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Research paper

Summer savory (*Satureja hortensis* L.) essential oil constituent oscillation at different storage conditions

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ABSTRACT

Storage situation had important role in active substances quantity and quality of medicinal and aromatic plants. A little research was focused in secondary metabolite changes after extraction. In this research, a factorial experiment based on completely randomized design with three replications was conducted. Treatments were three levels of storage temperature including room temperature (21 ± 2 °C), refrigerator temperature (4 °C) and freezer temperature (–20 °C) and three levels of storage time (T1: no storage, T2: three months storage, T3: six months storage). Essential oils after extraction by hydro-distillation method were analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The results showed that, in each of the three storage temperatures, some compounds such as α-Thujene, β-Pinene, α-Phlandrene, α-Pinene, α-Terpinene, Myrcene and along with γ-Terpinene as Carvacrol precursors were decreased. However, Carvacrol content increased after 6 months, significantly. Also the amount of ρ-Cymene, β-Phellandrene were increased. The major constituent of the *S. hortensis* essential oil such as Carvacrol was increased in all three storage temperatures, so that the highest and the lowest increasing occurred at freezer and room temperatures, respectively. Storage of Summer savory essential oil in the freezer enhanced the quality.

1. Introduction

Essential oils make up a large part of aromatic substances in plants. Generally, essential oils are considered as remnants of main processes of plants metabolism, especially in stressful conditions (Omidbaigi, 2005). They are made by medicinal and aromatic plants and known as secondary metabolites (Bakkali et al., 2008). Essential oils, are mainly composed from terpenoid compounds or combinations that have terpenes origin. They are not homogeneous in terms of chemical composition and they are observed as the different combinations. Usually, a set of different terpenoids, make essential oils of a plant or plant materials. Essential oils have usually a special odor and flavor depending on their total nature (Omidbaigi, 2005).

Essential oils are present in secretory cells and trichomes single or complex, secretory glands, secretory ducts in surface and internal parts of different organs like leaves, flowers, fruits, buds and shoots of plants. They have antimicrobial, anti-inflammation, anti-spasmodic, sedative, carminative, appetizer and sometimes expectorant properties, for this reason, they are used in various industries including the pharmaceutical, food and cosmetics. Essential oils have many applications in perfume industries and aromatherapy (Hajhashemi et al., 2003; Nychas et al., 2003; Perry et al., 2003; Silva et al., 2003; Omidbaigi, 2005; Bakkali et al., 2008).

Summer savory (*Satureja hortensis* L.) is one of the most important plants in Lamiaceae family, which its more than 30 species grow in the East Mediterranean (Hadian et al., 2008). It is one of the oldest plants that have been used as vegetables and medicinal plants and aromatic. Dried Summer savory has been introduced as one of the most pleasant spices and this plant are planting large areas of farmland in many countries, yearly (Omidbaigi, 2005). Generally, the aerial parts of it, that is usually harvested at flowering stage, has therapeutic effects such as facilitating digestion, stomach tonic, diuretic, astringent, carminative, anti-diarrhea and anti-worm. Summer savory essential oil is used in industries food (conserve and beverages) and pharmaceutical (Hajhashemi et al., 2000; Sefidkon et al., 2007). Various studies on *S. hortensis* essential oil has been shown that it contain high amounts of phenolic compounds like Carvacrol, γ-Terpinene, Thymol, *p*-Cymene, β-Caryophyllene, Linalool and other terpenoids. Although, reports based on difference in essential oil content and composition of *S. hortensis* in various species has been published (Rojas and Usubillaga, 2000; Viturro

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et al., 2000; Kurcuoglu et al., 2001; Baher et al., 2002; Baser et al., 2004; Sefidkon et al., 2006; Svoboda et al., 2006; Novak et al., 2006). Essential oils of savory species that contain higher Carvacrol and Thymol, have a stronger antimicrobial effect (Sefidkon et al., 2007) and are more suitable for use in pharmaceutical industry. However, presence of relatively appropriate levels of other essential oil composition have an important role in *S. hortensis* essential oil quality due to the strengthening antibacterial effect of Carvacrol (Ben Arfa et al., 2006).

Factors such as temperature, light and oxygen availability cause chemical changes in the essential oils components during storage (Nguyen et al., 2009). Active enzymes in making the changes are oxidases, peroxidase, hydrolases and isomerases and lead to change in the nature of essential oils so that, essential oils may have effects apart from their respective effects. For example, oxidized essential oils may cause allergic skin reactions (Pirila and Siltanen, 1958; Hausen et al., 1999; Matura et al., 2005; Omidbaigi, 2005; Hagvall et al., 2007; Bared-Christensson et al., 2009). Few studies have been conducted in relation to the effect of storage conditions on the stability of some medicinal plants essential oil, including Fennel (Toth, 1967), *Carum carvi*, *Cuminum cyminum* and *Pimpinella anisum* (El-Wakeil et al., 1986), Parsley (El-Nikeety et al., 1998), Biter fennel (Braun and Franz, 1999), Marjoram (Misharina et al., 2003), Laurel, Fennel and Coriander (Misharina and Polshkov, 2005), Lemon (Nguyen et al., 2009) and *Thymus fontanesii* (Haddouchi et al., 2011).

