

## EFFECT OF HARVESTING TIME ON THE PRODUCTION AND CHEMICAL COMPOSITION OF TULSI LEAVES ESSENTIAL OIL

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Abdel-Hamid<sup>1</sup>, M.F.; S. El-Hady<sup>1</sup>; M.E. Ibrahim<sup>2</sup> and Faten Ibrahim<sup>2</sup>

### ABSTRACT

Tulsi plant (Holy basil) is a promising medicinal and aromatic plant. Tulsi seeds (*Ocimum sanctum* L., Lamiaceae) imported from USA have been successfully adapted, propagated and cultivated under Egyptian climatic conditions. The present study was carried out to follow up the changes in the content and chemical composition of hydro-distilled tulsi leaf essential oil throughout the different physiological stages of plant life (vegetative, flowering and seed formation stages). Oil samples were analyzed using GC and GC-MS chromatographic technique. Data clearly reveal that physiological stages have influenced markedly the concentrations of the resultant oil together with its chemical composition (qualitatively and quantitatively). However one can conclude that flowering stage is the most profitable time of harvesting tulsi leaves for the following reasons. First of all, it exhibited the highest oil concentration (0.5%) and the highest numbers of terpenes (52 terpenic compounds) compared with vegetative stage (0.19% and 25 terpenes), and seed formation stage (0.2% and 20 terpenes). Secondly, the highest relative concentrations of eugenol, estragol and 1,8-cineol were attained during flowering stage and in turn increasing its biological activity.

**Key words:** Tulsi leaves (*Ocimum sanctum* L.), Essential oil, Vegetative stage, Flowering stage, Seed formation stage, GC, GC-MS, Eugenol,  $\beta$ -Bisabolene, Estragol, 1,8-Cineol.

### INTRODUCTION

Essential oil of tulsi has been used as a potent anti malarial drug. It raises the human body immunity by increasing the anti body productions. It is reputed to

possess anti-bacterial, anti-fungal, anti-viral, insecticidal and mosquito repellent properties. However, most of the medicinal, aromatic and biological activity of this oil is attributed to the existence of the main four terpenes in relatively high pro-

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- 1- Biochemistry Dept., Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt.
  - 2- Cultivation and Production of Medicinal and Aromatic Plants Department, National Research Center, Dokki, Cairo, Egypt.

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portion: eugenol, estragol, 1,8-cineol and  $\beta$ -bisabolene. Eugenol demonstrates antioxidant and anti-inflammatory activity (Kelm *et al* 2000). It showed potent anthelmintic activity (Asha *et al* 2001). Essential oils with high levels of eugenol exhibited the highest antioxidant and antimicrobial activity. Thus they appear most promising for food preservation (Juliani *et al* 2004). The biological activity of estragol has been studied (Albuquerque *et al* 1995) on skeletal muscles (Coelho-de-Soza *et al* 1997), on intestinal smooth muscles. The biological activity of 1,8-cineol has been proved (Saito *et al* 2004, a, b) as antioxidant (Satioh *et al* 2003) as it may play important role in the antidiarrhoeic effects of tea tree and rosemary (Savelev *et al* 2003) as fungicidal (Azuma *et al* 2003) as invitro antibacterial potent.  $\beta$ -bisabolene and other sesquiterpenes including  $\alpha$ -humulene,  $\beta$ -caryophyllene and  $\beta$ -caryophyllene oxide show promise as anti carcinogenic agents (Zheng *et al* 1992). Also Denyer *et al* (1994) isolated sesquiterpenes of antirhinoviral from ginger. The present study was carried out to reveal the changes in the content of tulsi leaf essential oil throughout the different developmental stages. Also, the changes in terpenic constituents were taken into consideration. However this may throw some light on the most profitable time of harvesting tulsi leaves having the highest content of essential oil with good quality.

