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## Research Article

# Hydrodistillation Essential Oil of *Michelia balansae* and its Characteristics from Xuan Son National Park, Vietnam

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## Abstract

**Background and Objectives:** *Michelia balansae*, belonging to the genus *Michelia*, consists of approximately 80 species including 26 species that were discovered in Vietnam. This genus was reported as a source of essential oils, which were principally collected in one season of the year. This study aimed to discover the variety of essential oils from leaves of *M. balansae* grown in Xuan Son National Park, Vietnam, collected various winter and summer. **Materials and Methods:** Essential oils from *M. balansae* leaves obtained during winter and summer were extracted by hydrodistillation method with optimal conditions (ratio solid:liquid 1:4 and 120 min of distillation time). The chemical composition of essential oil was analyzed by using the GC/MS method. **Results:** The optimal conditions including solid-liquid ratio (1:4 or 1:5) and distillation time (120-150 min) for the extraction of essential oil from *M. balansae* leaves were determined in this study. Moreover, the components of essential oil from *M. balansae* leaves collected during these two periods were identified. **Conclusion:** The present study provides further insights into the seasonal variation of essential oil compounds of *M. balansae*.

**Key words:** *Michelia balansae*, essential oil, seasonal variation, hydrodistillation, solid:liquid ratio, extraction time

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

*Michelia balansae* belongs to the genus *Michelia*, subfamily Magnolioideae (Magnoliaceae), which distributed in tropical and subtropical regions of Asia<sup>1</sup>. This genus consists of about 80 species including 26 species that were recorded in Vietnam<sup>2</sup>. Many chemical constituents were isolated from different *Michelia* species such as steroids, terpenoids, alkaloids, phenols, esters, aporphines, benzenoids, flavonoids and volatile constituents<sup>3</sup>. These compounds had important biological activities including anticancer, antibacterial activity, antiparasitic activity, anti-inflammatory, antidiabetic, antioxidants and flavouring agents Kumar *et al.*<sup>3</sup>. So, the *Michelia* plants have been recorded as traditional medicinal herbs that were used in the treatment of fever, colic, leprosy, postpartum protection, inflammation, gut, airways, eye and cardiovascular disorders<sup>3,4</sup>.

*Michelia* plants are resources of essential oils which possess many benefits. Essential oils have been extracted and analysed from various *Michelia* trees including *Michelia foveolata*<sup>5</sup>, *Michelia champaca*<sup>6-8</sup>, *Michelia alba*<sup>9-10</sup>, *Michelia balansae*<sup>11</sup>, *Manglietia fordiana*, *Manglietia conifera*, *Michelia mediocris* and *Michelia tonkinensis*<sup>12</sup>. The biological activities of *Michelia* essential oils were reported such as antibacterial, anti-fungal and antioxidant activities<sup>8,10,13-16</sup>. The present study aims to (1) Identify the essential oil components in leaves of *M. balansae* various seasons and (2) Indicate characteristics of the essential oil components in leaves of *M. balansae* by comparative to other plants.

## MATERIALS AND METHODS

**Study area:** This study was conducted at the greenhouse at the Faculty of Natural Sciences, Hung Vuong University and Institute of Natural Products Chemistry (INPC), Vietnam Academy of Science and Technology (VAST) from December, 2019-July, 2021.

**Plant materials:** Leaves of *M. balansae* grown in Xuan Son National Park, Tan Son District, Phu Tho Province, Northern Vietnam (21°9'N 104°56'E) were collected in June and December, 2020.

### Research procedure

**Hydrodistillation of essential oils:** The essential oils of the dry leaves, (500 g) were shredded and hydrodistilled for 2-3 hrs using a Clevenger-type apparatus (there was a change in the ratio of water to the material and the addition of NaCl salt with different concentration levels during the soaking process), then essential oils were separated and dried with anhydrous

Na<sub>2</sub>SO<sub>4</sub>. The obtained oils were stored at 5 °C in a refrigerator until being analyzed. The oil yield and all data were expressed as Means ± SD of 3 independent experiments.

