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Chemical constituents and antibacterial activity of essential oil of *Vitex rotundifolia* from Southern Vietnam

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Abstract. In present study, we identified the chemical composition of essential oil which was isolated from the leaves of *Vitex rotundifolia* using gas chromatography–mass spectrometry (GC–MS) analysis for the first time. A total of eighteen compounds were identified from essential oil, including sclareol (29.01 %); sandaracopimarinal (16.51 %); abietadiene (15.65 %); androsta–4,6–dien–3–one, 17–hydroxy–, (17β)– (8.12 %); abietal (6.45 %); dehydroabietan (5.02 %); verticiol (4.89 %) as major constituents. Furthermore, the antibacterial activity of the essential oils isolated from of the leaves of studied species has also been evaluated in the first time. The results proved that the essential oils could inhibit the growth of six pathogenic bacterial strains which the diameter of the growth inhibition zone of *S. aureus, B. cereus, P. aeruginosa, S. enteritidis, S. typhimurium, E. coli* were 27.3±0.6mm, 24.5±1.8mm, 24.3±0.8mm, 21.2±1.2mm, 8.8±0.3mm, 8.3±0.3mm, respectively.
Keyword: *Vitex rotundifolia; GC/MS; antibacterial activities; essential oil.*

Introduction

Medicinal plants have been used for centuries as remedies for human diseases. In recent years, traditional medicine is an important component of primary health care system in many developing countries like Vietnam because of its safety, effectiveness and inexpensive properties [PHAM, 2000]. With the improvement of science, the source of the medicinal properties associated with these treatments has been investigated. This led to an explosion in the last hundred years in the areas of isolation, biological activity, structural elucidation and the chemical synthesis of natural products [NICOLAOU *et al.*, 2000].

Essential oils are complex mixtures of volatile compounds that are produced by aromatic plants as secondary metabolites. Essential oils are characterized by the presence of bioactive compounds, mainly terpenoids, such as monoterpenes and esquiterpenes [ZAKI and SALLEH, 2020]. Many species of Lamiaceae family have diverse biological activities in their essential oils in which the *Vitex* genus has been subjected to the most abundant available studies on its ethnobotanical profiles to discover the priceless potentials. *Vitex* is the largest genus in the family Lamiaceae which comprises approximately 270 species distributed all over the world, especially in tropical and subtropical regions such as Brazil, Nigeria, Turkey, Thailand, Algeria, South Africa and Vietnam [GANAPATY and VIDYADHAR, 2005]. Many species of this genus have been used in medicine, including *V. agnuscastus, V. negundo, V. trifolia* and *V. rotundifolia* [AZHAR *et al.*, 2004; KIUCHI *et al.*, 2004; LI *et al.*, 2005; ONO *et al.*, 2008].

Vietnam is a biodiversity hot spot for the Lamiaceae_family in which 17 *Vitex* species have been recorded [PHAM, 2000].

Beach Vitex (*Vitex rotundifolia*) is a deciduous, sprawling shrub that typically grows to 10–40 cm tall. This species grows naturally along both sandy and

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rocky coasts and has a wide distribution in the world, including much of the Pacific Rim and many of the Pacific islands [MATTHEW *et al.*, 2017].

The traditional pharmacopeia's in Asian countries employed *V. rotundifolia* in the treatment of disease [HU *et al.*, 2008].

The fruits of *V. rotundifolia* have been used as a folk medicine for the treatment of headache, cold, migraine, eye pain, female hormonal disorders, asthma, chronic bronchitis, and gastrointestinal infections such as bacterial dysentery and diarrhea [ONO *et al.*, 2000; ONO *et al.*, 2001; HU *et al.*, 2007]. In Vietnam, *V. rotundifolia* has distribution in some regions, including Quang Tri, Bình Thuan, Kien Giang and Ba Ria–Vung Tau Provinces [PHAM, 2000; TRAN *et al.*, 2006].

This species has also been used in Vietnamese traditional medicine to treat a number of diseases such as headache, asthma and diarrhea [PHAM, 2000].

In Vietnam, especially southern Vietnam, information on the chemical constituents and the bioactivity of this species is limited, however.