## MATERIAL AND METHODS

### I- Experimental

**I-1- Plant material:** The plant of Tulsi (*Ocimum sanctum* L) was grown in Experimental Farm of Cultivation and Pro-

duction of Medicinal and Aromatic Plants Department of the National Research Center during two successive seasons, 2001 and 2002. The first season was for plantation while the second season was for analysis. The plants were propagated from seeds of *Ocimum sanctum* L., originated from U. S. A. (provided by, J.L. Hudson seedsman, P.O. Box 1058, Red wood city, California 94064, U. S. A.). The seeds were sown in nursery on 15<sup>th</sup> of March in the two seasons. Two months later after sowing, the seedlings were transplanted in 8 cm pots. The seedlings were planted in the field on 15<sup>th</sup> of April in hills 25 cm apart on rows 60 cm in-between. The leaves of tulsi were collected at three different times, at vegetative stage (May), at full flowering stage (June) and at seed formation stage (September). Voucher specimens have been deposited in the herbarium of Cultivation and Production of Medicinal and Aromatic Plants Department. The plants were kindly identified in Orman Botanical Garden.

**I-2- Essential oil:** The essential oil of the leaves collected from the different developmental stages was extracted by hydro distillation for 3 hr (according to Egyptian Pharmacopia, 1984). The extracted volatile oil was dehydrated over anhydrous sodium sulfate and stored in dark brown vials in refrigerator for analysis.

### II- Analysis

**II-1- Gas chromatography:** FID Hewlett-Packard (HP 5890) using DP5 (methylsilicone containing 5% phenyl groups) column 30 m x 0.25 mm i.d. was used. Temperature program: 2 min at 60°C, 60-

100°C (2°C/min) and 100-250°C (5°C/min). Helium was used as carrier gas at flow rate of 1.0 ml/min.

**II-2- Gas Chromatography Mass Spectrometry:** Hewlett-Packard (HP 5989 A) GC-MS system equipped with library software Wiley 138 and NBS75. Capillary GC conditions as mentioned above on DP5 column. Injection volume: 1.0µl at 1:50 split. Significant mass spectrometer (EI-MS 70eV) was used with scan mass range of 40-350u.

**II-3- Identification of components:** The chemical constituents of tulsi leaves volatile oil were identified based on the database of mass spectra from the MS library. The obtained data were confirmed by injecting the authentic samples of different components in EI-MS under the same conditions and in comparison also with data obtained from literatures.

## RESULTS AND DISCUSSION

Because of the importance of the chemical composition and the production of the Tulsi (*Ocimum sanctum* L) leaves essential oil, the important question is:

**What is the most convenient stage for harvesting tulsi leaves, having the highest content and good quality of hydro-distilled essential oil (the aim of this study)?**

In replying to this important question and give-satisfied answer, we harvested tulsi leaves at the three main different physiological stages of development at his life:

- 1- Vegetative stage (May)
- 2- Full flowering stage (June)
- 3- Seed formation stage (September)

Distilled leaf essential oils samples were subjected to GC and GC-MS. However, this study may reveal the following aspects:

- 1- Recommendation for the most convenient harvest time based on the highest essential oil content with high quality expressed as chemical composition.
- 2- Following up the differences in the chemical composition quantitatively and qualitatively of the extracted oils in relation to their stages of development.
- 3- Tracing the trend of the main terpenic constituents throughout the different stages.

Discussions of the obtained results in comparison with those in the previous literature could achieved the summarized following aspects:

- 1- Data clearly indicate that the maximum oil percentage was obtained in case of leaves harvested at flowering stage (0.5%) rather than at vegetative phase (0.2%) and seed formation phase (0.19%). However this may led one to recommend harvesting leaves at flowering stage from the commercial point of view (economically) i.e. maximum yield of the extracted essential oil. Anywise our findings are in good agreement with those obtained by several authors who reported that there is high degree of variation in herbage oil yield and composition depending upon ontogenetical stages of plant at the time of harvest (Gupta, 1996) dealing with *Ocimum* spp. including *Ocimum sanctum*. In this connection, it has been reported that basil essential oil content increased with plant development. The full flowering stage offered the most profitable time of harvest.

2- Following up the qualitative and quantitative differences in the chemical composition of the resultant oils in relation to stages of development, the data listed in Table (1) reveal the following aspects:

2-A- GC and GC-MS analysis reveal high degree of variation in the composition of tulsi leaf essential oil throughout the different developmental stages (the observation period extended for 150 days). Only 25 components were identified at vegetative stage, which increased dramatically to 52 compounds at flowering stage. Then tend to decrease and reached the least number i.e. 20 terpenic compounds at seed formation stage.

