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Effect of seasons, storage and distillation times on essential oil composition of *Melaleuca leucadendra* (L.)

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The effect of harvesting seasons, storage period and distillation time were studied on the essential oil composition of *Melaleuca leucadendra* (L.) growing in India, which were analysed using gas chromatography- flame ionisation detector (GC-FID) and gas chromatography-mass spectrometry method. Eighteen constituents were identified in each of the three experiments. The essential oils comprised mainly oxygenated sesquiterpenes(>88%), followed by sesquiterpene hydrocarbons and monoterpenoids. The main constituent reported of essential oils was (*E*)-nerolidol and β-caryophyllene. The essential oil content varied from 1.8–1.9%, with maximal in spring and minimal in the rainy season; (*E*)-Nerolidol (87.2-95.5%) and β-caryophyllene (0.1-3.5%) were major constituents. The leaves of the *Melaleuca* genus contain many oil glands, which make them a rich source of essential oil, and therefore, to extract maximum oil, distillation time was optimised for the complete extraction of leaf essential oil of *M. leucodendra*. Eight distillation time durations were tested in this study: 1, 2, 3, 4, 5, 6, 7, and 8 hours. Oil content increases with an increase in the distillation time. The best distillation time for maximum extraction of essential oil content was eight hours (1.6 mL) and five hours for higher major component (*E*)-Nerolidol value (96.1%). Results showed that essential oil extracted from dried leaves stored for one month to twelve months for storage experiments has no adverse effect on yield and quality in terms of its major constituent (≥90.0% of (*E*)-Nerolidol). The essential oil of *M. leucadendra* (L.) is promising for its marker aroma constituent, (*E*)-Nerolidol, for food flavour, fragrance, pharmaceutical, and cosmetic purposes.

Keywords: (*E*)-nerolidol, Distillation time, *Melaleuca leucodendra*, Seasonal variation, Shade drying

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Introduction

The genus *Melaleuca* L. (family Myrtaceae) comprises ca. 230 species distributed worldwide[1-2]. The genus comprises various well-known plants, viz. *M. alternifolia*, *M. cajuputi*, *M. quinquenervia*, *M. leucodendra*, *M. alsophila, M. bracteata, M. linarifolia, M. viridiflora*, and *M. argentea* as a source of industrially valuable essential oils and enriched aroma constituents[3]. Several studies showed that *Melaleuca* spp. possess manifold bioactivity, such as antioxidant, insecticidal, antifungal, antiviral, anti-inflammatory, and antimicrobial[4,5]. *M. leucadendra* is a medium-sized to tall tree distributed in Australia, New Guinea, and Indonesia[1,6]. This species contains three chemotypes; chemotype I contains methyl eugenol (>90%) with a lesser amount of (*E*)-methyl eugenol (<1%), whereas chemotype II contains eugenol (>70%) and methyl eugenol (<10%). In contrast, 1,8-cineole(>10%), p-cymene(>5%), α-pinene(>4%) and limonene(>3%) are compounds present in chemotype III[7]. The chemical composition of *M. leucadendra* has been studied from different ecological and geographical regions, reporting monoterpenoids and phenylpropanoids as main aroma constituents. 1,8-cineole (44.76-64.30%) was reported in *M. leucadendra* from Egypt and Java, Indonesian origin[8-10]. Phenylpropanoids were reported as major constituents in *M. leucadendra* from Australia and Brazilian origin, reporting methyl eugenol (99%) and (*E*)-methyl isoeugenol (~88%) as major constituent[6,11]. (*E*)-nerolidol (>90.0%) was reported as a marker constituent of the essential oil of *M. leucadendra* from northern Indian origin[4]. In comparison, in another study 1,8-cineole(19.9%), α-eudesmol (15.8%) and β-eudesmol (11.3%) were major constituents in *M. leucadendra* from northern India[12]. The major constituents in leaf oil of *M. leucadendra* from Cuba were viridiflorol (28.2%), 1,8-cineole (21.3%) and α-terpineol (10.1%)[13]; while terpinolene (29.21%), α-terpinene (22.55%), δ-2-carene (8.53%), α-phellandrene (7.61 %) were

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reported in leaf oil of *M. leucadendra* from Thailand[14]. The differences observed in the chemical composition of essential oils could potentially be attributed to genetic factors, environmental conditions, the timing of plant part harvesting (season), extraction methods, and storage practices[13,14]. Furthermore, there is no agreement in the literature regarding the optimum duration of distillation time for maximum oil content[15,16]. To observe some of these factors in the present experiment, the compositional variability of leaf essential oils of *M. leucadendra* have been studied with respect to seasonal variation, storage period, and distillation time for the first time from foots hills agroclimatic conditions of Uttarakhand.