**Essential oil analysis:** A Hewlett-Packard (HP) 6890 gas chromatograph was equipped with a Mass Spectrum Detector (MSD) Agilent Technologies 5975C and a HP-5 MS column (Agilent Technologies, Santa Clara, CA, USA). The column dimensions were 60 m × 0.25 mm, film thickness 0.25 µm. The injector was established at 250 °C. The temperature was automatically programmed at a 60 ramp of 4 °/min up to 240 °C. A flow rate for Helium as the carrier gas is 1 mL min<sup>-1</sup>. The split ratio was 100:1 and 1 mL of extract material was injected. The MSD circumstance was based on full scan modes under electron impact ionization voltage of 70 eV, emission current of 40 mA, acquisitions scan mass range 35-450 amu. GC-FID analytical procedure has been performed under the same condition as that of the GC-MS method. The determination of the EOs was performed by comparing their RI and MS data with those from HPCH1607, W09N08 libraries and NIST Chemistry Web-Book. The relative amount of each volatile individual was calculated based on the GC-FID peak area without any correction factor. Data were presented as Means ± SD of 3 independent replications.

**Statistical analysis:** Statistical analysis of all data was performed by using the ANOVA and means separated by Duncan's Multiple Range Test at the 5% level of significance (p = 0.05).

## RESULTS AND DISCUSSION

### Hydrodistillation extraction of essential oil of *M. balansae*:

Factors affecting the extraction of *M. balansae* essential oil, including solid-liquid ratio and distillation time, were examined in this study. First of all, the ratio of raw material to solvent (solid-liquid ratio) was investigated, ranging from 1:1 to 1:6, respectively. In addition, the extraction time (30-150 min) was also calculated.

### Effect of solid-liquid ratio on the extraction of essential oils:

The single factor test of the solid-liquid ratio was carried out to investigate the effect of the ratio on the extraction yield of essential oils (Fig. 1). The results showed that the solid-liquid ratio had an impact on the extraction yield. When the solid-liquid ratio was less than 1:16, the extraction yield of essential oil went up as the solid-liquid ratio increased. Essential oil yields were ranging from 0.67-1.08% in extraction experiments. The extraction rate of essential oils reached the highest at 1.33%.

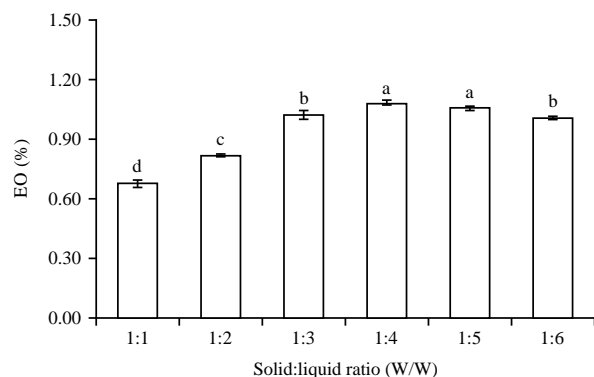


Fig. 1: Effect of solid:liquid ratio on essential oil yield obtained from leaves of *M. balansae*

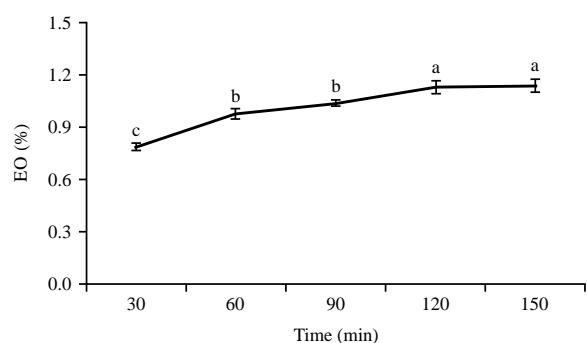


Fig. 2: Effects of extraction time on essential oil yield obtained from leaves of *M. balansae*

When the solid-liquid ratios were 1:4 and 1:5, their yields were 1.08 and 1.05%, respectively. On the contrary, when the ratios were 1:3 and 1:6, their yields fell at 1.02 and 1.00%, respectively. The extraction rate of essential oils was lowest when the solid-liquid ratio was 1:1. The result showed that the optimum solid-liquid ratio is 1:4 or 1:5.

**Effect of distillation time on the extraction yield:** The single factor test of distillation time was taken to investigate the effect of the ratio on the extraction yield of essential oils (Fig. 2). The results displayed that with the extension of distillation time, extraction yield levels increased or remained unchanged, with the maximum level achieved at distillation time between 120 (1.10%) and 150 min (1.16%). It was noticed that the extraction yield reached 0.78 at 30 min of distillation. The damage degree of the cell wall rose by heating with a longer distillation time, causing a significant increase in extraction yield. The maximum damage of the cell wall reached the highest level when the distillation time was 2 hrs, then the extraction yield was stable despite a longer distillation time. The optimal distillation time was 2 hrs when

energy-saving and extraction yield ensuring were considered. Zheljaskov<sup>17</sup> reported that the essential oil yield of lavender reached a maximum at 60 min of distillation duration.