In this work, chemical composition and antibacterial ability of essential oils extracted from the leaves of *V. rotundifolia* collected in Southern Vietnam were investigated and reported for the first time.

Material and methods

Plant materials

The specimens of Beach Vitex (*Vitex rotundifolia*) was collected from Binh Chau–Phuoc Buu Nature Reserve, Xuyen Moc District, Ba Ria–Vung Tau Province. This species grows on coastal sand dunes with location of about 10°31'11"N; 107°31'18"E, 21 m in elevation (Figure 1).

The specimens were collected by Mr. Van Son Le, a staff of Binh Chau–Phuoc Buu Nature Reserve, which vouchered numbers were VS Le 332 and 333.

All vouchered specimens were deposited at Herbarium of Binh Chau–Phuoc Buu Nature Reserve.

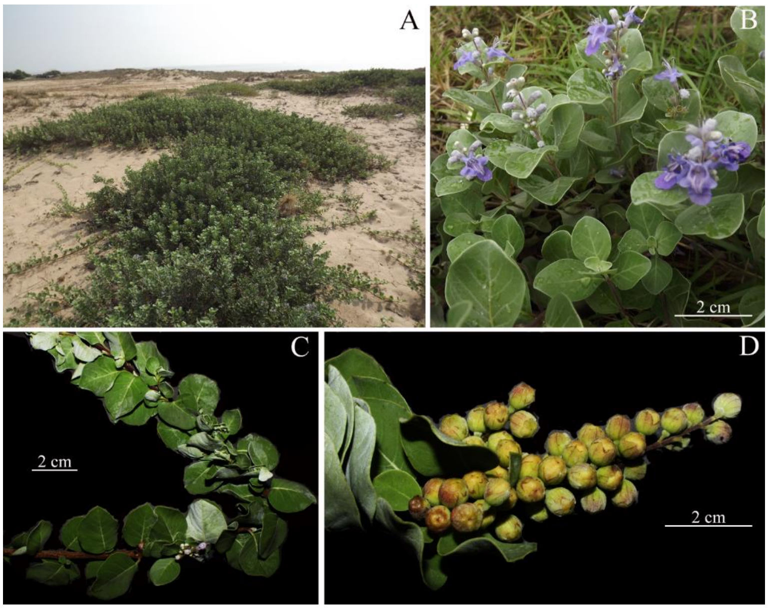

Figure 1. *Vitex rotundifolia*. A. Habitats, B. Flowers, C. Leaves, D. Fruits.

Bacterial strains

To clarify the antibacterial activity of the essential oils from the leaves of *Vitex rotundifolia*, the present study used six bacterial strains, including four Gram–negative bacteria (*Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enteritidis* (ATCC 13976), *Salmonella typhimurium* (ATCC 13311)) and two Gram–positive bacteria (*Bacillus cereus* (ATCC 11774), *Staphylococcus aureus* (ATCC 25923)).

The strains were maintained in 20 % glycerol solution at 20 ºC and activated by cultivation in Luria–Bertani broth at 37 ºC for 24 h before the antibacterial activity assay.

Distillation of the essential oils

Classical steam distillation using Clevenger apparatus was used to extract the essential oils from the leaves of the specimens. In this process, 500 g of washed and drained sample was placed in a distillation flask and filled with 1500 mL of distilled water. The flask was then subjected to Clevenger apparatus to extract the essential oils for 4 hours.

Essential oils were attracted by evaporated water to form the mixture of steam and essential oils, which is then condensed by refrigerant into liquid.

The yields of essential oil were calculated using the following formula

$$RO = M/B_m \cdot 100\%$$

(M: Mass of essential oils; B_m: Mass of sample).

