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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OILS FROM STEMS AND LEAVES OF *Michelia alba* D.C. GROWING IN VIETNAM

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ABSTRACT

The chemical composition of the essential oils obtained by hydrodistillation of the stems and leaves of *Michelia alba* D.C. growing in North Vietnam was analyzed using GC/MS. In total, 28 compounds were identified in both stem and leaf oils, accounting for 99.33% and 99.54% of the oils, respectively. Linalool was the major component of both oils (80.65% and 72.89%, respectively), and other 22 compounds were also present as the constituents of both oils with varying amounts except 1,10-di-epi-cubenol present only in stem oil, whereas borneol (=endo-borneol), α-copaene, α-selinene and ethyl 2-phenylhexanoate were found only in leaf oil. In the stem oil, (*Z*)-β-ocimene (1.94%), (*E*)-β-ocimene (2.28%), β-caryophyllene (1.26%), (*E*)-nerolidol (1.15%), caryophyllene oxide (2.26%) and epi-α-muurolol (=T-muurolol) (1.80%) were present in sizeable amounts. The minor components detected in the leaf oil were (*Z*)-β-ocimene (1.73%), (*E*)-β-ocimene (2.28%), *Cis*-β-elemene (3.03%), β-caryophyllene (4.04%), α-humulene (1.44%), (*E*)-nerolidol (4.42%) and caryophyllene oxide (1.36%). Antibiotic activity of the essential oil samples was tested against Gram positive bacteria *Staphylococcus aureus*, Gram negative bacteria *Escherichia coli*, and yeast *Candida albican* using an agar disk diffusion method. The antimicrobial activity of leaf oil was stronger than that of stem oil, although both oils have significant inhibitory activity against all three microorganisms tested with the inhibitory zone diameters ranging from 15.5 to 46.5 mm.

Keywords: *Michelia alba*, essential oil composition, stem oil, leaf oil, antimicrobial activity.

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INTRODUCTION

The genus *Michelia* belonging to family Magnoliaceae includes about 50 species. In Vietnam distribution of nearly 20 species were recorded. Some species among them contain essential oil used in fragrance and perfume production, some species are important sources of timber, and the leaves and stems of some species have been used as medicine to cure bronchitis and urologic diseases (Vo, 2004). *Michelia alba* D.C., belonging to the genus of *Michelia*, and commonly known as "Ngoc lan hoa trang" in Vietnam, is an evergreen tropical fast growing tree from Southeast Asia. It has medium-sized height (6–10 m) with an upright habit and attractive lime-green foliage. It is beloved for its wonderfully fragrant flower, one of the world most famous aromatic flowers. The orchid-like, white flowers appear from mid Spring through to Autumn. In Vietnam, *M. alba* is found wild growth or cultivated as ornamental tree for its fragrance.

The chemical compositions of essential oil from flower and leaf of *M. alba* in China (Ueyama et al., 1992); essential oil from flower of *M. alba* in Malaysia (Sanimah et al., 2008), in Thailand (Punjee et al., 2009; Pensuk et al., 2007), and in Vietnam (Ngo & Dau, 2017) have been reported. Various methods were used to extract essential oils or volatile constituents from the flowers of *M. alba* (Shang et al., 2002; Pensuk et al., 2007; Punjee et al., 2009)

The synergistic effect of two main components of essential oil of *M. alba*, linalool and caryophyllene at the 10:1 ratio in vapor was the key factor in enhancing the antifungal activity against mold *Aspergillus flavus* on brown rice and could extend the shelf-life of brown rice for up to 16 weeks compared to just 4 weeks for the control (Songsamoe et al., 2017). Volatile extract via solid-phase micro extraction (SPME) of *M. alba* leaves in China inhibited drinking and aggregation of imported red fire ant (Qin et al., 2018). Antimicrobial activities of essential oil of *Michelia foveolata* Merryll ex Dandy from Vietnam were investigated and it showed inhibition zones against many bacteria (Lesueur et al., 2007). However, to our knowledge, limited investigation has been done on the essential oil from stem and leaf of *M. alba* and its antimicrobial activity has not yet been published to date. In this study, we would like to report the results of an analysis and antimicrobial activity of the stem and leaf oils produced from *M. alba* growing in Vietnam.

