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Seasonal Variation of Essential Oils Composition of A Medicinal Plant - *Ocimum sanctum* (Purple)

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ABSTRACT

The present study deals with the extraction of total essential oils from medicinal plant *Ocimum sanctum* (Purple) in four different seasons of 2010 calendar year. Extraction of total essential oil content of plant materials was carried out by Soxhlet extraction whereas extraction of volatile oils by steam distillation using Clevenger type apparatus. Total essential oil and volatile oils are more in winter season (November month) whereas very less quantity in summer season (May month). Eugenol is the major constituent present in the plant. The percentage composition of eugenol in four seasons was found from gas chromatography analysis.

KEYWORDS

Ocimum sanctum (Purple), Soxhlet extraction, Clevenger apparatus, Eugenol, Gas chromatography.

INTRODUCTION

The plants are indispensable to man for his life. Nature has provided a complete store house of remedies to cure several ailments of mankind. Medicinal plants form the backbone of traditional system of medicine in India. Essential oils are volatile natural complex secondary metabolites characterized by a strong odour and have a generally lower density than that of water [1,2].

The Lamiaceae family (Labiatae) is one of the largest and most distinctive families of flowering plants, with about 220 genera and almost 7000 species worldwide [3]. *Ocimum sanctum* also known as *Ocimum tenuiflorum* is commonly known as tulsi or holy basil or "the incomparable one" in India. The plant grows wild in India but is also widely cultivated in home and temple gardens and is used for household remediation [4]. The more exuberantly flavoured red holy basil (red or purple variety) has dark green leaves with reddish purple stems and a purplish cast on the younger leaves known as Krishna tulsi. Holy basil has a strong anise like, slightly musky and lemony taste with a camphoraceous aroma. The dominant aroma component in holy basil is eugenol.

The stem and leaves of holy basil contain a variety of constituents that may have biological activity, including saponins, flavonoids, triterpenoids and tannins [5]. Several constituents have been identified in leaf oil of *Ocimum sanctum*

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(Purple). Eugenol, a monoterpene, has been identified as the dominant volatile constituent [6-9]. Different parts of tulsi plant have been used, by traditional medical practitioners, as expectorant, analgesic, anticancer, antiasthmatic, antiemetic, diaphoretic, antidiabetic, antifertility, hepatoprotective, hypotensive, antistress, analgesic, antihyperlipidemic, antioxidant potentials in experimental animals [10-15]. The therapeutic potential of the essential oils extracted from fresh leaves of *Ocimum sanctum* L. has been found to be largely due to eugenol [10,16].

EXPERIMENTAL

Ocimum sanctum (Purple) was grown in the spacious domestic home garden at Gavaravaram village, Elurumandal in West Godavari district of Andhra Pradesh is situated between 16.7° North 18.1° East, elevation 22 mts 72 feet. Collections were made, Vouchers ofspecimens were deposited at the Botany department. Each specimen was labelled, numbered, annotated with the date of collection.

In the present study the specimen numbers are F2, M2, A2 and N2. The specimen was subjected for identification at plant systematic laboratory, Kakatiya University, Warangal, Andhra Pradesh, India.

1 kg of leaves of the sample in each season was collected, dried under shade, finely powdered in an electric blender (80 mesh) and stored in air tight containers at room temperature in the dark until used.

Soxhlet extraction: Extraction of total essential oil content of plant materials was carried out by Soxhlet extraction method [17]. 5 g of dry powder was subjected to Soxhlet extraction with 250 mL methanol as solvent, extraction was carried out for 3 h, 10 cycles and temperature was maintained at 65 °C.

Steam distillation: Extraction of volatile oils from the plant materials was carried out by steam distillation using Clevenger type apparatus [18]. 100 g of powdered sample was water distilled by using a Clevenger oil arm fitted with condensers through which cooled water was circulated to prevent low volatiles from escaping. The temperature was maintained at 60 °C. The volatile oil was collected and dried over anhydrous sodium sulphate and stored at -4 °C. 1 mg of volatile extract was dissolved in 1 mL of methanol, from that solution 10 µL was taken and made up to 100 µL with methanol. This solution was used for GC analysis.

Gas chromatography analysis: The essential oils were analyzed using a Shimadzu gas chromatograph model 17A Japan (2014), equipped with flame ionization detector (FID) and DB-Wax capillary column (30 m × 0.32 mm, film thickness 0.5 µm). Injector and detector temperatures were set at 240 and 250 °C, respectively. Column oven temperature was programmed from 40 to 220 °C at the rate of 8 °C min^{-1}; initial and final temperatures were held for 3 and 10 min, respectively. Helium was used as a carrier gas with a flow of 1.5 mL min^{-1}. A sample of 0.1 µL was injected using slit mode (split ratio 1:20). Quantification was completed by built-in data-handling software supplied by the manufacturer (spin chrome CFR) of the gas chromatograph. The results (composition) were reported as a relative percentage of the total peak area.