Materials and Methods

Plant material collection and identification

Fresh leaves were collected from March (2019) of *M. leucadendra* tree grown at CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Pantnagar, Uttarakhand, India (29° N, 79.38° E, at an altitude of 243.84 m). The plant was identified by one of the authors, Dr Amit Chauhan, a Taxonomist. The voucher specimen (No. CIMAP/PNT/ML-10) was deposited in the Herbarium of CSIR-CIMAP, Research Center, Pantnagar.

Extraction of essential oils

The essential oils were extracted by hydro-distillation using a Clevenger-type apparatus and dried using Na_2SO_4. As per the objectives of the experiment on seasonal variation, the essential oil extracted from fresh leaves in spring (March-April), summer (May-June), rainy (July-August), autumn (October-November), and winter seasons (December-January) of year 2019-20. Moreover, to optimize the distillation time, fresh leaves were distilled with eight different distillation times (1 to 8 h). However, to find out the effect of storage periods, the leaves were dried in the shade for 1, 2, 3, 4, 5, 6, and 12 months prior to distillation.

Analysis of the essential oil

The essential oil was analyzed for their chemical composition by using GC-FID and GC-MS analysis. GC-FID analysis was carried out with a Thermo Scientific Trace1300 Gas Chromatograph coupled to a flame-ionization detector (FID), while GC-MS analysis was done by PerkinElmer Turbomass Mass Spectrometer (Shelton, USA) system fitted with Equity-5 fused silica capillary column (Supelco Bellefonte, PA, USA) as per standard protocol[4,17].

Results and Discussion

The essential oil yield and composition variation of *M. leucadendra* according to their retention indices and as per experimental objectives, viz. seasons, distillation time, and storage periods, are summarized in Tables 1-3. The essential oil yield varied from 1.4–1.6% in *M. leucodendra* leaves harvested at different seasons, with maximal in the spring season (1.6%) and minimal in the rainy and winter season (1.40%).

The chemical composition of essential oil of *M. leucodendra* from different seasons was determined by GC-FID and GC-MS analysis, which resulted in the identification of eighteen compounds forming 91.2–97.4% of the total oil composition (Table 1). The major constituents identified were (*E*)-Nerolidol (87.2–94.0%) followed by β-caryophyllene (0.3–2.0%). The maximum content of (*E*)-Nerolidol was reported in the spring season (94.0%), followed by winter (93.6%), autumn (93.1%) and summer season (92.2%), and the lowest content of (*E*)-nerolidol was obtained in the rainy season (87.2%). The contents of the second major constituent, β-caryophyllene were in the following order: rainy (2.0%)> summer (1.3%), winter (1.3%) > autumn (1.0%) > spring season (0.3%). Harvesting season has an impact on essential oil yield and content.

Several studies have reported the impact like on *Rosmarinus officinalis* L.[18], *Thymus linearis*[19], *Mentha longifolia*[20], *Ocimum* species[21], *Artemisia nilgirica*[22], and *Thymus serpyllum*[23]. These studies also have suggested that every plant has a season most suitable for its essential oil content and composition. The changes in essential oil content and composition of *M. leucadendra* with respect to storage period under shade conditions are summarized in Table 2 and were classified into four classes based on their terpene structures. The essential oil yield of dried leaves of *M. leucadendra* was found to show variation in order 1 and 2-month stored leaves (4.2%) > 3-month stored leaves (4.1%) > 4 and 6-month stored leaves M1 (4.0%) and minimal in 9 and 12-month stored leaves (3.8%). The percentage of major constituents (*E*)-nerolidol varied from 91.6–95.5%, with the maximum in the essential oil of 9-month stored leaves and minimum in the essential oil extracted from 1 and 2-month stored leaves (91.6%). Results showed only slight quantitative variation in

Table 1 — Seasonal variation on essential oil composition of *Melaleuca leucadendra* L.

