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The Effect of Different Harvest Stages on the Quality and Quantity of the Essential Oil of Tulsi (*Ocimum sanctum* L.)

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Abstract

This study was carried out at the research field of Tarbiat Modares University, Peykan Shahr, Tehran to determine the changes in the content and chemical compositions of essential oil of tulsi (*Ocimum. sanctum* L.) at three different growth stages including vegetative, flower bud formation and full flowering stages. The essential oils of the aerial parts were extracted by hydrodistillation method using Clevenger apparatus, and analyzed by GC and GC-MS. The hydro-distillation of the aerial parts of *Ocimum. sanctum* at the vegetative, flower bud formation, and full flowering stages yielded 0.95%, 0.87% and 1.3% (w/w) EO, respectively. The main constituent of the EO from the vegetative stage were 1, 8-Cineole and β -bisabolen, while eugenol was found as the main compound of flower bud formation and full flowering developmental stages.

Keywords: *Ocimum. sanctum* L.; Essential Oil; 1, 8-Cineole; β -bisabolen; Eugenol.

Introduction

Ocimum sanctum L. (Tulsi in Sanskrit or Reyhan-e- Moghaddas in Farsi) belonging to Lamiaceae family, is native to Asia, and Central and western parts of Africa. Tulsi is traditionally used as a medicinal plant (Prakash and Gupta 2005). Pharmacological studies and clinical practices have demonstrated that this species possesses anti-oxidative (Shah and Verma 2012, Ahmad *et al.*, 2012) and antimicrobial functions including antibacterial (Prakash and Gupta 2005, Agarwal *et al.*, 2012), antifungal (Kumar *et al.*, 2010, Balakumar *et al.*, 2011), antimalarial (Prakash and Gupta 2005) and anti-helminthic (Asha *et al.*, 2001). It has been also recommended to treat diabetes, bronchitis, diarrhea, dysentery, dyslipidemia, hypertension and skin diseases (Prakash and Gupta 2005, Pattanayak *et al.*, 2010, Singh *et al.*, 2001).

Chemical and biological diversity of the aromatic and medicinal plants differ significantly depending on the factors such as cultivation area, climatic conditions, genetic modification, different plant parts, developmental stages and collection time (Franz *et al.*, 1993, Saharkhiz *et al.*, 2009). In the recent years, numerous publications have reported the chemical compositions of the EOs of medicinal and aromatic plants demonstrating that the growth stage and harvesting time have a major impact on the EO content and compositions (Saharkhiz *et al.*, 2009, Ghani *et al.*, 2009). Therefore, it is necessary to determine the proper time and plant growth phase to harvest by

analyzing the EO and its compositions at various growth and developmental stages. To the best of authors' knowledge, literature pertaining to the EO content and composition of Tulsi from Iran is not available. Moreover, there is no report on EO compositions of Tulsi at different collection time such as vegetative, flower bud formation and flowering stages. It is, therefore, imperative to determine the appropriate harvesting time by analyzing the oil yield and composition of the plant.

The current study aimed to assess the EO content and chemical constituents of three different growth and developmental stages of *Ocimum sanctum* L.

Materials and Methods

Plant Material

The experiment was carried out at the research field of Tarbiat Modares University, Peykan Shahr, Tehran. Average annual precipitation at the site is 122.2 mm, minimum air temperature is -5° C and maximum air temperature is 40.4° C. The dominant winds at the area blow from Northeast. Some chemical characteristics of the experimental soils are shown in Table 1. *Ocimum sanctum* aerial parts were collected from the cultivated plants at three stages of growth and development during June and July 2013. The samples were harvested at vegetative, flower bud formation and full flowering stages.

Table 1: Some physical and chemical characteristics of the experimental soil

EC ds m ⁻¹	pH	OC ^a (%)	TN ^b (%)	P (mg kg ⁻¹)	K(mg kg ⁻¹)	Silt (%)	Sand (%)	Clay (%)
1.04	7.7	1.73	0.06	14	275	12	78	10

^a Organic matter (OC), ^bTotal Nitrogen (TN)

Essential Oils Preparation

All samples were shade-dried (during 15 days). EO was extracted by subjecting flowers and leaves together (50 g) to hydrodistillation for 2 h using an all glass Clevenger-type apparatus (Goldis, Tehran, Iran), according to the method outlined by the European pharmacopoeia (Anonymous 1996). EO yield was expressed as percentage w/w on dry matter basis. The oils were dried over anhydrous Na₂SO₄ and stored in sealed vials at low temperature (4°C) before gas chromatography (GC) and gas chromatography/mass spectrometry (GC-MS) analysis.