2-B- All terpenic fractions responded greatly due to the developmental stages, but at different extents. However, the highest number of terpenic compounds were observed at flowering stage compared with the other developmental stages, e.g. 9 monoterpene hydrocarbons were traced at flowering stage compared with 6 and 5 at vegetative and seed formation stages, respectively. Oxygen containing monoterpenes showed high respondency, as 18, 7 and 8 components were identified throughout flowering, vegetative and seed formation stages, respectively. Also sesquiterpene hydrocarbons exhibited the highest number i.e. 13 components at flowering stage compared with 6 and 9 components at seed formation and vegetative stages, respectively. The most pronounced impact was noticed in case of oxygen containing sesquiterpenes, as at vegetative stage, only one component (*d*-germancrene-4-ol) was detected, then the formation and accumulation of new compounds was enhanced and reached to five components at flowering stage, then finally they disappeared

completely at seed formation stage (150 days observation period).

2-C- Various compounds including other aldehydes, ketones, esters, acids and diterpene (phytol) showed the same trend, as only one component (ethyl pentanoate) existed at vegetative stage compared with 8 compounds appeared at flowering stage then most of them disappeared at seed formation stage, as only 2 compounds were identified.

Depending upon the fore-mentioned findings, one can conclude that the prevailed physiological conditions at flowering stage of Tulsi plant may favor the biosynthesis and accumulation of terpenic compounds, rather than vegetative and seed formation stages.

Oxygen containing terpenes responded greatly to plant age, however these compounds are of high importance as they are responsible for the important biological activities of the tulsi leaf essential oil as will mention below for eugenol 33, estragol 25 and 1,8- cineol 13.

3- Tracing the trend of main components throughout the different stages of tulsi plant age was illustrated in Table (1). The obtained data reveal the following statements:

3-A- At all developmental stages, four predominant compounds have been identified. Three of them are oxygen containing monoterpenes i.e. eugenol 33 (27.57-31.80%), estragol 25 (6.037-10.88%) and 1,8-cineol 13 (4.30-9.82%). The fourth component is  $\beta$ -bisabolene 46 (13.25-16.74%), which is considered as sesquiterpene hydrocarbon.

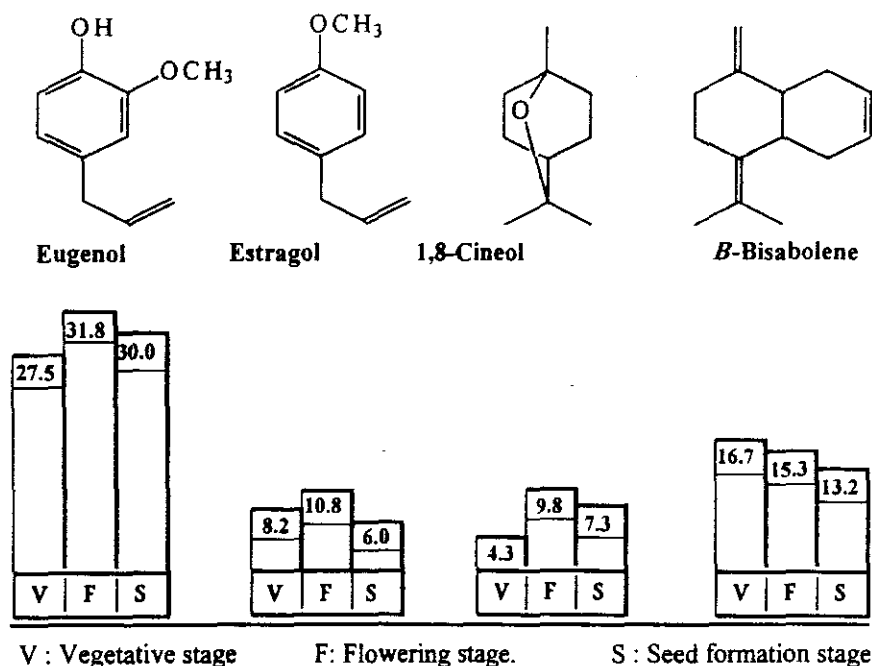
3-B- Flowering stage exhibited the highest values of oxygen containing