S. No	Compound[a]	RI[b]	RI[c]	Content (%)				
				Spring	Summer	Rainy	Autumn	Winter
1	α-Pinene	934	932	t	0.3	0.3	0.2	0.1
2	Sabinene	965	969	0.1	0.1	0.1	t	t
3	Limonene	1026	1024	0.5	0.3	t	0.1	0.2
4	Linalool	1101	1095	0.9	0.2	0.1	0.2	0.4
5	Camphor	1145	1141	0.6	t	0.1	t	t
6	Terpinen-4-ol	1177	1174	t	t	t	0.1	0.1
7	α-Terpineol	1188	1186	0.3	t	0.1	t	0.1
8	Neral	1232	1235	t	0.1	t	0.1	t
9	Linalyl acetate	1256	1259	0.3	0.2	t	t	t
10	β-Caryophyllene	1423	1417	0.3	1.3	2.0	1.0	1.3
11	α-Humulene	1454	1452	t	0.2	0.1	0.1	0.1
12	(*E*)-β-Farnesene	1456	1454	t	0.1	0.1	0.1	t
13	Germacrene D	1488	1484	0.1	0.2	0.2	t	0.1
14	**(*E*)-Nerolidol**	1565	1561	**94.0±0.5**	**92.2±2.3**	**87.2±5**	**93.1±0.5**	**93.6±0.8**
15	Caryophyllene oxide	1583	1582	0.3	0.3	0.7	0.5	0.4
16	Viridiflorol	1602	1592	t	0.1	0.1	t	0.1
17	β-Eudesmol	1648	1649	t	t	t	t	0.1
18	(Z)-Nerolidyl acetate	1673	1676	t	t	0.1	t	0.1
Monoterpenes hydrocarbons				0.6	0.7	0.4	0.3	0.3
Oxygenated monoterpenes				2.1	0.5	0.3	0.4	0.6
Sesquiterpene hydrocarbons				0.4	1.8	2.4	1.2	1.5
Oxygenated sesquiterpene				94.3	92.6	88.1	93.6	94.3
Total identified				**97.4**	**95.6**	**91.2**	**95.5**	**96.7**
Essential oil content (%)[d]				1.6±0.04	1.5±0.00	1.4±0.04	1.5±0.03	1.4±0.06

[a]Mode of identification: Retention Index (RI), MS (GC-MS); [b]Experimental retention index determined on DB-5 gas chromatography capillary column (30 m × 0.32 mm) using n-alkanes; [c]Retention index from the literature (Adams, 2007); [d]Fresh weight basis (v/w); t = trace (<0.05%)

composition with respect to harvest in different seasons as well as in different storage periods. Several previous studies have evaluated the effect of drying and storage on the EO yield, such as *Ocimum basilicum* L.[24], *Stachys lavandulifolia*[25], *Origanum vulgare*[26], *Cymbopogon distans*[27], *Rosmarinus officinalis*[28], *Melaleuca alternifolia*[29]. These studies also have suggested shade drying as one of the most suitable methods of drying herbs. This might be attributed to the lower temperature of shade drying, which results in lesser evaporation of fragrant compounds present in the essential oil. This data has significant implications for profitable oil production.

The essential oil content and relative percentage of the identified constituents of essential oils from the fresh leaves of *M. leucadendra* using different distillation times are tabulated in Table 3 using the GC-MS method. As per the experimental design, the extracted oil from samples was used for optimizing the distillation duration for maximum recovery of essential oils, for which the fresh leaves were hydro-distilled for 1, 2, 3, 4, 5, 6, 7, and 8 h respectively, by hydro-distillation to produce oil by the recommended method. Results showed that distillation duration had a significant effect on essential oil content, varying from 0.8–1.6%. The highest oil content was observed when the leaves of *M. leucodendra* were hydro-distilled for 8 h (1.6%) followed by 7 h (1.5%) > 6 and 5 h (1.4%)> 4 h (1.2%) > 3 h (1.1%) and minimum (0.8%) at 1 h. The essential oils of different distillation duration (1-8 h) were analysed by GC-FID and GC-MS techniques.