### Seasonal variation of essential oil composition of *M. balansae*:

The current study presented the EO composition of summer and winter leaves of *M. balansae* which were collected in Xuan Son National Park, Viet Nam in June (summer) and December (winter), 2020. The EO yield from the steam distillation of leaves of *M. balansae* in the summer was 1.05% while it was estimated to be approximately 1.18% in the winter. The composition displayed by the mean percentage of total identified compounds of essential oils collected through the 2 seasons was showed in Table 1. A total of 30 and 28 compounds, accounting for 98.80 and 98.95% of the total content, were identified in the essential oils obtained from summer and winter leaves, respectively. In general, the sesquiterpene hydrocarbons fraction in two essential oils samples dominated that in monoterpene hydrocarbons.

Significant levels of monoterpene hydrocarbons in the essential oils were found only in the winter (43.75%). Limonene was the most prominent component of monoterpene hydrocarbons. By contrast, the level of monoterpene hydrocarbons in the essential oils collected in summer was only 0.63%. Moreover, limonene was not detected in the EO collected in this season. The content of sesquiterpene hydrocarbons in the essential oils was significantly higher during the summer (95.39%) and lower during the winter (54.60 %). Additionally, the oils collected in winter and summer showed notable differences in major compounds. In the summer crops, the major constituents were  $\alpha$ -Cadinene (23.85%), Bicyclogermacrene (20.19%),  $\beta$ -Caryophyllene (15.13%) and  $\beta$ -Selinene (11.26%). However, the prominent components in the winter oils were found to be Limonene (39.05%), Germacrene D (26.76%). This oil also held  $\delta$ -Cadinene (6.95%),  $\beta$ -Caryophyllene (7.16%) and  $\beta$ -Selinene (0.62%) but with the lower amount. Similarly, the summer oils included Germacrene D (4.77%) (Table 2). Levels of oxygenated sesquiterpenes were higher during the winter than those in the summer. Conversely, oxygenated monoterpene, including Linalool and  $\alpha$ -Terpineol, was not detected in the summer oil. The variation between the composition of the *Michelia* spp. essential oils in winter and that in summer was shown by the presence of many compounds which appeared only in one season. For example, Sabinene, Myrcene, Limonene, Terpinolene,  $\alpha$ -Ylangene,  $\beta$ -Copaene,  $\beta$ -Bourbonene,  $\beta$ -Cubebene,  $\beta$ -Gurjunene, Muurola-4(14),5-diene and Germacrene B were detected only in winter

Table 1: Seasonal variation in content and chemical composition of essential oils from leaves of *M. balansae*

EO constituents	RI (Winter)	RI (Summer)	EO composition in winter (%)	EO composition in summer (%)
<b>Monoterpene hydrocarbons</b>			43.75	0.63
α-Pinene	938	938	1.04	0.16
Sabinene	977	977	0.11	-
β-Pinene	983	984	2.65	0.47
Myrcene	990	990	0.71	-
Limonene	1034	1034	39.05	-
Terpinolene	1093	1093	0.19	-
<b>Sesquiterpene hydrocarbons</b>			54.6	95.39
δ-Elementene	1348	1346	2.02	0.48
α-Cubebene	1358	1360	0.19	0.18
α-Copaene	1387	1389	1.82	4.72
cis-β-Elementene	1401	1402	0.71	3.76
β-Caryophyllene	1435	1437	7.16	15.13
α-Guaiene	1451	1451	-	0.78
α-Humulene	1469	1470	2.1	1.93
9-epi-(E)-Caryophyllene	1476	1478	0.64	0.28
γ-Murolene	1489	1490	0.94	1.79
β-Selinene	1502	1504	0.62	11.26
Germacrene D	1497	1497	26.76	4.77
α-Selinene	1512	1512	-	0.42
Bicyclogermacrene	1511	1514	1.72	20.19
α-Bulnesene (=δ-Guaiene)	1520	1520	-	1.71
δ-Cadinene	1527	1529	0.27	0.54
α-Cadinene	1535	1537	6.95	23.85
trans-Cadina-1,4-diene	1547	1547	-	0.13
α-Calacorene	1559	1559	-	0.2
(E)-Nerolidol	1569	1569	-	0.82
Scapanol	1590	1592	0.26	0.18
Spathulenol	1596	1596	-	2.27
α-Ylangene	1383	1383	0.12	-
β-Copaene	1433	1433	0.24	-
β-Bourbonene	1389	1389	0.41	-
β-Cubebene	1400	1400	0.71	-
β-Gurjunene (=Calarene)	1443	1443	0.43	-
Muurolo-4(14),5-diene	1507	1507	0.3	-
Germacrene B	1574	1574	0.23	-
<b>Oxygenated sesquiterpenes</b>			0.17	2.78
Caryophyllene oxide	1603	1603	-	0.7
epi-α-Cadinol (=Tau-Cadinol)	1655	1657	0.17	0.61
α-Muurolo-4(14),5-diene	1661	1661	-	0.19
α-Cadinol	1671	1671	-	0.73
neo-Intermedeol	1674	1674	-	0.55
<b>Oxygenated monoterpene</b>			0.43	0
Linalool	1100	1100	0.32	-
α-Terpineol	1196	1196	0.11	-
<b>Total</b>			98.95	98.80

