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Influence Of Distillation Time On The Essential Oil Yield And Eugenol Content In *Ocimum Sanctum* L (CIM-Ayu)

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Abstract

Ocimum sanctum L, commonly known as Holy basil, is a biennial or triennial shrub described as a sacred medicinal plant in ancient Hindu literature. The essential oil from the areal part of *O. sanctum*, variety CIM-Ayu, possess significant biological activates due to the presence of a major volatile fragrant compound Eugenol. Eugenol is wieldy used in the manufacture of several pharmaceutical drugs. To show the influence of distillation time (DT) on the yield of essential oil and its Eugenol content, four different hydro-distillation experiments were conducted with 30 min. of varying time durations i.e., 0-30min, 0-60min, 0-90min and 0-120min respectively. The yield of essential oil was observed highest (0.16%) in the 0-30 min. experiment whereas, Eugenol was found to be highest in 0-60 min DT (66.348%). The second major component β Caryophyllene showed a decreasing trend (14.777 to 13.773%) from first to last experiment. From the above, it infers that the enriched Eugenol fraction can be collected in first one hour of distillation.

Key words: *Ocimum sanctum*, CIM-Ayu, Essential oil, Eugenol, Gas Chromatography

1. Introduction

Ocimum sanctum Linn (Family: Lamiaceae), commonly known as holy Basil, is an ancient medicinal (Bariyah, 2013) herb, found throughout the world and commonly cultivated in gardens and holy places in India. The leaves of *O. sanctum* are traditionally used to treat asthma, fever, cold & cough, diarrhea, skin diseases, eye pain, insect bite etc., (Saroj and Krishna, 2017). The herb also exhibited radioprotective activity (Pingalea et al., 2012) owing to its high antioxidant activity and presence of some active polysaccharides. Essential oil from the areal parts of *O. sanctum* is an important flavor and fragrance source in the food and pharmaceutical industry (Denys and James, 1990).

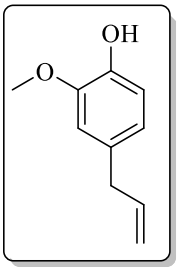

Fig. 1: Chemical structure of Eugenol; Density: 1.06 g/cm^3; Boiling Point: 254 °C (489 °F); Molecular formula: $C_{10}H_{12}O_2$; Molecular weight: 164; Color: Pale Yellow; CAS No: 97-53-0.

Eugenol (4-allyl-2-methoxyphenol; Fig 1), one of the major chemical constituents of *O. sanctum* essential oil (Saroj and Krishna, 2017), has shown many potent pharmacological activities such as antitumoral (Manikandan et al., 2010), hypotensive (Lahloua et al., 2004), convulsive (Sayyah et al., 2002) and hypothermic (Won et al., 1998) etc. Eugenol also has shown wider applications in the flavor & fragrance industries (Chaieb et al., 2007), in cosmetic & food products as an antimicrobial, and antioxidant agent (Yanishlieva et al., 2001; Suppakul et al., 2003). Semi-Synthetic derivatives of Eugenol such as 1,2,3-triazole chalcone (**I**), mannich adducts (**II**) showed significant anti-tumor activity against hepatocellular carcinoma cell lines (Komuraiah et al., 2021). Dimerization of eugenol (**III**) showed good DPPH radical scavenging activity (Bortolomeazzi et al., 2009). Recently, thermally stable resins (**IV**) (Hu et al., 2015) and high-performance flame-retardant epoxy resins (**V**) (Wan et al., 2015) were synthesized using Eugenol as starting ingredient (Fig. 2).