This is the first report on the effect of distillation time on oil composition. Distillation time showed significant change in essential oil yield and composition of plants, such as lavender[30], palmarosa,

Table 2 — Effect of storage period on essential oil composition of *Melaleuca leucadendra* L.

S. No	Compound[a]	RI[b]	RI[c]	Content (%)						
				1M	2M	3M	4M	6M	9M	12M
1	α-Pinene	934	932	0.1	0.1	0.1	0.2	0.1	0.1	0.1
2	Sabinene	965	969	0.4	0.4	0.5	0.3	0.4	0.3	0.4
3	Limonene	1026	1024	0.1	t	t	0.	t	0.1	0.1
4	Linalool	1101	1095	0.1	t	t	t	0.1	0.1	0.1
5	Camphor	1145	1141	t	t	t	t	t	0.1	0.1
6	Terpine-4-ol	1177	1174	t	0.1	t	0.1	0.1	0.1	t
7	α-Terpineol	1188	1186	t	t	t	t	0.1	t	0.1
8	Nerol	1232	1235	t	0.1	0.1	0.1	0.2	t	0.1
9	Linalool acetate	1256	1259	0.1	0.1	t	0.1	0.1	t	t
10	β-Caryophyllene	1423	1417	0.1	1.1	1.1	1.0	1.0	1.0	1.0
11	α-Humulene	1454	1452	0.2	0.1	0.2	0.2	0.1	0.2	0.2
12	(*E*)-β-farnesene	1456	1454	t	0.1	0.1	0.1	0.1	0.1	0.3
13	Germacrene-D	1488	1484	t	t	t	t	0.1	0.1	0.1
14	**(*E*)-Nerolidol**	1565	1561	**91.6±5.7**	**95.0±0.4**	**94.9±0.3**	**95.0±0.1**	**92.0±5.6**	**95.5±0.3**	**92.0±0.2**
15	Caryophyllene oxide	1583	1582	t	0.2	0.3	0.1	0.1	0.1	1.1
16	Viridiflorol	1602	1592	t	0.1	0.3	0.1	0.3	0.2	0.1
17	β-Eudesmol	1648	1649	0.1	0.4	0.4	0.4	0.2	0.3	1.1
18	(*Z*)-Nerolidyl acetate	1673	1676	t	0.2	0.2	0.2	0.3	0.1	t
Monoterpenes hydrocarbons				0.6	0.5	0.6	0.5	0.5	0.5	0.6
Oxygenated monoterpenes				0.2	0.3	0.1	0.3	0.6	0.3	0.4
Sesquiterpene hydrocarbons				0.3	1.3	1.4	1.3	1.3	1.4	1.6
Oxygenated sesquiterpene				91.7	95.9	96.1	95.8	92.9	96.2	94.3
Total identified				**92.8**	**98.0**	**98.2**	**97.9**	**95.3**	**98.4**	**96.9**
Essential oil content (%)[d]				4.2±0.10	4.2±0.04	4.1±1.1	4.0±0.30	4.0±0.08	3.8±0.80	3.8±0.30

[a]Mode of identification: Retention Index (RI), MS (GC-MS); [b]Experimental retention index determined on DB-5 gas chromatography capillary column (30 m × 0.32 mm) using n-alkanes; [c]Retention index from the literature (Adams, 2007); [d]Dry weight basis (v/w) t = trace (<0.05 %); 1M to 12M showed storage period month wise prior to distillation

Table 3 — Effect of distillation time on essential oil composition of *Melaleuca leucadendra* L.