Table 1. GC-MS Profile of Distilled Tulsi Leaf Essential Oil at Different Stages of Plant Life

Peak No.	Compound	Vegetative Stage	Flowering Stage	Seed Formation Stage
1	2-Hexanal	--	0.084	--
2	2-Heptanone	--	0.025	0.038
3	Ethyl pentanoate	1.110	3.124	2.001
4	<i>n</i> -Nonane	--	0.097	--
5	2-methyl-4-heptanone	--	0.064	--
6	Tricyclene	--	0.021	--
7	$\alpha$ -Thujene	0.020	0.018	0.259
8	$\alpha$ -Pinene	0.525	0.956	0.071
9	Sabinene	0.221	0.458	0.039
10	<i>B</i> -Pinene	1.858	1.265	--
11	3-Octanone	--	0.015	--
12	Myrcene	--	0.020	--
13	<b>1,8-Cineol</b>	<b>4.302</b>	<b>9.821</b>	<b>7.391</b>
14	<i>Trans</i> - $\beta$ -Ocimene	3.942	4.000	2.574
15	$\gamma$ -Terpinene	0.254	0.968	0.187
16	<i>Cis-p</i> -Menth-2-en-1-ol	0.775	1.509	1.923
17	Terpinolene	--	0.183	--
18	Mw154 C <sub>10</sub> H <sub>18</sub> O	--	1.805	--
19	<i>Trans-p</i> -Menth-2-en-1-ol	1.915	2.789	1.834
20	<i>Cis</i> -Sabinene-hydrate	--	0.272	--
21	Camphor	--	0.087	--
22	<i>Trans</i> -Sabinene-Hydrate	--	2.003	--
23	Borneol	0.101	0.099	0.062
24	$\alpha$ -terpineol	--	0.165	0.133

Table 1. Cont.

Peak No.	Compound	Vegetative Stage	Flowering Stage	Seed Formation Stage
25	<b>Estragol</b>	<b>8.259</b>	<b>10.88</b>	<b>6.037</b>
26	Nerol	--	0.022	--
27	Geraniol	--	0.013	--
28	Linalyl-acetate	0.060	0.003	--
29	Geranial	--	0.001	--
30	Bornyl- acetate	--	0.003	--
31	Carvacrol	--	0.002	--
32	$\alpha$ -Cubebene	0.107	0.004	--
33	<b>Eugenol</b>	<b>27.57</b>	<b>31.80</b>	<b>30.08</b>
34	$\beta$ -Cubebene	--	0.001	--
35	<i>B</i> -Elemene	0.971	1.168	1.470
36	$\alpha$ -Cedreene	0.030	--	--
37	$\alpha$ -Bergamotene	--	1.420	--
38	<i>B</i> -Caryophyllene	0.435	0.307	0.564
39	<i>Trans</i> - $\alpha$ -Bergamotene	--	0.032	--
40	$\alpha$ -Humulene	1.807	2.826	2.235
41	<i>Allo</i> -Aromadendrene	0.050	0.015	--
42	Acoradiene	0.198	0.111	--
43	$\gamma$ -Muurolene	--	0.099	--
44	<i>D</i> -Germacrene	0.700	0.097	0.931
45	Valencene	0.009	tr.	0.229
46	<b><i>B</i>-Bisabolene</b>	<b>16.74</b>	<b>15.38</b>	<b>13.25</b>
47	<i>T</i> -Nerolidol	--	0.033	--
48	<i>D</i> -Germacrene-4-ol	0.829	0.575	--
49	Torreyol	--	0.004	--
50	<i>B</i> -Eudesmol	--	0.125	--
51	$\alpha$ -Bisabolol	--	0.077	--
52	Phytol	--	0.018	--
<b>Total</b>		<b>72.37%</b>	<b>95.86%</b>	<b>71.30%</b>



monoterpenes i.e. eugenol 33, estragol 25 and 1,8-cineol 13. On the other hand,  $\beta$ -bisabolene 46 showed its maximum consonance with that reported by Gupta, (1996), dealing with *Ocimum sanctum* grown under Indian conditions.