MATERIALS AND METHODS

Plant material: Leaves and stems of *Michelia alba* D.C. growing in the Me Linh Station for Diversity, Institute of Ecology and Biological Resources, Ngoc Thanh commune, Phuc Yen city, Vinh Phuc province, northern Vietnam (21°23.052’N 105°42.745’E) were collected in April 2018. The plant was identified by Dr. T.C. Nguyen and Dr. Q.N. Vu individually, and a voucher specimen (VP1804) has been deposited at the Herbarium of Institute of Ecology and Biological Resources (HN), Vietnam Academy of Science and Technology. Respective 1.6 kg and 2.6 kg samples of the fresh leaf and stem materials were shredded and hydrodistilled for 3 and 4 hours using a Clevenger type apparatus, and then essential oils were separated and dried with anhydrous $MgSO_4$. The obtained oils were stored at (-)5°C until analysis.

***Microbial strains*:** Evaluation of the antimicrobial activity of the essential oils was performed using 1 strain each of Gram positive test bacteria *Staphylococcus aureus* (ATCC 13709), Gram negative test bacteria *Escherichia coli* (ATCC 25922) and yeast *Candida albican* (ATCC 10231). These strains were obtained from ATCC (American Type Culture Collection).

Gas Chromatography–Mass Spectrometry: Analysis of the essential oils was carried out by GC/MS using an Agilent GC7890A system with Mass Selective Detector (Agilent 5975C). A HP-5MS fused silica capillary column (60 m × 0.25 mmid. × 0.25 μm film thickness) was used. Helium was the carrier gas with a flow rate of 1.0 mL/min. The inlet temperature was 250°C and the oven temperature program was as follows: 60°C to 240°C at 4°C/min with an interphase temperature of 270°C. The split ratio was 1:100, the detector temperature was 270°C and the injection volume was 1 μL. The MS interface temperature was 270°C, MS mode, E.I. detector voltage 1,200 V, and mass range 35–450Da at 1.0 scan/s. Identification of components was achieved based on their retention time indices and by comparison of their mass spectral fragmentation patterns with those stored on the MS library (HPCH1607, NIST08, Wiley09). Component relative contents were calculated based on total ion current without standardization. Data processing software used was MassFinder 4.0.

Screening of antimicrobial activity: The agar disk diffusion method was performed to test the antimicrobial activity of essential oil (Bauer et al., 1966; Jorgensen & Ferraro, 2009; Balouiri et al., 2016). Testing media included Mueller-Hinton Agar (MHA) used for bacteria and Sabouraud Agar (SA) used for fungi. Microorganisms were stored at (-)80°C and activated prior to testing by culture to reach concentration of 1.0×10^6 CFU/ml in the medium. For testing, 100 µl *inoculum solution* was taken and spread evenly over the surface of the agar. Using an aseptic technique, 2 holes (about 6mm in diameter of each hole) were made on each agar plate and 50µl essential oil was pipetted in each hole. The petri dishes were kept at room temperature for 2–4 hours and then incubated at 37°C for 18–24 hours. The presence or absence of growth around each antimicrobial disk on each plate culture was observed. A ruler with millimeter markings was used to measure the diameters of inhibition growth zones values, and the values were recorded in the chart. The zone of inhibition is the point at which no growth is visible to the unaided eye. An inhibition zone of 14 mm or greater (including diameter of the hole) was considered as high antibacterial activity (Mothana & Lindequist, 2005; Philip et al., 2009).

RESULTS AND DISCUSSION

Chemical composition of *Michelia alba* essential oils

Both essential oils extracted from the stems and leaves of *M. alba* by hydrodistillation were yellow liquid having densities lighter than water. The chemical compositions of the essential oil from the stems and the leaves of *M. alba* from Vietnam are summarized in table 1, figures 1 & 2.

Table 1. Compositions of the stem and leaf essential oils of *Michelia alba* D.C.