Gas chromatography/mass spectrometry (GC/MS) analysis

Experiments were performed on an Agilent 7890A GC–5975C MSD system (Agilent Technologies, Santa Clara, CA) using a DB5–MS+10m Duraguard Capillary Column (30 m × 250 µm × 0.25 µm) as the stationary phase. The GC parameters used were as follows: split injection (1.0 µL sample at 100.0 °C, 1.0 min—split ratio of 10:1); He carrier gas (40 cm s^{-1} at constant velocity); 275.0 °C transfer line temperature; oven temperature program: 1.0 min at 100 °C, increased 20.0 °C min^{-1} to 200.0 °C, then increased 15.0 °C min^{-1} to 325.0 °C and held for 5.0 min, MS parameters: electron impact ionization at 70 eV, filament source temperature of 230.0 °C, quadrupole temperature of 150.0 °C, m/z scan range 50–600 at 2 spectra s^{-1}.

Mass spectral signals were recorded after a 6.10 min solvent delay to avoid derivatization interferents, and turned off between 10.0 and 13.0 min to avoid saturation of the detector due to the high content of monosaccharides.

A blank sample with the FAME standard mixture (FAME std) was also injected under the same GC conditions.

Antibacterial activity assay

The six bacterial strains were cultured in Luria–Bertani Broth until 0.5 McFarland turbidity standard was reached. This bacterial culture was used to test the antibacterial activity of essential oils in the process called disc diffusion test, in which 0.1 mL of bacterial culture was spread on Petri plate containing Mueller Hinton Agar medium. The sterile paper discs containing 10 µL of the essential oil solution were placed on the surface of the Petri dish spread with bacteria. The plate was then incubated at 37ºC for 16–18 hours.

Gentamycin antibiotic disc (Nam Khoa, Vietnam) was used as a positive control for the experiments. Zone of inhibition was measured after 16–18 hours of incubation to evaluate the resistance of essential oils against the bacterial strains [BAUER *et al.*, 1996].

The experiments were performed in triplicates. The results were presented as mean ± standard deviation (SD) from triplicate analyses, and the differences among experimental groups were determined by Fisher's least significant difference (LSD) procedure using Statgraphics Centurion XV software (Statpoint Technologies Inc, Virginia, USA) with the criterion of statistical significance was set as $p < 0.05$.

Results and discussion

The essential oil from the leaves of *V. rotundifolia* was obtained in a yield of 0.09% calculated on a dry weight basis.

The essential oil from the leaves of *V. rotundifolia* in this study had a total of 18 substances, majority of which included 7 components, including sclareol (29.01 %); sandaracopimarinal (16.51 %); abietadiene (15.65 %); Androsta–4,6–dien–3–one, 17–hydroxy–, (17β)– (8.12 %); abietal (6.45 %); dehydroabietan (5.02); verticiol (4.89 %) (Figure 2 and Table 1).

As aforesaid, *V. rotundifolia* is a common species, essential oil composition of which has been studied by several previous studies.

However, those specimens were collected in different geographical region of Vietnam or other countries [TRAN *et al.*, 2006; KIM *et al.*, 2014].

Note that, the concentrations of chemical compositions of plant essential oils were found to vary depending on the geographical regions where they are cultivated [HASSIOTIS *et al.*, 2010; DEVKOTA *et al.*, 2013].

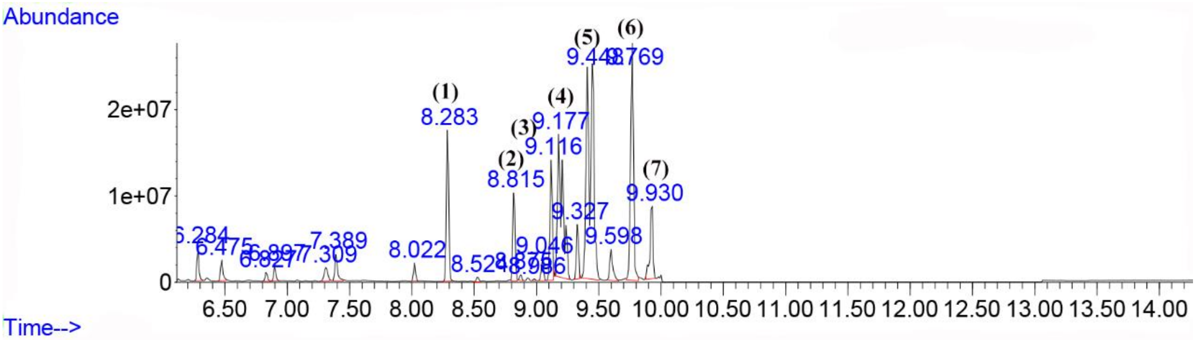

Figure 2. Gas chromatogram of essential oil from *V. rotundifolia* leaves with major components. (1) Androsta–4,6–dien–3–one, 17–hydroxy–, (17β)–; (2) verticiol; (3) abietal; (4) sandaracopimarinal; (5) sclareol; (6) abietadiene; (7) dehydroabietan.