At close, the overall abovementioned results clearly indicate that harvesting tulsi leaves at flowering stage resulted in the highest essential oil concentration with high quality due to the highest relative values of its main terpenic components. Also, high numbers of terpenic components have been noticed; even at relatively low concentration, rather than the other two stages. Anywise this may indicate that oil accumulation and composition may be controlled by the physiological conditions along the plant life and

content at vegetative (earlier stage), then it tended to decline up to seed formation stage. However, our findings are in the most suitable physiological conditions have prevailed at flowering stage rather than vegetative (earlier stage) and seed formation (later stage). Thus the flowering stage may offer the most profitable time of harvesting leaves from tulsi plant grown under Egyptian conditions for producing high quantity of essential oil with high quality.

The medicinal and aromatic properties of tulsi essential oil together with its biological activity may be ascribed to its main terpenic components, eugenol 33,  $\beta$ -bisabolene 46, estragol 25 and 1,8-cineol 13. However, the available literature could be summarized as follows:

**Eugenol**, it is well known that it is used in perfumeries flavoring, essential oils and medicine. It is used in the production of isoeugenol for the manufacture of vanillin. It has been used in dental practice because of its analgesic and antiseptic properties. Interestingly, eugenol exhibits irritant action in addition to analgesic effect (Sneddon and Gelw, 1973) which reminiscent of the effect of capsaicin (Szallasi and Blumberg, 1999). It acts as capsaicin dose in sensory neurons because both of them contain venially moiety (Tominaga *et al* 1998). Eugenol demonstrates food antioxidant and anti-inflammatory activity, which support traditional uses of *Ocimum sanctum* (Kelm *et al* 2000). The essential oil of *Ocimum sanctum* and eugenol showed potent anthelmintic activity (Asha *et al* 2001). Eugenol inhibited the oxidative mutagenesis by *ter-buty*-hydroperoxide (TBH) in *Escherichia coli* (Ramos *et al* 2003). Essential oils with the highest levels of eugenol exhibited the highest antioxidant and antimicrobial activity, thus they appear most promising for food preservation (Juliani *et al* 2004). Thymol and eugenol were capable of inducing bacterial cell lysis as both cell wall and membrane were significantly damaged (Rhayour *et al* 2003). Dip *et al* (2004) referred to the ability of eugenol to reduce edema induced by *dieffenbachia picta* schott in mice. Varel *et al* (2004) concluded that plant oils thymol and eugenol might offer solutions to controlling various environmental problems associated with livestock wastes. Finally it is interesting to mention that eugenol is not only produced by medical and aromatic plants, but it is also synthesized through genetically engineered *Sac-*

*charomyces cerevisiae* (Soher *et al* 2003).

**Estragol**, which represents one of the important constituents of tulsi leaf essential oil, its biological activity has been studied by (Albuquerque *et al* 1995) on skeletal muscles and by (Coelho-de-Soza *et al* 1997) on intestinal smooth muscle. In this connection, Bianchi-santamaria *et al* (1993) reported that tarragon oil with an estragol content of 60% showed genotoxicity whereas 2 basil oils from *A. dracunculus L.* and *Ocimum basilicum L.* with estragol content of 8.1 and 16.5 % respectively were not genotoxic.

**1,8-cineol**, its biological potency has been proved by (Saito *et al* 2004, a, b) as antioxidant against the oxidation of lenoleic acid and by (Satioh *et al* 2003) as it may play an important role in the antilussive effects of tea-tree and rosemary, while by (Savelev *et al* 2003) as fungicidal, in addition to (Azuma *et al* 2003), as in vitro antibacterial potent.

Concerning  $\beta$ -Bisabolene and other sesquiterpenes, the presence of  $\beta$ -bisabolene in remarkable quantities in addition to  $\alpha$ -humulene (1.807-2.826%) and  $\beta$ -caryophyllene (0.307-0.564%) is of great interest since these sesquiterpenes together with  $\beta$ -caryophyllene oxide and  $\alpha$ -humulene epoxide show promise as anti carcinogenic agents (Zheng *et al* 1992). Also, Denyer *et al* (1994) isolated sesquiterpenes of antirhinoviral from ginger (*Zingiber officinale*).