Nº	Components	RI^S	Stem oil (%)	RI^L	Leaf oil (%)
1	β-Pinene	984	0.43	985	0.13
2	(*Z*)-β-Ocimene	1038	1.94	1039	1.73
3	(*E*)-β-Ocimene	1049	2.28	1050	2.28
4	*Trans*-Linalool oxide	1078	0.43	1079	0.21
5	*Cis*-Linalool oxide	1094	0.38	1095	0.20
6	Linalool	1107	80.65	1107	72.89
7	Borneol (=Endo-Borneol)	1178	-	1178	0.13
8	α-Terpineol	1201	0.47	1201	0.25
9	Methyl chavicol (=Estragole)	1206	0.32	1207	0.20
10	Geraniol	1258	0.36	1258	0.17
11	α-Copaene	-	-	1390	0.21
12	*Cis*-β-Elemene	1404	0.81	1404	3.03
13	Methyl eugenol	1409	0.86	1410	0.71
14	(*E*)-Caryophyllene (=β-Caryophyllene)	1437	1.26	1438	4.04
15	α-Humulene	1472	0.49	1473	1.44
16	Germacrene D	1498	0.64	1499	1.27
17	(*E*)-Methyl isoeugenol	1505	0.86	1505	1.25
18	α-Selinene	-	-	1514	0.86
19	γ-Cadinene	1534	0.21	1534	0.15
20	δ-Cadinene	1537	0.32	1537	0.48
21	(*E*)-Nerolidol	1571	1.15	1572	4.42
22	Caryophyllene oxide	1606	2.26	1606	1.36
23	Humulene Epoxide II	1633	0.33	1633	0.21
24	1,10-di-epi-Cubenol	1649	0.29	-	-
25	Ethyl 2-phenylhexanoate	-	-	1652	0.47
26	epi-α-Muurolol (=T-Muurolol)	1663	1.80	1663	0.91
27	α-Cadinol	1676	0.36	1676	0.18
28	neo-Intermedeol	1679	0.43	1679	0.36
	Total		**99.33**		**99.54**

Note: RI: Retention time indices; ^{S}: Stem oil; ^{L}: Leaf oil

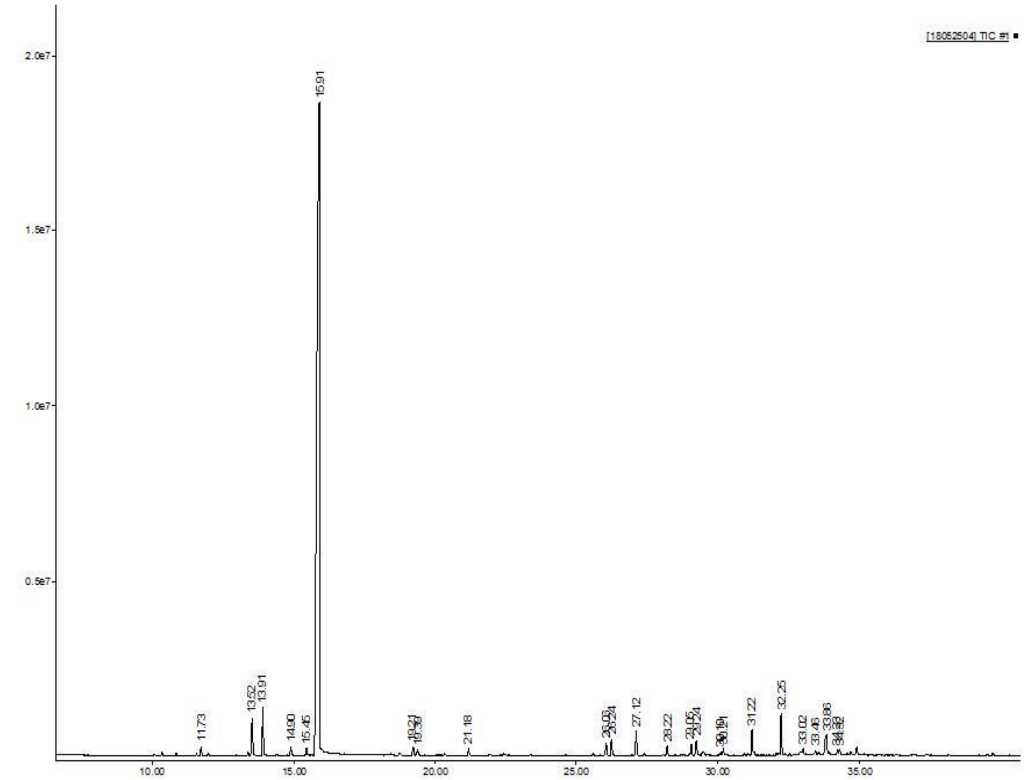

Figure 1. Spectra of stem essential oil constituents of *Michelia alba* D.C.