Furthermore, the studied materials which used in *present study* were difference compared to those in previous studies countries [TRAN *et al.*, 2006; KIM *et al.*, 2014].

For instance, Tran and collab. demonstrated that the essential oil from the fruits of *V. rotundifolia* collected in Quang Tri Province, Vietnam contained 1,8 cineol (19.38 %), camphen (15.51 %), sabinen (12.79 %) and α–pinen (10.33 %) as major constituents [TRAN *et al.*, 2006].

Similarly, Hu and collab. showed that linoleic acid (47.46 %) and palmitic acid (5.18 %) were the major constituents of the essential oil isolated from the stem of *V. rotundifolia* which collected in Jiangxi Province, in China [HU *et al.*, 2007].

Table 1.

Chemical compositions in the essential oils from the leaves of *Vitex rotundifolia*.

Retention Time	Compound Name	Molecular Formula	Peak area (%)
6.284	Germacrene D	$C_{15}H_{24}$	1.58
6.475	β–Cadinene	$C_{15}H_{24}$	1.17
6.827	1–Cyclohexene–1–butanal, α,2,6,6–tetramethyl–	$C_{14}H_{24}O$	0.54
6.897	Germacrene D–4–ol	$C_{15}H_{26}O$	0.73
7.309	tau–Muurolol	$C_{15}H_{26}O$	1.15
7.389	α–Cadinol	$C_{15}H_{26}O$	1.63
8.022	15,16–Dinorlabd–12–ene, 8,13–epoxy–	$C_{18}H_{30}O$	0.83
8.283	Androsta–4,6–dien–3–one, 17–hydroxy–, (17β)–	$C_{19}H_{26}O_2$	8.12
8.524	Trachyloban–18–al, (4α)–	$C_{20}H_{30}O$	0.33
8.815	Verticiol	$C_{20}H_{34}O$	4.89
9.046	Thunbergen	$C_{20}H_{32}$	1.12
9.116	Abietal	$C_{20}H_{30}O$	6.45
9.177	Sandaracopimarinal	$C_{20}H_{30}O$	16.51
9.327	Khusimyl methyl ether	$C_{16}H_{26}O$	2.76
9.448	Sclareol	$C_{20}H_{36}O_2$	29.02
9.598	Manool oxide	$C_{20}H_{34}O$	2.16
9.769	Abietadiene	$C_{20}H_{32}$	15.65
9.930	Dehydroabietan	$C_{20}H_{30}$	5.02
Total			99.66

Furthermore, Kim and collab. proved the essential oil isolated from the stem of *V. rotundifolia* collected in Jeju Island, South Korea included manoyl oxide (14.3 %), α–Terpineol (13.1 %), α–Pinene (10.0 %), dehydroabietane (5.9 %) and 1,8–Cineole (4.9 %) as major constituents [KIM *et al.* 2014].

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The bioactivities of some compounds of the essential oil identified from the leaves of *V. rotundifolia* in this study have been documented in previous studies [ZHANG *et al.*, 2017].

For instance, sclareol, the most abundant constituent from the essential oil isolated from the leaves of studied species (29.02 %), could inhibited tumor cell growth through the upregulation of Cav1 and provides a potential therapeutic target for cervical cancer [DIMAS *et al.*, 2006].

Furthermore, this compound had the effect on the human breast cancer cell lines MN1 and MDD2 derived from the parental cell line, MCF7. MN1 cells express functional p53 [DIMAS *et al.*, 2006].