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## تأثير وقت الحصاد على كمية والتركيب الكيميائي للزيت الطيار لأوراق التولزى

[١]

محمد فتحى عبد الحميد<sup>١</sup> - سمير الهادى<sup>١</sup> - محمد السيد إبراهيم<sup>٢</sup> -

فاتن ابراهيم<sup>٢</sup>

- ١- قسم الكيمياء الحيوية - كلية الزراعة - جامعة عين شمس - شبرا الخيمة - القاهرة - مصر  
٢- قسم زراعة وانتاج النباتات الطبية و العطرية-المركز القومى للبحوث- الدقى - القاهرة - مصر

تمت بنجاح زراعة نبات التولزى من بذور التولزى المستوردة من أمريكا تحت الظروف المناخية المحلية المصرية فى مصر. وقد تم استخلاص الزيت الطيار من الأوراق فى ثلاث مراحل مختلفة من عمر النبات بواسطة التقطير بالبخار وذلك من الأوراق أثناء مرحلة النمو الخضرى فى شهر مايو وكذلك من الأوراق أثناء مرحلة الأزهار التام فى شهر يونيو وأخيراً من الأوراق أثناء مرحلة تكوين البذور فى شهر سبتمبر وكان الزيت المستخلص مميز الرائحة وذو لون أصفر فاتح. تم دراسة

الزيت مثل الأيوجينول والأستراجول وبيتا-بايسابولين و ٨١-سينيول والتي يعزى إليها أيضا الأهمية البيولوجية لزيوت التولزي فقد وجد أن مرحلة الأزهار هي أفضل المراحل العمرية للنبات والتي يمكن من خلالها الحصول على أفضل زيت من حيث تركيبه الكيميائي وأحتوائه على أعلى تركيز نسبي للمركبات الهامة الرئيسية السابقة بالإضافة الى أنه وجد أن الزيت في هذه المرحلة يحتوى على أكبر عدد من المركبات (٥٢ مركب بالمقارنة ب ٢٥ مركب في مرحلة النمو الخضري و ٢٠ مركب في مرحلة تكوين البذور).

بناء على النتائج المتحصل عليها من خلال هذه الدراسة : يمكن اعتبار أن مرحلة الأزهار هي أفضل المراحل العمرية لنبات التولزي لجمع الأوراق للحصول على أعلى نسبة زيت طيارعالي الجودة حيث يحتوى على أعلى نسبة من المركبات الفعالة السابق ذكرها بالإضافة الى أكبر عدد من المركبات يمكن وجوده في الزيت.

التركيب الكيميائي للزيت بواسطة مطياف الكتلة وكذلك جهاز الفصل الكروماتوجرافي الغازى المتصل بجهاز مطياف الكتلة وذلك لمعرفة التركيب الكيميائي والمكونات الأساسية للزيت وكذلك تقدير كمية الزيت وذلك فى كل الأوراق الخاصة بالمراحل العمرية المختلفة للنبات موضوع الدراسة وكان الهدف من الدراسة:

١- تحديد وتتبع كمية الزيت والتركيب الكيميائي له أثناء المراحل الفسيولوجية المختلفة فيما يفيد فى تحديد أنسب الفترات لجمع الأوراق لاستخلاص أعلى كمية زيت ذات الجودة العالية.

وقد أثبتت النتائج المتحصل عليها فى هذه الدراسة أن:

- ١- أعلى نسبة زيت يمكن الحصول عليها من الأوراق فى مرحلة الأزهار (٥٠,٥%) فى حين أعطت الأوراق فى المراحل العمرية الأخرى أقل من نصف كمية الزيت الناتجة من مرحلة الأزهار (٠,٢ و ٠,١٩%).
- ٢- بالنسبة لجودة الزيت وذلك من خلال دراسة وتتبع المركبات الرئيسية فى

تحكيم: أ.د نجاح الشحات على  
أ.د صلاح عبد العزيز ترك