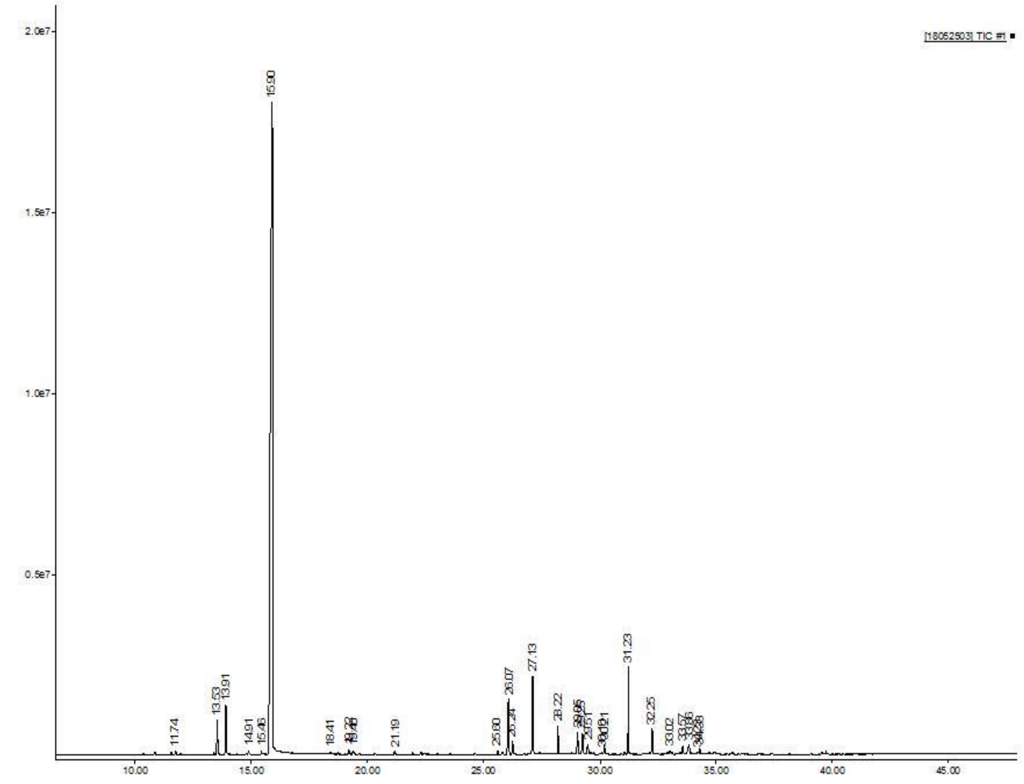

Figure 2. Spectra of leaf essential oil constituents of *Michelia alba* D.C.

The oil yield from the stems was 0.106% (v/w) calculated on a dry weight basis. In this essential oil a total of 24 constituents representing 99.33% of the compositions were identified. Linalool was the major component accounting for 80.65%. The most abundant minor components were (*Z*)-β-ocimene 1.94%; (*E*)-β-ocimene 2.28%; β-caryophyllene 1.26%; (*E*)-nerolidol 1.15%; caryophyllene oxide 2.26% and epi-α-muurolol (=T-muurolol) 1.80%. The rest of the components were present at the amount ranging from 0.21% to 0.86%. A total number of hydrocarbon compounds in the stem essential oil of *M. alba* were 9 compounds accounting for 8.38%, while a total number of compounds containing oxygen were 15 compounds accounting for 90.95%. Monoterpenes consisted of 9 compounds that accounted for 87.26%, sesquiterpenes included 13 compounds accounting for 10.35%, and compounds containing benzene were 2.04%.

The oil yield from the leaves was 0.373% (v/w) calculated on a dry weight basis. In this essential oil, a total of 27 constituents representing 99.54% of the compositions were identified. Similar to the stem oil, linalool was the most abundant component of leaf oil accounting for 72.89%, smaller amount compared with that in the stem oil. (*Z*)-β-ocimene 1.73%; (*E*)-β-ocimene 2.28%; *Cis*-β-elemene 3.03%; β-caryophyllene 4.04%; α-humulene 1.44%; (*E*)-nerolidol 4.42% and caryophyllene oxide 1.36% were also present in sizeable amount. The rest of the components accounted for from 0.13 to 1.27%. The amounts of some constituents present in leaf and stem oils were quite different from each other, for example, *Cis*-β-elemene, (*E*)-caryophyllene (=β-caryophyllene), α-humulene, germacrene D and (*E*)-nerolidol in leaf oil accounted for 3.03%, 4.04%, 1.44%, 1.27%, and 4.42%, respectively, whereas their amount in stem oil was 0.81%, 1.26%, 0.49%, 0.64%, and 1.15%, respectively. Conversely, caryophyllene oxide and epi-α-muurolol (=T-muurolol) were 2.26% and 1.80% in stem oil but only 1.36% and 0.91% in leaf oil. A total number of hydrocarbon compounds in *M. alba* leaf oil was 11 compounds accounting for 15.62%, while a total number of compounds containing oxygen were 16 compounds accounting for 83.92%. Monoterpenes consisted of 10 compounds that accounted for 78.19%, sesquiterpenes included 14 compounds accounting for 18.92%, and compounds containing benzene were 2.63%.