S. No	Compound[a]	RI[b]	RI[c]	Content (%)							
				1H	2H	3H	4H	5H	6H	7H	8H
1	α-Pinene	934	932	0.4	0.1	0.3	0.3	0.3	0.2	0.2	0.2
2	Sabinene	965	969	0.1	t	t	t	t	-	-	t
3	Limonene	1026	1024	0.4	0.1	t	0.2	0.2	0.1	0.1	0.1
4	Linalool	1101	1095	0.1	t	0.1	0.1	0.1	-	0.3	0.1
5	Camphor	1145	1141	0.9	0.2	t	0.2	0.3	0.1	0.2	0.1
6	Terpine-4-ol	1177	1174	t	0.1	-	0.1	-	t	t	-
7	α-Terpineol	1188	1186	0.1	t	-	-	t	-	-	t
8	Nerol	1232	1235	0.2	t	0.1	-	-	-	-	-
9	Linalool acetate	1256	1259	0.1	0.1	-	-	-	-	-	0.2
10	β-Caryophyllene	1423	1417	1.0	1.0	1.0	1.4	1.0	0.9	1.0	1.0
11	A-Humulene	1454	1452	0.1	0.3	0.3	0.2	0.2	0.2	0.1	0.1
12	(E)-B-Farnesene	1456	1454	0.1	t	0.1	0.1	0.1	0.2	0.1	0.1
13	Germacrene-D	1488	1484	0.1	0.2	0.1	0.1	t	-	0.1	-
14	**(*E*)-Nerolidol**	1565	1561	**93.9±0.9**	**95.5±0.4**	**95.5±0.3**	**95.3±0.8**	**96.1±0.8**	**95.3±0.9**	**94.7±2.0**	**95.7±0.7**
15	Caryophyllene Oxide	1583	1582	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.2
16	Viridiflorol	1602	1592	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2
17	β-Eudesmol	1648	1649	0.2	0.1	0.1	t	t	0.1	0.2	t
18	(Z)-Nerolidyl acetate	1673	1676	0.1	0.1	-	t	0.1	0.1	0.1	-
Monoterpenes hydrocarbons				0.9	0.2	0.3	0.5	0.5	0.3	0.3	0.3
Oxygenated monoterpenes				1.4	0.4	0.2	0.4	0.4	0.1	0.5	0.4
Sesquiterpene hydrocarbons				1.3	1.5	1.5	1.8	1.3	1.3	1.3	1.2
Oxygenated Sesquiterpene				94.5	96	95.8	95.5	96.4	95.8	95.4	96.1
Total identified				**98.1**	**98.1**	**97.8**	**98.2**	**98.6**	**97.5**	**97.5**	**98.0**
EO content (%)[d]				0.8	0.9	1.1	1.2	1.4	1.4	1.5	1.6

[a]Mode of identification: Retention Index (RI), MS (GC-MS); [b]Experimental retention index determined on DB-5 gas chromatography capillary column (30 m × 0.32 mm) using n-alkanes; [c]Retention index from the literature (Adams, 2007); [d]Fresh weight basis (v/w) t = trace (<0.05 %); 1H to 8H showed the distillation time in hours.

lemon-grass, peppermint[31], cumin seed oil[32], *Acorus calamus*[33], and *Agastache astromontana*[34]. Regardless of the distillation time, the isolated oils of different distillation duration did not show significant differences in their chemical composition as the content of major constituents (*E*)-nerolidol varied from 93.3–96.1%, with a maximum 96.1% in essential oil distilled for 5 h, and lowest 93.3% in essential oil distilled for 1 h. However, essential oil content increases with an increase in distillation time duration.Variations in oil yield and composition with respect to harvesting seasons, distillation duration and post-harvest storage prior to essential oil extraction have been reported by various researchers in several other aromatic plants[18-35].

Conclusion

The current research has significant results for the production of (*E*)-Nerolidol-rich essential oil of *Melaleuca leucadendra*, which is known for its floral and fruity aroma and used for food flavouring, fine fragrances, perfumery, cosmetics and pharmaceutical products. Oxygenated sesquiterpenes(>88%) were the major class of compounds, followed by sesquiterpene hydrocarbons and monoterpenoids. The main constituent reported of essential oils was (*E*)-nerolidol and β-caryophyllene. Current research provides a way to extract maximum essential oil content in the spring season, one month of storage and eight-hour long distillation. This is the first-ever report to study the essential oil composition of leaves of *M. leucadendra* with respect to seasons (seasonal variation), storage and distillation time grown in the foothills of northern India. Furthermore, it is suggested that season, storage conditions, and distillation time have a significant impact on the recovery of essential oils, and slight modifications in existing distillation practices may increase the oil return per tree.

Conflict of interest

The authors declare that they have no known conflicting interests that could have appeared to influence the work reported in this paper.

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