oils. By contrast, α-Guaiene, α-Selinene, α-Bulnesene, trans-Cadina-1,4-diene, α-Calacorene, (E)-Nerolidol and Spathulenol were found only in summer oils. The variation in the composition of essential oils collected from leaves at different seasons was reported for *Mentha longifolia* leaves grown in Tunisia<sup>18</sup>. Similarly, these results are in agreement with those of Heikal *et al.*<sup>19</sup>, who described essential oil composition variation obtained from *Eucalyptus cinerea* leaves at different seasons.

In comparison with previous studies, the contents of Limonene, Germacrene D and δ-Cadinene of *M. balansae* grown in Xuan Son National Park, Vietnam, were significantly higher than those of other *Michelia* cultivars (Table 2), including *M. balansae* (A.DC.) Dandy<sup>11</sup>, *M. tonkinensis* and *M. mediocris*<sup>12</sup>, *M. alba*<sup>9-10</sup>, *M. foveolata*<sup>5</sup>, *M. compressa* var. *formosana*<sup>20</sup>. For α-Pinene, the compound was found in significantly lower quantity in comparison with *M. balansae* (A.DC.) Dandy (18.41%)<sup>11</sup> and *M. tonkinensis*<sup>12</sup> from Nghe An

Table 2: Summary of the chemical composition of essential oils from leaves of *M. balansae*

Essential oil constituents	<i>M. balansae</i> (winter)	<i>M. balansae</i> (summer)	<i>M. balansae</i> (A.D.C.) Dandy <sup>11</sup>	<i>M. foveolata</i> (summer) <sup>5</sup>	<i>M. mediocris</i> (summer) <sup>12</sup>	<i>M. tonkinensis</i> (summer) <sup>12</sup>	<i>M. alba</i> (summer) <sup>10</sup>
<b>Monoterpene hydrocarbons</b>	43.75	0.63	24.95	0.5	1.7	52.5	0.13
α-Pinene	1.04	0.16	18.41	0.3	0.2	40.3	-
Sabinene	0.11	-	0.83	-	0.2	-	-
β-Pinene	2.65	0.47	1.58	-	0.4	7.4	0.13
Myrcene	0.71	-	3.88	0.2	0.9	4.8	-
Limonene	39.05	-	-	-	-	-	-
Terpinolene	0.19	-	0.25	-	-	-	-
<b>Sesquiterpene hydrocarbons</b>	54.6	95.39	40.55	67.4	59.7	23.4	14.67
δ-Elementene	2.02	0.48	5.11	-	-	-	-
α-Cubebene	0.19	0.18	-	-	-	0.3	-
α-Copaene	1.82	4.72	0.25	0.2	0.5	1.2	0.21
cis-β-Elementene	0.71	3.76	0.99	1.4	-	-	3.03
β-Caryophyllene	7.16	15.13	3.47	37.1	2.0	6.9	4.04
α-Guaiene	-	0.78	-	-	-	-	-
α-Humulene	2.1	1.93	1.66	2.5	2.3	3.6	-
9-epi-(E)-Caryophyllene	0.64	0.28	-	-	-	-	-
γ-Murolene	0.94	1.79	-	-	-	-	-
β-Selinene	0.62	11.26	-	-	7.2	-	-
Germacrene D	26.76	4.77	17.98	1.8	-	1.0	1.27
β-Selinene	-	0.42	-	-	-	-	0.86
Bicyclgermacrene	1.72	20.19	7.55	23.3	-	7.3	-
α-Bulnesene (=δ-Guaiene)	-	1.71	0.19	-	-	-	-
γ-Cadinene	0.27	0.54	-	-	-	-	0.15
α-Cadinene	6.95	23.85	0.18	0.6	10.9	0.6	0.48
trans-Cadina-1,4-diene	-	0.13	0.2	-	-	-	-
α-Calacorene	-	0.2	-	-	0.4	-	-
(E)-Nerolidol	-	0.82	-	0.5	36.4	-	4.42
Scapanol	0.26	0.18	-	-	-	-	-
Spathulenol	-	2.27	0.24	-	-	0.7	-
α-Ylangene	0.12	-	0.54	-	-	-	-
β-Copaene	0.24	-	-	-	-	-	0.21
β-Bourbonene	0.41	-	-	-	-	-	-
β-Cubebene	0.71	-	-	-	-	1.8	-
β-Gurjunene (=Calarene)	0.43	-	-	-	-	-	-
Muurolo-4(14),5-diene	0.3	-	-	-	-	-	-
Germacrene B	0.23	-	2.19	-	-	-	-
<b>Oxygenated sesquiterpenes</b>	0.17	2.78	0.69	2.4	5.7	0.3	1.54
Caryophyllene oxide	-	0.7	0.21	1.4	-	-	1.36
epi-α-Cadinol (=Tau-Cadinol)	0.17	0.61	0.48	-	-	-	-
α-Muurolo (=δ-Cadinol)	-	0.19	-	-	-	-	-
α-Cadinol	-	0.73	-	1.00	5.7	0.3	0.18
Neo-Intermedeol	-	0.55	-	-	-	-	-
<b>Oxygenated monoterpene</b>	0.43	0	0.28	0	1.6	0.3	73.14
Linalool	0.32	-	0.28	-	1.5	0.3	72.89
α-Terpineol	0.11	-	-	-	0.1	-	0.25