In summary, the yields of essential oils from stems and leaves of *M. alba* were 0.106% and 0.373%, respectively. Chemical compositions of these oils had the same pattern with linalool was major constituent (80.65% in stem oil and 72.89% in leaf oil) and the same presence with various amounts of other 22 compounds constituents except 1,10-di-epi-cubenol in stem oil; and borneol (=endo-borneol), α-copaene, α-selinene and ethyl 2-phenylhexanoate in leaf oil.

In the previous studies, the yields of essential oil of *M. alba* extracted by steam distillation were 0.12% from stem material (Huang et al., 2009) and 0.15–0.49% from leaf material (Ueyama et al., 1992; Huang et al., 2009). Comparative analysis of the present results with previously reported data on stem oil samples of *M. alba* revealed some marked differences. Huang et al. (2009) reported the main component of stem essential oil of *M. alba* was linalool 69.62%; followed by the minor components such as (*E*)-ocimene 2.07%; β-cubebene 1.81%; caryophyllene 3.35%; germacrene D 4.49%; isoledene 1.66%; nerolidol 1.59%; α-asarone 2.65%. The composition of leaf essential oil of *M. alba* collected in Fukien, China contained linalool as the main component representing 80.1%. The minor components present with rather high amount were β-Elemene 1.78%; β-caryophyllene 3.0%; caryophyllene oxide 1.68%; and nerolidol 1.19% (Ueyama et al., 1992). Volatile components analysis of *M. alba* leaves in China showed that linalool was the highest contents in volatiles from fresh, fallen, and dried leaves accounting for 26.10%, 40.52%, and 36.52%, respectively (Qin et al., 2018). Huang et al. (2009) showed the leaf essential oil of *M. alba* contained linalool as the major component with 63.31% and the most abundant minor components were (+)-2-bornanone 1.86%; *Trans*-citral 2.02%; β-cubebene 2.60%; caryophyllene 4.41%; α-humulene 1.78%; nerolidol 7.40%; isoaromadendrene epoxide 3.53%; and α-cadinol 1.63%. These variable results suggested that variability of the stem and leaf oil composition in different population of the same plant species might be due to genetic diversity (Skoula et al., 1999). Previous report of some plant species also showed different chemical compositions among the same species growing in different places under different conditions (Skoula et al., 1996; Yu et al., 2003).

Essential oil from flowers of *M. alba* contained different components in comparison to the oil from stems and leaves, and its variation particularly depends on the extract methods and the distribution area of harvested species. The major components of flower oil identified included indole (1H-indole) (35.49%) and hexadecanoic acid (13.18%) by the enfleurage method; linalool (66.92%) by steam distillation; linalool (28.92%) and 2-methylbutanoic acid (33.01%) by hexane (Pensuk et al., 2007); linalool (72.8%) by petroleum ether (Ueyama et al., 1992); linalool (85.78%, 91.74% and 83.38%) by water distillation, water-steam distillation, and steam distillation, respectively (Punjee et al., 2009); indole (67.89%) by cold enfleurage; linalool (91.00%) by hot enfleurage; phenylethyl alcohol (39.10%) and indole (25.98%) by hexane; phenylethyl alcohol (34.93%) and linalool (34.86%) by petroleum ether (Punjee et al., 2009); linalool (88.5%) by steam distillation (Ngo & Dau, 2017). The different stages of development is also one of the reasons that makes the variation in major compositions of flower essential oils of *M. alba* that were dihydrocarveol (43.8–64.5%), linalool (59.1–79.4%) and butanoic acid-2-methyl, methyl ester (6.4–20.4%). (Sanimah et al., 2008).

