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Essential oil content and composition of *Vitex pseudo-negundo* in Iran varies with ecotype and plant organ

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ABSTRACT

Medicinal plants are rich in secondary metabolites that constitute the composition of many drugs. The quantity and quality of these valuable materials are affected by climatic factors and ontogenetic growth stages. In the present study, the effects of climatic factors and different plant parts (leaf, flower and fruit) were examined on the quantity and quality of vitex essential oil (EO) (*Vitex pseudo-negundo* (Hausskn.) Hand.-Mzt.) in eight different regions where the plant grows. Analysis of EO samples was performed by using GC and GC–MS. Results show that the main EO compounds in vitex are α-pinene (29.5–48.9%), limonene (9.2–14.3%), α-terpinyl acetate (0.6–22%) and (E)-caryophyllene (8.1–17.7%) in different plant organs and ecotypes. The principal component analysis (PCA) was applied to develop a model of the distribution of total EO content in different plant organs based on a complex of environmental factors. The main components were the longitude, latitude, altitude, annual average precipitation and annual average temperature. These components explained about 67% of the variations. Temperature, rainfall and elevation did not show significant independent regression relationships ($P \leq 0.05$) with the EO content, but had a significant correlation with some of the compounds in the EO. In different regions where the plant grows, the variations among EO contents ranged from 0.21 to 0.76% in different plant organs. The maximum and minimum EO contents were found in the leaves and fruits, respectively.

1. Introduction

The Lamiaceae family is richly diverse and constitutes an important part of edible vegetation in the human diet, especially in dry and arid environments. Secondary metabolites are commonly produced by these plants and thus play important ecological and biological roles. Biological activities are diverse among the EOs of species in the Lamiaceae family. This may be a manifestation of their rich chemical diversity, and in part explains the extensive amount of research that have already investigated the chemical composition of plants in this family, and its relevance with taxonomy, biogeography and medicine (Giuliani and Bini, 2008; Lakušić et al., 2012). Vitex or the chaste tree grows in the wild in different parts of the world, ranging from Mediterranean regions to Central Asia and Southern Europe (Mozdianfard et al., 2012). *Vitex pseudo-negundo* (Hausskn.) Hand.-Mazz. is a synonym for *Vitex agnus-castus* L. (A working list of all plant species http://www.theplantlist.org/).

The genus *Vitex* (Lamiaceae) includes almost 250 species (Kosovac et al., 2016) while some of them have been widely used for medicinal purposes throughout the world (Senatore et al., 1996). Different species of the genus *Vitex* are prevalently used as traditional medicines across the globe (Kuruüzüm-Uz et al., 2003). Over the past few years, research has revealed that *V. agnus-castus* affects the female hormonal system, and this finding has received considerable attention so far, since clinical preferences encourage the search for non-aggressive, alternative hormonal therapies with little or no side effects (Villella, 2016). The dried fruits of this plant are known to be raw materials when preparing against pre-menstrual disorders stemming from primary and secondary insufficiencies of the corpus luteum, or mastodynie and menopausal distress. The fruits were previously a substitute for pepper from Italy to Eastern Georgia, where it is still being consumed to some extent (Novak et al., 2005). The fruit has a taste and aroma similar to that of pepper, and its infusion is traditionally practiced (about 2–5% w/v) for its diuretic properties in Turkey where the fruit is also used as a sedative

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and digestive agent (Senatore et al., 2003). The fruits of *V. agnus-castus* are popular phytomedicines in Europe (Pal et al., 2013). In recent years, several reports have described investigations in the realm of phytochemistry which claim that plants in this genus produce diterpenoids, flavonoids, terpenoids, steroids and iridoids (Pal et al., 2013). Physical factors like temperature, atmospheric pressure, wind velocity, prolific precipitation and altitude can all affect plant growth (Körner, 2007), plant physiology and morphology (Hoch and Körner, 2003), and differentiation, from a chemical perspective, among and within species (Djerrad et al., 2015). Furthermore, it is frequently observed that the chemical composition of EOs mostly correlates with geographic variation (Chalchat et al., 1993; Djouahri et al., 2015; Jamshidi et al., 2009), environmental and agronomic conditions (Moghtader and Afzali, 2009), the phenological stage of plants (Ruberto and Baratta, 2000) and harvest time (Yesil-Celiktas et al., 2007). There are two different sets of chemical compositions reported in the literature for the EO of *V. pseudo-negundo* which are different depending on the locality of plant distribution in northern humid-subtropical and central semi-arid regions of Iran (Safaei-Ghomi and Meshkatalsadat, 2011). The diversity indicates that chemical composition depends greatly on climate. Generally, the leaves contain higher amounts of EOs, while the inflorescences and fruits also contain large quantities of EO, in which the main compounds are 1,8-cineole, (E)-β-farnesene, sabinene, β-pinene, α-terpineol, α-terpinyl acetate, β-caryophyllene and bicyclogermacrene (Senatore et al., 1996; Sørensen and Katsiotis, 2000).