In 2008, Ali and collab. demonstrated that the essential oil of *Boswellia elongata* contained verticiol as a major compound (52.4 %) and the essential oil of this species could be used as a rich source of natural antioxidants and anticholinesterase inhibitors [ALI *et al.*, 2008 NASSER *et al.*, 2008].

Antibacterial activity

Data stated in Table 2 and Figure 3 showed that the essential oil from the leaves of *V. rotundifolia* was able to resist against six tested bacteria.

Accordingly, the diamètres of inhibition zones of the essential oil from the studied specimens against *S. aureus, B. cereus, P. aeruginosa, S. enteritidis, S. typhimurium, E. coli* were 27.3±0.6 mm, 24.5±1.8 mm, 24.3±0.8 mm, 21.2±1.2 mm, 8.8±0.3 mm, 8.3±0.3 mm, respectively.

On the other hand, the antibacterial effect of essential oil leaves of *V. rotundifolia* against *S. aureus, B. cereus, P. aeruginosa, S. enteritidis* was much stronger than that of positive control whereas the diameter of the growth inhibition zone of *S. typhimurium* and *E. coli* in tested sample was smaller than that of positive control (Table 2).

Table 2.
Inhibition zone of the essential oils isolated from the leaves of *Vitex rotundifolia* against six bacterial strains

Tested bacteria	Growth inhibition zone (mm)	
	Studied sample	**Positive control**
Bacillus cereus	24.5±1.8^{b}	18.8±1.0^{a}
Escherichia coli	8.3±0.3^{a}	19.6±0.6^{b}
Pseudomonas aeruginosa	24.3±0.8^{b}	16.5±0.5^{a}
Salmonella enteritidis	21.2±1.2^{b}	17.3±0.6^{a}
Salmonella typhimurium	8.8±0.3^{a}	13.3±0.6^{b}
Staphylococcus aureus	27.3±0.6^{b}	17.6±1.2^{a}

^{a,b}Different superscript lower–case letters in the same row denote significant difference ($p<0.05$).

These results indicated *V. rotundifolia* essential oil as the potential antibacterial agents. Together of analysis of oil composition, the antibacterial activity of the oil could be offered the presence of bioactive compounds of *V. rotundifolia* leaf essential oil, including sclareol (29.02 %), sandaracopimarinal (16.51 %) and abietadiene (15.65 %). The *antimicrobial* activities of these major components have been well documented in several reports [JASSBIA *et al.*, 2002; CHOUDHARY *et al.*, 2006]. For instance, Jassbi and collab. and Choudhary and collab. showed that sclareol was able resist against three bacterial strains such as *Bacillus subtilis, Staphylococcus aureus* and *Shigella flexneri* [JASSBIA *et al.*, 2002; CHOUDHARY *et al.*, 2006].

Recently, Popova and collab. demonstrated that sclareol was not only highly effective against a set of medicinally important yeasts such as *Candida albicans, C. glabrata, C. parapsilosis, C. tropicalis* but could also inhibit the growth of the bacterial pathogens, including *Bacillus cereus, Escherichia coli*, *Salmonella abony*, *Pseudomonas putida, P. aeruginosa*, *Staphylococcus aureus, Proteus mirabilis* and *P. vulgaris* [POPOVA *et al.*, 2020, STOLERU, *et al.*, 2014, CARUSO, *et al.*, 2019].

In another study, Tsujimura and collab. showed that *Propionibacterium* *acnes*, a gram–positive human skin commensal, could be inhibited by sandaracopimarinal [TSUJIMURA *et al.*, 2019].

Furthermore, the essential oil isolated from *Anisomeles indica* (Lamiaceae family) contained abietadiene as major compound (20.5 %) was able to resist against five fungal and bacterial strains, including *Klebsiella pneumoniae, Agrobacterium tumefaciens. Staphylococcus aureus, Pasteurella multocida* and *Aspergillus flavus* [ANAND *et al.*, 2016, KURZBAUM, *et al.*, 2019, PETRACHE, *et al.*, 2014].