The composition of the stem and leaf essential oils of *M. alba*, investigated in the present study, differs from those of the other species of *Michelia*. Indeed, the essential oils from aerial parts of *M. foveolata* in North Vietnam are largely dominated by sabinene (32.4%), terpinen-4-ol (13.7%) (Lesueur et al., 2007). The other study indicated that β-caryophyllene (37.1%), bicyclogermacrene (23.3%) were the major compounds of leaf oil, and β-caryophyllene (26.4%) was the major compound of stem bark oil of *M. foveolata* in Central Vietnam (Do et al., 2016). On the other hand, α-eudesmol (49.70%), eudesma-4 (15), 7-dien-1-β-ol (13.74%) were identified as the major components in leaf oil of *M. foveolata* in South China (Zhong et al., 2006). It was reported that the essential oils from *M. yunnanensis* contained mainly bornyl acetate (12.4%) (Li & Li, 2000). The main component of leaf oil of *M. montana* in India was asaricin (81.8%) (Genderen, van et al., 1999). In the recent study, essential oils from leaves of *M. mediocris* and *M tonkinensis* in Central Vietnam contained δ-cadinene (10.9%), (*E*)-nerolidol (36.4%); and α-pinene (40.3%) respectively (Do et al., 2016). The volatile components of the leaf oil of *M. balansae* from Vietnam included α-pinene (18.4%), α-phellandrene (17.3%) and germacrene D (17.9%) (Nguyen et al., 2005).

Antimicrobial activity of essential oils

Antimicrobial activity of the essential oils extracted from the stems and leaves of *M. alba* was examined using the standard agar disk diffusion method against three test microorganisms. The results were obtained 18–24 hours after incubation and are presented in table 2, figures 3 & 4.

Table 2. Anti-yeast and antibacterial activity of stem and leaf essential oils of *Michelia alba* D.C. (average ± standard deviation, n = 2)

Nº	Sample	Inhibition zones (mm)		
		Staphylococcus aureus	***Escherichia coli***	***Candida albican***
1	Stem oil	15.5 ± 0.71	18.0 ± 1.41	46.0 ± 1.41
2	Leaf oil	18.0 ± 1.41	30.5 ± 2.12	46.5 ± 0.71

Both of essential oils of *M. alba* investigated showed strong inhibition (Mothana & Lindequist, 2005; Philip et al., 2009) against all three microorganism strains tested with inhibition zones of more than 14.0 mm. The stem essential oil showed strong inhibitory activity against *Staphylococcus aureus* and *Escherichia coli*, and very strong activity against *Candida albicans* with the inhibitory zone diameters of 15.5, 18 and 46 mm, respectively. The leaf essential oil exhibited strong activity against *S. aureus* and very strong activity against *E. coli* and *C. albicans* with the inhibitory zone diameters of 18, 30.5 and 46.5 mm, respectively. Antimicrobial activity of the leaf essential oil was higher than that of stem essential oil of *M. alba*. *Staphylococcus aureus* is known as a bacterium causing pains, burns, sore throats, pus infections on the skin and internal organs; *Escherichia coli* can cause some gastrointestinal diseases, such as gastritis, colitis, enterocolitis, bacillary dysentery; *Candida albicans* also can cause baby thrush in children and gynecological diseases. The assay results will be the basis to open the new research of the antimicrobial activity of this plant.