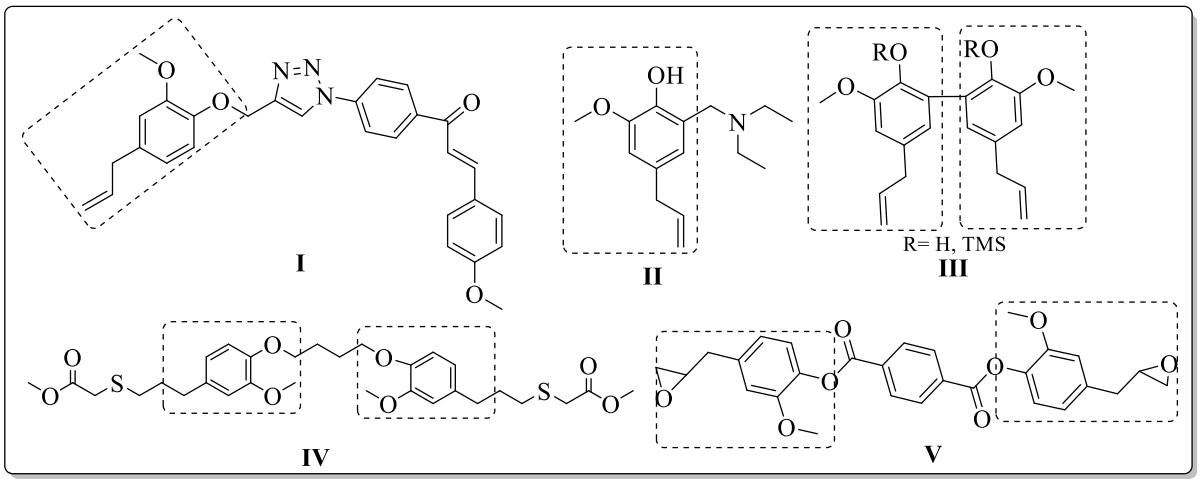

Fig. 2: Semi-Synthetic Analogues of Eugenol.

Eugenol, being one of the leading ingredients in the food, cosmetic, flavor, fragrance and pharmaceutical industries, a high demand exists for its natural source. Research on improving process parameters to optimize and enrich Eugenol content in the post-harvest processing of herbs is the need of the day. Here, we report influence of distillation time (DT) on yields and chemical composition of Essential oil from *O. sanctum var.* CIM-Ayu (developed by CSIR-CIMAP through natural selection), with an aim to standardize optimum process parameters for enrichment of Eugenol.

2. Materials and Methods

2.1. Plant Material

CIM-Ayu aerial parts were collected from experimental fields of CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre, Boduppal, Hyderabad, India in June, 2019 (542 m above sea level with a geographical position bearing of 78°8' E longitude and 17°32' N latitude). The mean annual rainfall of this region is generally 750 mm in June, 2019.

2.2. Hydro-distillation

200 grams of each fresh foliage of CIM-Ayu aerial parts were charged along with 1 L water in four different Clevenger-type apparatus (C1-C4). The apparatus was heated over a heating mantle and vapours generated from the vessel were condensed and collected in a graduated glass receiver. The essential oil samples from C1÷ (0-30 min),; C2÷ (0-60 min), C3÷ (0-90) min and C4÷ (0-120) min DT experiments were collected, measured, dried over an anhydrous sodium sulphate and used for further analysis.

2.3. Analysis of Essential pols

The essential oils were analyzed on a Varian CP-3800 model Gas Chromatography with Galaxy software equipped with flame ionization detector (FID) and electronic integrator; separation of the compounds was achieved using a Varian CP-Sil 5 CB capillary column (ID: 50 m X 0.25 mm; film thickness 0.25 µm). Nitrogen was used as the carrier gas at a constant flow rate of 0.5 mL/min. The column temperature was programmed from 120°C (held for 2 min.) to 240°C (held for 5 min.) at a rate of 8°C/min. The injector and detector temperature were set at 250°C and 300°C respectively. Samples of 0.2 µL were injected with a 20:100:20 split ratio. Retention indices were generated with a standard solution of *n*-alkanes

(C_6-C_{19}). The composition was reported as a relative percentage of the total peak area without FID response factor correction.

2.3.1. Identification of Essential Oil Constituents

The identification of chemical constituents in the essential oil was done on the basis of their retention indices (RI), determined with reference to a homologous series of *n*-alkanes (C_9–C_{24}, Polyscience Corp., Niles, IL, USA) under identical experimental conditions) in both polar and nonpolar columns, co-injection with standards (Aldrich and Fluka), a mass spectra library search (NIST/EPA/NIH Version 2.1 and Wiley Registry of Mass Spectral Data, 7th edition), and by comparison with the mass spectral literature data. The relative amounts of the individual components were calculated based on computer calculated GC peak areas without correction for flame ionization detection response factors.

3. Results and Discussion

3.1. Effect of DT on Essential Oil Yields

The DT's have significantly affected the yields and chemical composition of essential oils. The essential oil yields obtained from the four different DT experiments (C1-C4) varied significantly from 0.16 to 0.44%. A maximum of 13.55% oil yield was noted in the first 0-30 min experiment (C1) and the later experiments recovered varied and unstable oil yields as shown in Table 1.