Essential Oils Analysis by Gas Chromatography/Mass Spectrometry (GC/MS)

The EOs were analyzed by GC-MS (Agilent, USA). The analysis was carried out on a Thermoquest-Finnigan Trace GC/MS instrument equipped with a DB-5 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 mm). The oven temperature was programmed to increase from 60 to 250°C at a rate of 4°C/minute and finally held for 10 minutes; transfer line temperature was 250°C. Helium was used as the carrier gas at a flow rate of 1.1 mL/minute, with a split ratio equal to 1:50. The quadrupole mass spectrometer was scanned over the 35-465 amu with an ionizing voltage of 70 eV and an ionization current of 150 mA.

GC-FID analysis of the oil was conducted using a Thermoquest-Finnigan instrument equipped with a DB-5 fused silica column (60 m x 0.25 mm i.d., film thickness 0.25 mm). Nitrogen was used as the carrier gas at the constant flow of 1.1 mL/minute; the split ratio was the same as that of GC/MS. The oven temperature was raised from 60 to 250°C at a rate of 4°C/minute and held for 10 minutes. The injector and detector (FID) temperatures were kept at 250 and 280°C, respectively. Semi-quantitative data were obtained from FID area percentages without the use of correction factors.

Identification of EO Components

Retention indices (RI) were calculated by using retention times of n-alkanes (C₆-C₂₄) that were injected after the oil at the same temperature and conditions. The compounds were identified by comparison of their RI with those reported in the literature and their mass spectrum was compared with the Wiley Library.

Results and Discussion

The hydro-distillation of the aerial parts of *Ocimum sanctum* L. at the vegetative, flower bud formation, and full flowering stages yielded 0.95%, 0.87% and 1.3% (w/w) EO, respectively. The composition of EOs at different growth stages is shown in Table 2, in the order of their elution from a DB-5 column. GC/MS analyses showed that the main constituents of the EO from the vegetative stage were 1, 8-Cineole and β -bisabolen, while eugenol was found as the main compound of flower bud formation and full flowering developmental stages.

Table 2: Chemical Components of the Essential Oils Distilled From Three Developmental Stages of *Ocimum sanctum* L.

No.	Compound	RI	Vegetative	Flower Bud Formation	Full Flowering
1	Ethyl Isovalerate	847	-	-	0.79
2	α -Pinene	947	0.72	0.37	0.62
3	Sabinen	973	0.51	0.40	0.45
4	β -Pinene	1007	1.04	1.49	1.69
5	Myrcene	1050	0.49	1.124	0.89
6	1,8-Cineole	1056	20.74	19.37	20.41
7	Linalool	1101	0.18	0.15	0.11
8	Terpinen-4-ol	1176	-	0.24	-
9	α -Terpineol	1203	-	0.33	0.38
10	Estragol	1221	11.45	10.57	11.36
11	Eugenol	1374	15.66	37.11	24.59
12	α -Cis-	1460	3.09	1.26	2.65
13	Bergamotene	1475	2.30	1.25	1.23
14	α -Humlene	1537	20.95	13.25	18.72
15	β -Bisabolen γ -Elemene	1558	10.43	7.3	7.4

abbreviation: RI, retention index.

EOs and their components are generally recognized as safe (GRAS) for human and animal consumption under US Federal Regulations. The compositions of EOs might be affected by the developmental stage of the plant (Saharkhiz *et al.*, 2011, Saharkhiz *et al.*, 2009). Although some authors reported Methyl Chavicol (Khan *et al.*, 2010, Amber *et al.*, 2010) as the major compounds of the EO, others reported eugenol as the main constituent of the EO (Kumar *et al.*, 2010, Asha *et al.*, 2001). In the present study, β -bisabolen was the dominant compound of the oil at the vegetative stage which declined gradually from 20.95% to 13.25% at the flower bud formation stage. Eugenol which reached its maximum level at the flower bud formation stage was identified as the main compounds of flower bud formation and full flowering stages. The lower concentration of eugenol in this study, compared with that of some previous reports (Kumar *et al.*, 2010, Asha *et al.*, 2001), may reflect variations due to geographical location. During the various developmental stages of *Ocimum sanctum* L., the concentration of 1, 8-Cineole gradually declined from 20.74% to 20.41%.

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