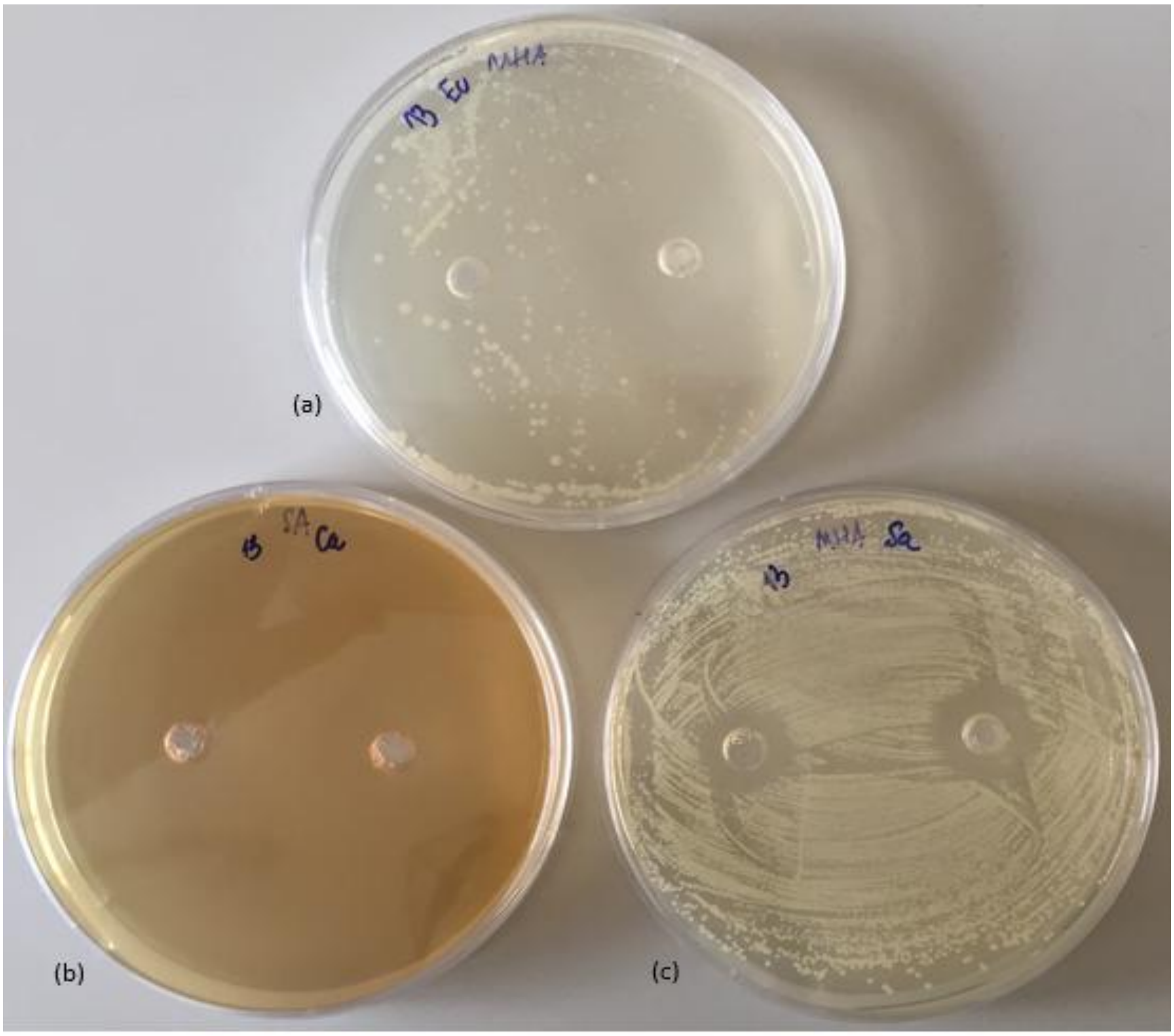

Figure 3. Antimicrobial activity of stem essential oil of *Michelia alba* D.C. (Note: (a): *Escherichia coli*; (b): *Candida albicans*; (c): *Staphylococcus aureus*)

In the previous research of Qin et al. (2018), volatile extracts of *M. alba* fresh leaves in China via Solid-Phase Micro extraction (SPME) showed the highest inhibitory activity on drinking and aggregation of imported red fire ant in comparison to the one of fallen leaves and dried leaves. The antimicrobial activity of stem and leaf essential oil from *M. alba* may be attributed to the presence of linalool as major component. This constituent has been shown to have synergistic effect enhancing the antifungal activity against mold *Aspergillus flavus* when caryophyllene was also present in the oil with the ratio 10:1 (Songsamoe et al., 2017). Moreover, components in lower amounts could also contribute to the antimicrobial activity of the essential oils, maybe due to synergism with the other active compounds (Marino et al., 2001).