To date, the *antibacterial* activities of the essential oil of *V. rotundifolia are limited. However, the antimicrobial* assays of other *Vitex* species have been conducted by *previous reports* [NYILIGIRA *et al.*, 2004; KHOKRA *et al.*, 2008; GHANNADI *et al.*, 2012; GONÇALVES *et al.*, 2017].

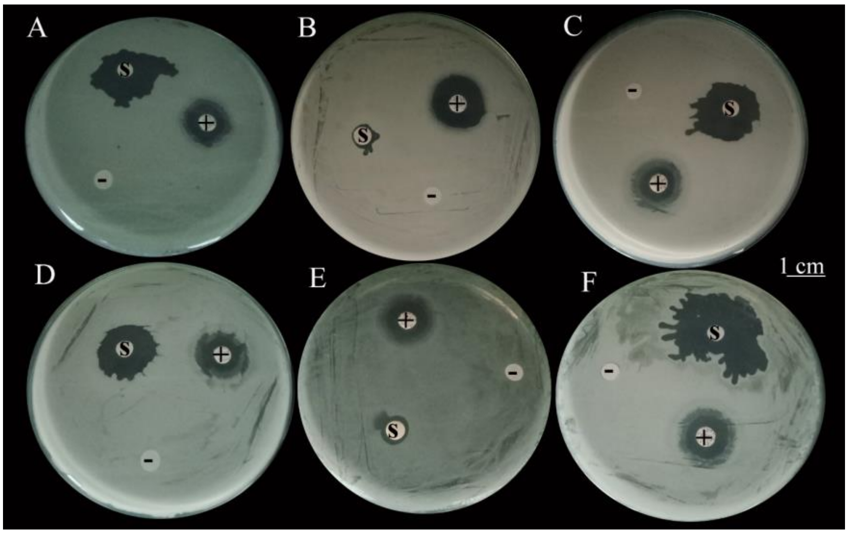

Figure 3. Antibacterial activity of the essential oils extracted from the leaves of *Vitex rotundifolia* against 6 bacterial strains. A. *Bacillus cereus,* B. *Escherichia coli*, C. *Pseudomonas aeruginosa*, D. *Salmonella enteritidis*, E. *Salmonella typhimurium*, F. *Staphylococcus aureus*. (–) Negative control with sterilized distilled water, (+) Positive control with discs containing gentamicin.

For instance, the essential oil from the seeds of *V. agnus–castus* could inhibit antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Salmonella enteritidis* [GHANNADI *et al.*, 2012]. Similarly, essential oil extracted from the leaves of *V. agnus–castus* was demonstrated that it had promising activity against *Streptococcus mutans, Lactobacillus casei* and *Streptococcus mitis* [GONÇALVES *et al.*, 2017].

Furthermore, the essential oil isolated from the aerial parts of two subspecies of *V. obovate* and three other *Vitex* species, including *V. rehmannii, V. zeyheri, V. pooara, V. obovata* subsp. *obovata* and *V. obovata* subsp. *wilmsii* were able to resist against *S. aureus, B. cereus* and *E. coli* [NYILIGIRA *et al.*, 2004]. In another study, three essential oils, including fruit, leaf and flower oils of *V. negundo* were evaluated for antibacterial potential against *Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* [KHOKRA *et al.*, 2008].

Conclusions

In this study, eighteen chemical compounds of essential oil of *Vitex rotundifolia* leaf were firstly investigated in which sclareol (29.01 %); sandaracopimarinal (16.51 %); abietadiene (15.65 %); Androsta–4,6–dien–3–one, 17–hydroxy–, (17β)– (8.12 %); abietal (6.45 %); dehydroabietan (5.02); verticiol (4.89 %) were major constituents.

Furthermore, the antibacterial effect of essential oil of studied specimen against *S. aureus, B. cereus, P. aeruginosa, S. enteritidis* was much stronger than that of positive control which the diameter of the growth inhibition zone was 27.3±0.6 mm, 24.5±1.8 mm, 24.3±0.8 mm, 21.2±1.2 mm, respectively whereas the diameter of the growth inhibition zone of *S. typhimurium* and *E. coli* in tested sample was smaller than that of positive control.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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