Owing to the increasing tendency of human to natural compounds like essential oils, it seems necessary to further study for their biological activities for novel applications in different fields such as human health, agriculture and environment. Some of them can are replaced by similar chemical compounds, without side effects (Carson and Riley, 2003). Little research has been conducted on the chemical changes of essential oils during storage while, it is necessary for consumers and producers in various industries, to ensure their effectiveness and quality of manufactured products from essential oils. For this reason, the aim of this study was to evaluation of chemical changes of *S. hortensis* essential oil during storage at different conditions.

2. Materials and methods

2.1. Plant material and isolation procedure

In order to ensure uniformity of plant material, *S. hortensis* cv. Saturn was cultivated at Karaj (Research Station of Horticultural Science, University of Tehran, altitude 1320 m, longitude 51°E, latitude 35°48′N) during growing season of 2014–2015. The plants were harvested at full flowering stage, when they have the highest essential oil, and were dried in oven at 40 °C. The essential oils of dried samples were isolated by hydro distillation for 3 h, using a Clevenger-type apparatus (British Pharmacopoeia, 1988).

2.2. Treatments and essential oils storage conditions

For studying the effect of storage circumstance on summer savory essential oil constituents, a factorial experiment (including two factors: storage time and storage temperature) based on completely randomized design with three replications was conducted. Treatments were consists of three levels of storage temperature consist of room temperature (21 ± 2 °C), refrigerator temperature (4 °C) and freezer temperature (–20 °C) and three levels of storage time (T1: no storage, in this treatment components were identified by GC–MS after the essential oils were taken immediately, T2: three months storage, T3: six months storage). Essential oil samples were stored in glass flasks in the dark for 6 months at room temperature (21 ± 2 °C), refrigerator (4 °C) and freezer (–20 °C) and after the end of each storage time period, the essential oil composition of different treatments were measured.

2.3. Essential oils analysis and identification

To identify the components of the essential oils was used Agilent Technologies-7890A gas chromatograph. Type, length, diameter and thickness of column were HP-5-MS, 30 m, 0.32 mm and 0.25 µm respectively. Temperature program of column was 60–210 °C at the rate of 4 °C/min. Nitrogen carrier gas was at a flow rate of 0.5 ml/min. Gas chromatograph connected to a mass spectrometer (GC–MS) was an Agilent Technologies-5975C model. Column type was HP-5MS with 30 m in length, 0.25 mm in diameter and 0.25 µm in thickness. Temperature program was 280 °C and Helium carrier gas was at a flow rate of 1 ml/min.

According to the area under the curve of each chromatogram peaks and compared to the total area under the curve relative percentage of each of the components of oil were detected. Identifying spectra was done using a data bank of mass, retention time, Kovats index, study of mass spectra of each of the essential components and patterns of spectra refraction, compared to standard spectra and use of reputable sources (Adams, 2001).

2.4. Statistical analysis

All experiments were carried out in triplicate. Statistical analysis was performed by JMP software (version 8) and the averages obtained were compared by using Tuky tests (HSD) at 5% level of probability. Relationships between parameters were determined by Pearson's correlation test.

3. Results

The analysis of variance showed that temperature storage, time storage and their interaction effect had significant effect on many essential oil composition of *S. hortensis* (Table 1). Simple and interaction effects of treatments were not significant on some essential oil composition such as Camphene and Aromadendrene ($P > 0.05$), also effect of temperature storage were not found to be significant on Limonene, 1,8-Cineol and β-Phellandrene ($P > 0.05$) (Table 1). Interaction effect of temperature and time storage were not significant on Limonene, 1,8-Cineol and Terpinene-4-ol ($P > 0.05$), while treatments effect were significant on other essential oil composition ($p < 0.01$ and $p < 0.05$) that the most important compounds will be described (Table 1).

3.1. α-Thujene and α-Pinene

Under different storage conditions, the lowest of α-Thujene content (1.03%) was measured in freezer temperature at 6 months. Most of the changes during storage, was happened at freezer temperature. Changes in this compound at refrigerator, was not significant and it was almost constant. α-Thujene content initially rose slightly at room temperature and was reduced at 6 months significantly (Table 2).

Changes in α-Pinene content in different storage conditions was significant only in the freezer temperature so that minimum of α-Pinene content (0.72%) was observed in the freezer temperature at the 6 months storage and the other treatments were in the same group (Table 2).

3.2. β-Pinene and α-Phellandrene

β-Pinene decreased at the room temperature during the time, however the difference between the no storage and three months storage was not significant. Changes in β-Pinene content was not significant at the refrigerator temperature during storage while this composition decreased at freezer temperature over time, however, the difference between the no storage and three months storage was not significant, also the difference between 3 and 6 months storage was not