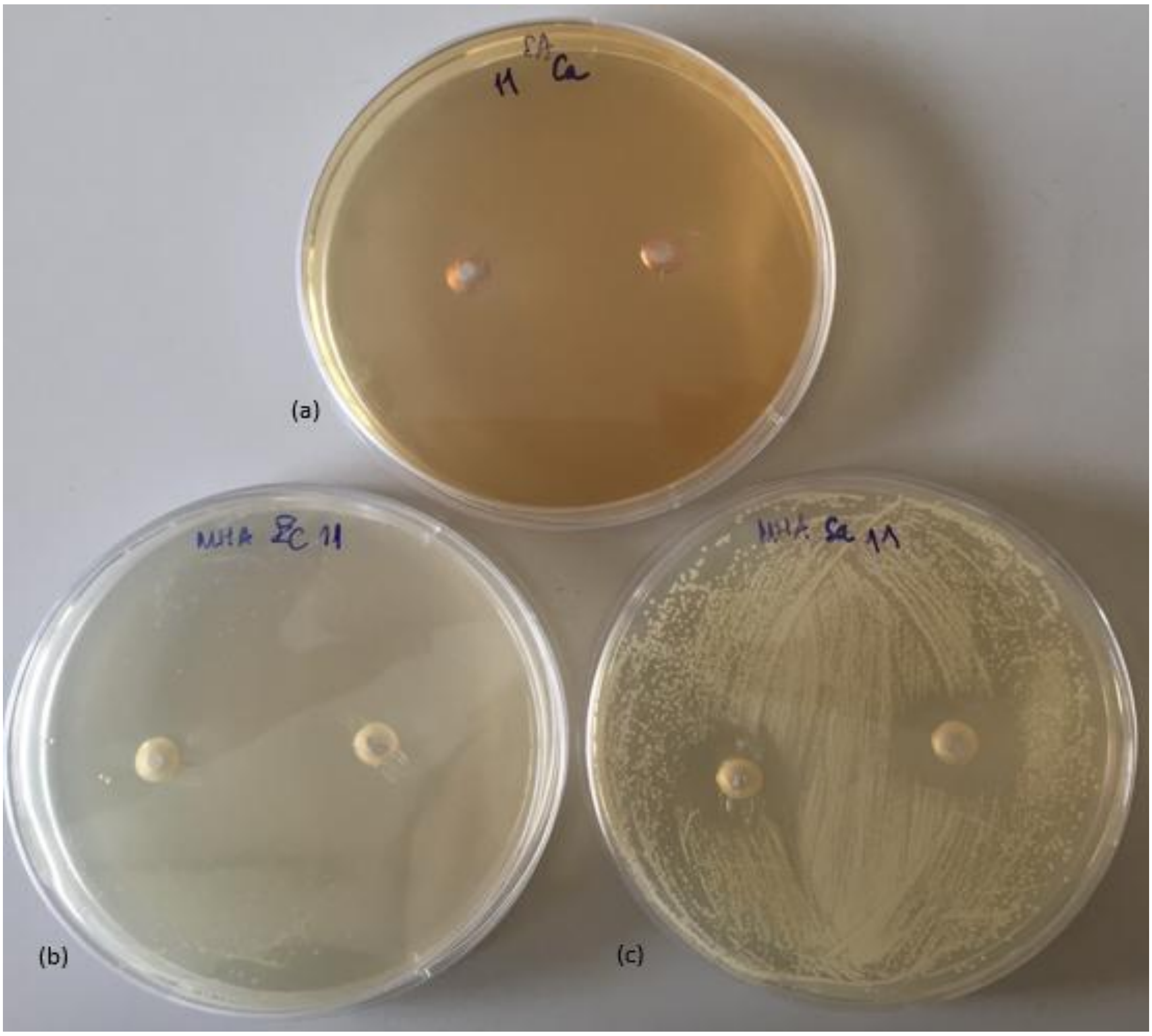

Figure 4. Antimicrobial activity of leaf essential oil of *Michelia alba* D.C. (Note: (a): *Candida albicans;* (b): *Escherichia coli*; (c): *Staphylococcus aureus*)

CONCLUSION

The yields of stem and leaf essential oils of *M. alba* extracted by hydrodistillation were 0.106% (v/w) and 0.373% (v/w), respectively, calculated on a dry weight basis. A total of 24 and 27 constituents were identified in the stem and leaf oil, in which 23 constituents were present at both oils. It is in accordance with previous researches that the major component of stem and leaf essential oils of *M. alba* was linalool, which accounted for 80.65% and 72.89% of the compounds, respectively, in this study. The amount of linalool in the investigated samples was in the normal range in commercial essential oils (New directions aromatics, 2018). In general, antimicrobial activity of leaf oil was stronger than that of stem oil, and both oils exhibited the strong inhibition against all 3 strains of test microorganisms including *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* with inhibitory zone diameters ranging from 15.5 to 46.5 mm. The results of this study showed that stem and leaf essential oils of *M. alba* from Vietnam are potentially useful raw materials in the pharmaceutical, flavor and fragrance industries.

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