Table 1: Experimental data for hydro-distillation of CIM-Ayu aerial parts

Fraction	Time (min)	Essential oil yield (%)		Variation (%)		Recovery (%)
		Individual	Total			
Clevenger-1 (C1)	0-30	0.16	0.16	--	0.16	13.55
Clevenger-2 (C2)	0-60	0.22	0.38	C2-C1	0.06	05.08
Clevenger-3 (C3)	0-90	0.36	0.74	C3-C2	0.14	11.86
Clevenger-4 (C4)	0-120	0.44	1.18	C4-C3	0.08	06.77

3.2. Essential oil composition as influenced by distillation time

The CIM-Ayu essential oils obtained from the C1-C4 experiments were analyzed for their chemical composition by GC-FID technique. A total of eight compounds (Limonene, Linalool, Camphor, Methyl Chavicol, Eugenol, Methyl Eugenol, β-Elemene and β-Caryophyllene) accounted for 89.239 to 93.315% of the total essential oil. The major components with their percentage expression in each experiment are presented in Table 2. A higher percentage of Eugenol content 66.348% was noted in the essential oil collected in C2 experiment i.e., 0-60 min DT duration and the oil sample collected in C~~2~~3 i.e., 0-90 min DT has shown the lowest (47.695%). Thus, it may conclude that the concentration of eugenol significantly increased from C1-C2 (55.877 to 66.348 %) but later showed a marginal decreasing (C3: 47.695%) and increasing (C4: 53.449%) pattern. Another major hydrocarbon β-Caryophyllene expressed maximum (14.799%) in the C1 but shown a gradual decrease in the remaining C2-C4 experiments (14.362 to 13.773 %). (Fig. 3).

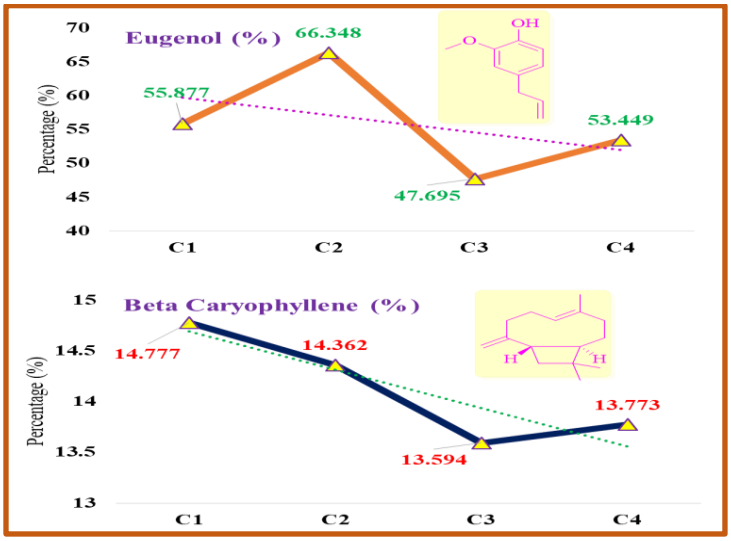

Fig. 3: Variations of Major Marker compounds

The essential oil in second Clevenger (C2) collected during the initial one hour (0-60 min) of distillation duration time, accounting to 66.348% of eugenol in total oil, may be collected separately to get enriched eugenol fraction. These fractions enriched with eugenol require lesser effort for further purification during downstream processing thereby reducing the time and production cost. Rest of the fractions (C3 and C4) can be used to enrich eugenol further by fractional distillation, if so required.

Table 2: GC Analysis Report of *O. sanctum* (CIM-Ayu) Essential Oils

Component	RI Values	Percentage of Chemical Composition (%)			
		C-1 (0-30 min)	C-2 (0-60 min)	C-3 (0-90 min)	C-4 (0-120 min)
Limonene	1030	0.675	0.361	0.368	0.287
Linalool	1105	1.010	0.69	0.439	0.364
Camphor	1130	0.111	0.052	0.052	0.043
Methyl chavicol	1186	2.166	1.345	0.894	0.764
Eugenol	1340	55.877	66.348	47.695	53.449
Methyl Eugenol	1376	5.332	0.424	20.638	15.687
β- Elemene	1396	9.291	8.877	8.928	8.948
Beta Caryophyllene	1422	14.777	14.362	13.594	13.773
Total		**89.239**	**92.461**	**92.608**	**93.315**

The other hydrocarbons like Limonene (0.675-0.287), Linalool (0.364-1.01%), Camphor (0.111-0.043), Methyl Chavicol (2.166-0.764%), Methyl Eugenol (0.424 -15.687%) and , β- Elemene (8.877 -9.291%) have shown a different but specific range pattern from C1-C4 experiments. Limonene, Linalool, Camphor and Methyl Chavicol have shown a gradual decreasing pattern from C1-C4. Whereas, methyl eugenol was decreased, increased and decreased pattern was noticed.

