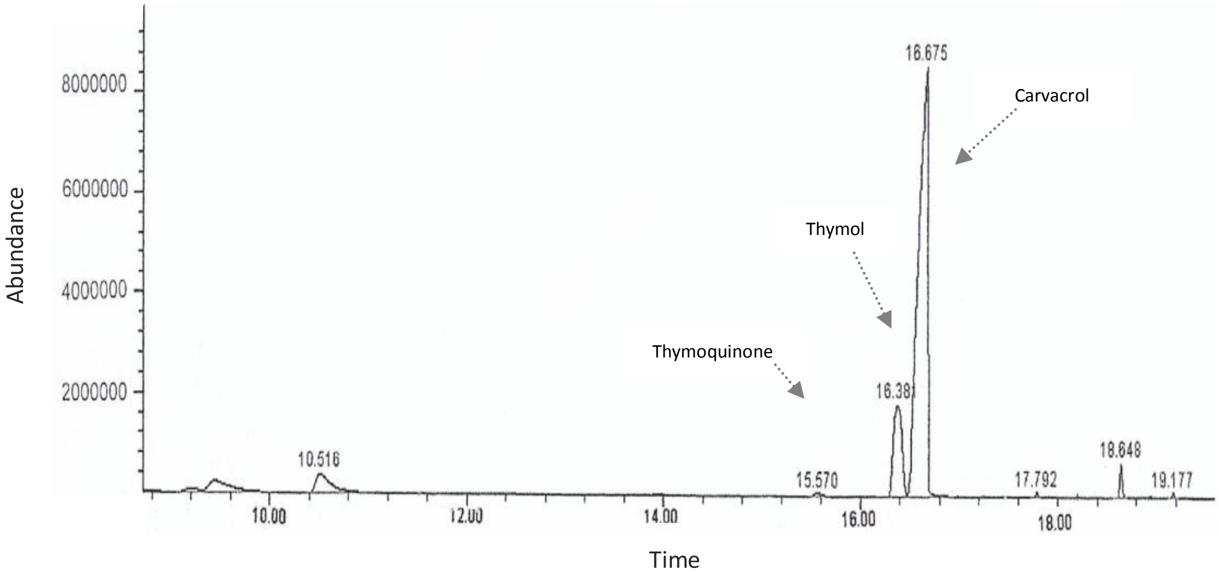

Figure 3. Chromatogramm of thymol and carvacrol obtained after injection of essential oil from wild *O. syriacum* harvested in February 2010.

34.6 and 50%, whereas average thymol values range between 7.5 and 31.5% (Table 1). In our previous study, wild *Origanum* in the same region was of thymol type, and cultivated samples were of carvacrol type (Zein et al., 2011). In many studies of *O. syriacum* of Mediterranean region, one of these two major compounds was over dominating. Data about this change in phenolic type was not available. So, polymorphism studies may explain this slight difference and may predict the phenolic type of *Origanum*.

On the other hand, the content of essential oils in thymol and carvacrol increase progressively from February to April, where it reaches 81% in cultivated population and 40% in wild population. Then, the content decrease in May and the average values were 37 and 58%, respectively in wild and cultivated populations (Figure 2). Our previous study shows comparative results, where the poor content of thymol and carvacrol in February and March was accompanied by increased rate of γ-terpinene and p-cymene, which are the precursors of thymol and carvacrol. This finding may help researchers and industrials about optimal harvesting time, allowing yielding oil with high content in thymol and carvacrol. The optimal harvesting time was in April just before flowering. In this study, thymoquinone was detected as a trace amount only in samples harvested in February. This promising anticancer molecule was found in essential oil of *O. syriacum* in previous studies.

It was detected as a trace amount in autumn, and its level peaked in January (9.36%) in Turkish thyme (Toncer et al., 2010). Also, from the Syrian varieties harvesting in full bloom in July, thymoquinone present in the samples was in a wide range between 0.04 and 24% (Lukas et al., 2009). Palestinian thyme was found to contain thymoquinone in samples harvested in January and February (Abu-Lafi et al., 2008). Thymoquinone was cited as one of component of essential oils of *O. syriacum* (Skoula and Harborne, 2002; Peter 2004). It was a dominant aglucone component from glycoside bound volatiles, within the genus *Origanum*, and was not present in essential oil in Algerian *Origanum* population (Belhattab et al., 2005). In previous studies, thymoquinone was not found in Lebanese varieties. Composition in thymoquinone may vary significantly among different genotypes. With regard to the importance of bioactive natural compound from *O. syriacum*, further studies should be applied, that addresses annual harvesting time, polymorphism and other factors that may affect production of these substances.

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