province, Vietnam. High contents of β-Caryophyllene and Bicyclgermacrene were identified in *M. foveolata*<sup>5</sup>. The high contents of Linalool were found in *M. alba* D.C. from China<sup>9</sup>, but less quantity in this study. Compared to the composition of *Michelia* essential oils collected in Vietnam, which were collected in summer<sup>5,10-12</sup>, many compounds such as Limonene (39.05%) and other components with little quantity were discovered 1st time in this study, including 9-epi-(E)-Caryophyllene, Scapanol, β-Bourbonene, β-Gurjunene (=Calarene), Muurolo-4(14),5-diene and α-Muurolo (=δ-Cadinol) (Table 2).

Based on the findings and results of previous studies, the components of *M. balense* essential oils disclosed a variation in its constituents depending on geographical and harvest time. Naturally, the plant adaptive metabolism could affect the quality, quantity and chemical composition of the plant essential oils. It also holds a unique and specific chemical composition. This result was in line with those of Zouari-Bouassida *et al.*<sup>18</sup>, who presented a variety of essential oil compositions collected from *Mentha longifolia* leaves at different seasons. Moreover, the result of this study supports the findings in the research conducted by Heikal *et al.*<sup>19</sup>, who

reported variation of essential oil components obtained from *Eucalyptus cinerea* leaves at various seasons. The variation of essential oil components could be a mechanism that adapts to changing seasons. Therefore, investigation on essential oil components of *M. balansae* or other plants between regions with various seasons is necessary for future studies. Besides, the described variation of essential oil components of *M. balansae* leaves between summer and winter could suggest the favourable collection time for different uses of essential oil of this tree.

### CONCLUSION

This study described the optimal conditions such as solid-liquid ratio (1:4 or 1:5) and distillation time (120-150 min) for the extraction of *M. balansae* essential oil. This study also investigated the chemical composition of essential oils of *M. balansae* collected from Xuan Son Nation Park, Vietnam, in winter and summer. A total of 28 and 30 compounds, accounting for 98.95 and 98.80% of the total content, were described in the essential oils collected from winter and summer leaves, respectively. Moreover, the variation in quality and quantity of components of essential oils was reported to depend on the time of harvest and growing location.

### SIGNIFICANCE STATEMENT

This study showed the influence of solid-liquid ratio and extraction time on essential oil yield collected from leaves of *M. balansae* grown at Xuan Son Nation Park, Vietnam. Additionally, These results released the variation of chemical compounds of essential oil in the plant from winter and summer. Some components such as limonene and other compounds at little amount were firstly reported from *M. balansae* essential oil collected during winter. This study helps the researchers to uncover the critical areas of extraction techniques that many other researchers did not explore.

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