(Fig. 4 & 5)

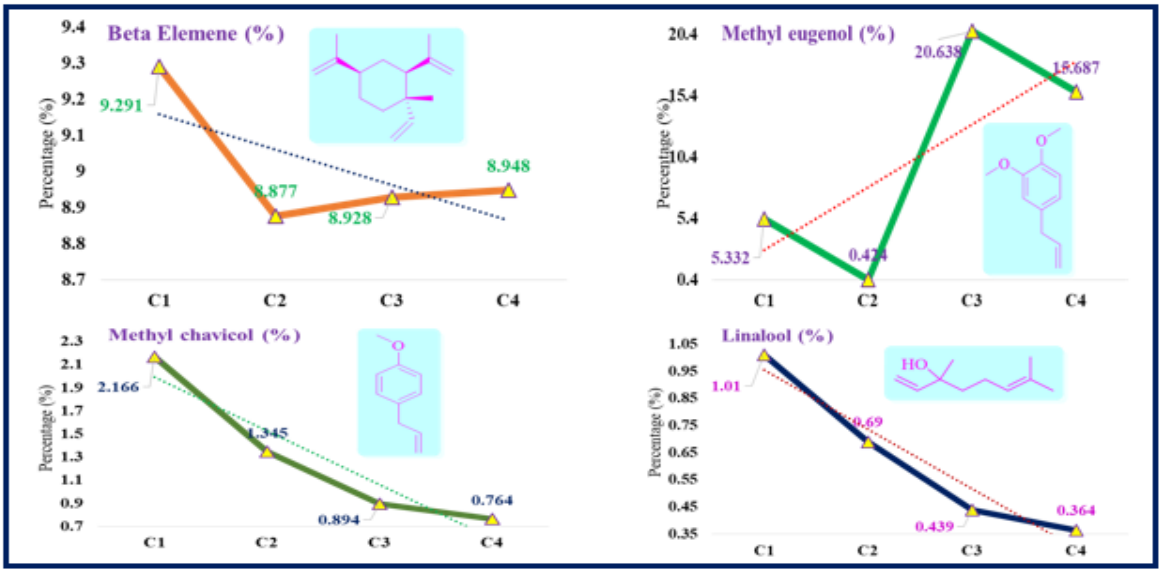

Fig. 4: Variations of Minor Marker compounds in different clevengers.

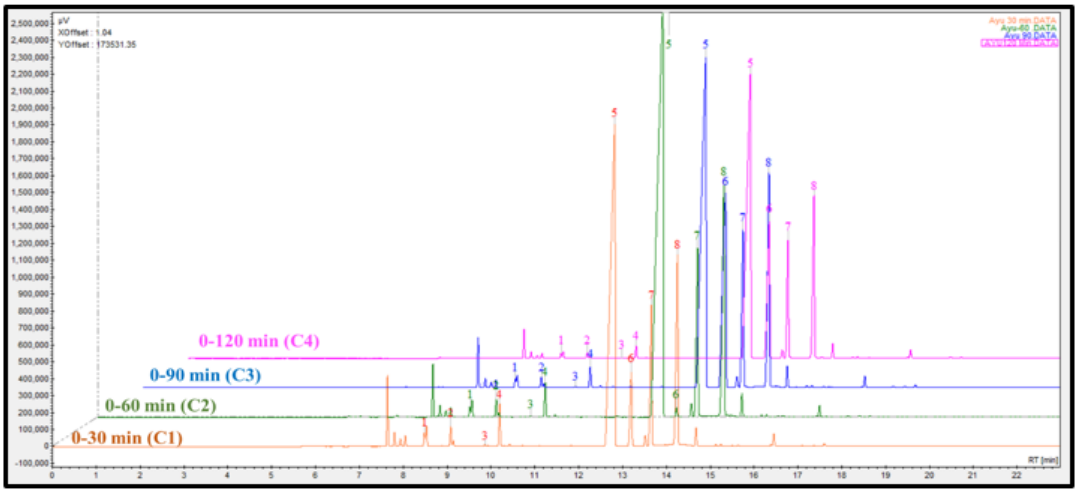

Fig. 5: Comparative GC Chromatograms of Essential oils [1-Limonene; 2- Linalool; 3- Camphor; 4- Methyl Chavicol; 5-Eugenol; 6- Methyl Eugenol; 7- Beta Caryophyllene; 8-Caryophyllene] from C1-C4.

4. Conclusion

The essential oil yields of CIM-Ayu were higher in first half an hour of the fresh foliage therefore first half an hour was crucial time to get good quantity of essential oils from CIM-Ayu. The composition data inferred that the enriched eugenol fraction can be collected within one hour of distillation. The foregoing studies would serve as leads to distillers in optimizing the distillation process for extraction of essential oil from CIM-Ayu. Further, the large potential application of eugenol rich essential oils would also encourage farmers for extensive plantation of *O. sanctum* (CIM-Ayu) to realize the demands of essential oil in food, cosmetic and chemical industries.

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