2. Material and methods

2.1. Plant collection area

This study was carried out in two years, from 2014 to 2015, at eight experimental sites in the Fars Province of Iran (Fig. 1). The climate and geographical data of collection sites are shown in Table 1.

2.2. Plant material

Fruits, leaves and flowers of vitex were collected from eight different populations in the wild, and the timespan of plant collection started from December 2014 to June 2015 in the Fars Province of Iran. Leaves, flowers and fruits were separated from branches and were air-dried at room temperature (less than 25 °C) in a shady location for 14 days.

The plant species was identified and authenticated by A. Khosravi, a plant taxonomist at Shiraz University, Shiraz, Iran. The voucher specimens (55006) were deposited in the herbarium.

2.3. Extraction and analysis of essential oil

The EO extraction was carried out by hydrodistilltion using an all glass Clevenger-type apparatus to extract the EO (W/W%) according to the method recommended by the European Pharmacopoeia (Formisano et al., 2015; Mechergui et al., 2016; Singh et al., 2017). For this, dried aerial parts of the leaves (100 g), fruits (60 g) and flowers (50 g) were placed in 1100 mL distilled water in a round bottomed flask (2000 mL). The extraction process took 3 h and had 3 replications for each sample. The yellow EO was dried over anhydrous sulfate and was stored in sealed vials in the dark at 4 °C prior to GC and GC–MS analysis (Duymuş et al., 2014).

2.4. Essential oil analysis procedure

The components of volatile oil from the plant samples were identified using GC and GC–MS analyses. The GC analysis was performed using an Agilent gas chromatograph series 7890-A equipped with a flame ionization detector (FID). The analysis was carried out on fused silica capillary HP-5 column (30m × 0.32 mm i.d.; film thickness 0.25 μm). The sample volume injected into the GC was 0.2 μL pure EO. The temperature of injector and detector was set at 250 °C and 280 °C, respectively. Nitrogen was used as carrier gas at a flow rate of 1 mL min; the oven temperature program was 60–210 °C at the rate of 4 °C.min, which was then programmed to 240 °C at the rate of 20 °C.min, and finally, held isothermally for 8.5 min. The split ratio was 1:50. The GC–MS analysis was carried out by the use of Agilent gas chromatograph equipped with fused silica capillary HP-5MS column (30m × 0.25 mm i.d.; film thickness 0.25 μm) coupled with 5975-C mass spectrometer. The sample volume injected into the capillary column was 0.1 μL pure EO in the split mode (1:50). Helium was used as carrier gas with the ionization voltage of 70 eV. The temperature of ion source and interface was 230 °C and 280 °C, respectively. Mass range was from 45 to 550 amu. The oven temperature program was the same as for the GC. The retention indices for all components were determined according to the method using *n*-alkanes as standard.

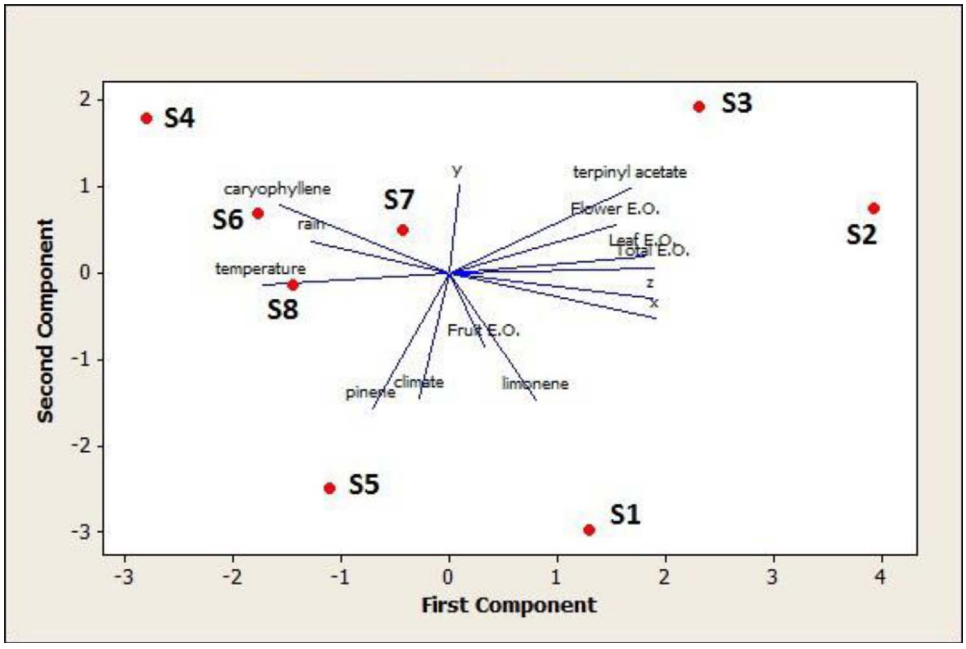

Fig. 1. Different sampling locations of *Vitex pseudo-negundo* plants from Fars province.

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