The chromatogram is observed as a series of peaks where each peak represents a chemical compound. The X-axis represents the time scale and the time at which the peak is recorded is called the retention time (Rt). The peak height and peak area is an indication of the quantity of the compound in the mixture. The peak area is integrated as a percentage of the total.

RESULTS AND DISCUSSION

Table-1 shows the yield of total essential oil and percentage composition of eugenol in *Ocimum sanctum* (Purple) in four different seasons of 2010 calendar year. It shows that the yield was affected by seasonal changes. The highest amount of the oil in these plants was found in winter *i.e*. in November and very low in summer *i.e*. in May. In *Ocimum sanctum* (Purple) the percentage composition of essential oil is high in November (2.0 %) and low in May (1.52 %). In India the temperature is very high in summer ranging 35-42 °C. Low essential oil yield in summer might be attributed to the high temperature and partial evaporation of some constituents of oil can be expected.

It was found that the dry weight of a plant is inversely proportional to the total essential oil content of that plant. For example in the month of November the dry weight of the plant material is low and the total essential oil content for the plant is high. Also, the percentage composition of eugenol in *Ocimum sanctum* (Purple) was high in the month of November (61.538 %) and very low in the month of May (37.969 %)

Several constituents have been identified in oil from the leaves of *Ocimum sanctum* (Purple), The leaves of *Ocimum sanctum* contain 0.7 % volatile oil comprising about 71 % eugenol and 20 % methyl eugenol (Fig. 1) and the oil also contains carvacrol and sesquiterpine hydrocarbon caryophyllene [19]. Eugenol a monoterpene has been identified as the dominant volatile constituent [6-9]. Asha and Prasath [20] reported that the oil of *Ocimum sanctum* posses eugenol (53.10 %) as the main compound.

Conclusion

Thus the plant materials have to be collected from November to February in order to have high yield of essential oils and eugenol. From the above data it was concluded that in *Ocimum sanctum* (Purple) eugenol was the major chemical constituent. Eugenol (l-hydroxy-2-methoxy-4-allylbenzene), is a phenolic compound having large number of applications chemically and biologically. The clove buds are rich sources of eugenol

TABLE-1
TOTAL ESSENTIAL CONTENT AND PERCENTAGE COMPOSITION OF EUGENOL IN *Ocimum sanctum* (Purple)

Month and year	Specimen numbers	Wet weight (g)	Dry weight (g)	Moisture content (g)	Composition of essential oil (%)	Composition of eugenol (%)
February 2010	F2	1000	232.0	768.0	1.85	49.000
May 2010	M2	1000	254.5	745.5	1.52	37.969
August 2010	A2	1000	208.0	792.0	1.61	42.448
November 2010	N2	1000	191.0	809.0	2.00	61.538

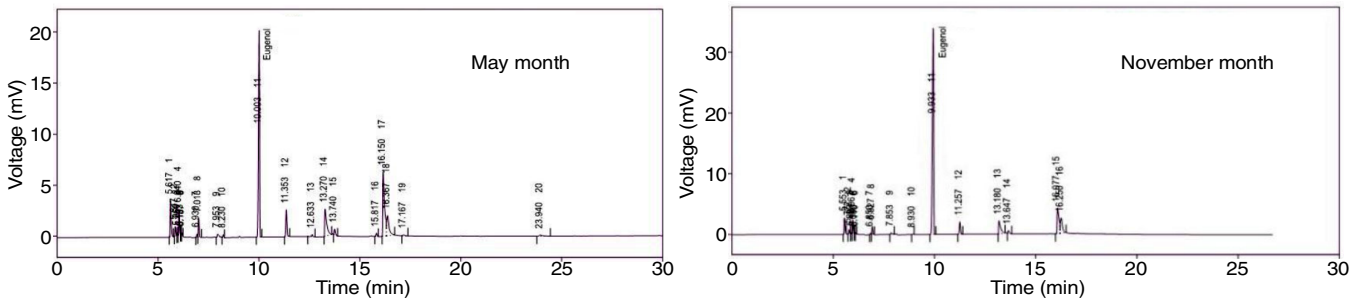

Fig. 1. Chromatograms of *Ocimum sanctum* (Purple) isolated in different seasons

containing about 70-85 %. Although this plant source is rich in eugenol but because of it's higher prices the commercial extraction of eugenol from that plant is costly. In contrast to these sources *Ocimum sanctum* (Purple), is the low-cost sources for commercial extraction of eugenol. The aerial parts (leaves, flowers and stem) of the plant contain essential oils with good percentage of eugenol. Thus it is of the opinion that *Ocimum sanctum* (Purple) is quite useful and commercially viable to extract the costly chemical constituents like eugenol.

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