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Article

Studies on Asteraceae: Chemical Compositions of Essential Oils and Antimicrobial Activity of the Leaves of *Vernonia patula* (Dryand.) Merr. and *Grangea maderaspatana* (L.) Poir. from Vietnam

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Abstract: Essential oils from the leaves of *Vernonia patula* (Dryand.) Merr. and *Grangea maderaspatana* (L.) Poir. collected from Pu Hoat Nature Reserve, Nghe An Province were studied for their chemical constituents and antimicrobial activity. The main constituents of *V. patula* were the sesquiterpenes namely β -caryophyllene (28.5 %), caryophyllene oxide (16.6 %), α -copaene (9.0 %), and α -humulene (7.1 %). On the other hand, a mixture of monoterpenes and sesquiterpenes hydrocarbons represented by myrecene (27.7 %), α -humulene (19.7 %), and germacrene D (15.8 %) were the main compounds of essential oils from the leaves of *G. maderaspatana*. Both essential oils displayed antimicrobial activity against the tested Gram-positive and Gram-negative bacteria with the minimum inhibitory concentrations (MIC) less than 50.0 $\mu\text{g/mL}$ (8.67-24.56 $\mu\text{g/mL}$). The leaf essential oil of *V. patula* was the most potent towards *Enterococcus faecalis* (ATCC 299212) with MIC value of 8.67 $\mu\text{g/mL}$. The essential oils also showed anti-candidal properties with MIC values of 15.99 $\mu\text{g/mL}$ (*V. patula*) and 65.67 $\mu\text{g/mL}$ (*G. maderaspatana*). The chemical components and antimicrobial activity of the *V. patula* leaf essential oil was reported for the first time.

Keywords: *Vernonia patula*, *Grangea maderaspatana*, essential oil, terpenes, *Enterococcus faecalis*, *Candida albicans*.

Introduction

Vernonia is a genus of about 100 species of forbs and shrubs in the family Asteraceae. *Vernonia patula* (Dryand.) Merr. (Synonym *V. chinensis* Less.) is known locally as Nút áo tím in Vietnam

¹. The plant is an erect annual weed about a meter high with a stiff slightly branched stem ¹. The leaves are 2.5-5 cm long, variable in shape, broadly elliptic or lanceolate, obtuse or acute, irregular toothed or shallowly crenate-serrate. The pinkish

violet flowers are about 6 mm in diameter². The decoction of the leaves and roots are used to treat colds, diarrhea, cough fevers, and convulsions³. Extracts from *V. patula* were reported to displayed anti-inflammatory action⁴, anti-oxidative effects⁴, thrombolytic and membrane stabilizing activities⁵, anti-nociceptive potential^{6,7}, sedative activity⁶, and anti-diarrheal effect⁷.

The phytochemical compounds isolated from *V. patula* included the bauerenyl acetate, friedelin, epifriedelanol, and 20(30)-taraxastene-3- β ,21- α -diol⁸. These compounds show no inhibitory effect on the growth of PC-12 cells⁸. The flavonoids apigenin, luteolin and chryseriol, and one triterpene betulinic acid were found in *V. patula*⁴. Variety of compounds such as incaspitolide D, (S)-N-benzoylphenylalanine-(S)-2-benzamido-3-phenylpropyl ester, indole-3-carboxylic acid, and diosmetin were also isolated from the plant⁹. The phenolic compounds characterized by *V. patula* were gallic acid, vanillic acid, caffeic acid, quercetin, kaempferol⁶, and chlorogenic acid¹⁰. Till the moment, no report exists on the chemical composition and biological activity of essential oil from this species.

Grangea maderaspatana (L.) Poir. (Synonym *Artemisia maderaspatana* L.) of the family, Asteraceae is a hairy, branched annual aromatic herb that spreads from the roots and grows up to 70 cm in height. The buds are white and woolly. The leaves are pale green of about 1-9.5 cm long and 0.3-4 cm wide. The leaves are alternate, stalkless, deeply cut, and divided into toothed lobes. The yellow flowering heads are borne opposite the leaves, and are short-stalked, rounded, and 8-10 mm across. The flowers are small in size and numerous. *G. maderaspatana* is globally found in Indo-Malaysia and Africa¹¹. The plant is known in the Vietnamese language as Rau cóc¹. Extracts from *G. maderaspatana* possess favorable analgesic^{12,13}, anti-inflammatory¹³, and antiarthritic¹³ activities in experimental models. This plant is pharmacologically studied for estrogenicity¹⁴, anti-fertility¹⁴, antioxidant¹⁵, antimicrobial¹⁵, hepatoprotective¹⁶ and diuretic activities¹⁷. Phytochemical screening of the non-volatile extracts from various parts of *G. maderaspatana* yielded 3-hydroxy-8-methoxy flavone-7-O- β -L-arabinofuranosyl-(1 \rightarrow 4)-O- β -

D-galactopyranoside¹⁸. Frullanolide, a compound purified from *G. maderaspatana* exhibited strong anti-breast cancer activity against MDA-MB-468 (IC₅₀, 8.04 \pm 2.69 μ g/mL) cancer cells¹⁹. Phytochemical investigation of the ethanolic extract of *G. maderaspatana* led to the isolation of gramaderins A-D²⁰. Moreover, 5,7-dihydroxy-3,6,3',4',5'-pentamethoxyflavone, 5,3'-dihydroxy-3,6,7,4',5'-pentamethoxyflavone and 8-acetoxypentadeca-1,9Z,14-trien-4,6-diyne-3-ol which exhibited significant anti-inflammatory activity²⁰ were also isolated from the plant. A review on the pharmacological potentials and chemical constituents of *G. maderaspatana* was recently published²¹.

Preliminary chemical screening of essential oils of *G. maderaspatana* from countries other than Vietnam has been performed earlier. γ -Gurjunene (26.5 %), terpinyl acetate (20.8 %) and hinesol (11.6 %) were the main compounds described from steam distilled aerial parts of *G. maderaspatana* from India¹⁵. This oil sample exhibited antioxidant activity, as well antimicrobial (*Streptomyces candidus*, MIC 5 mL/L) and anticandidal action (MIC 5 mL/L)¹⁵. Aerial sample of *G. maderaspatana* analysed also from India²² had an abundance of α -humulene (46.3 %), β -caryophyllene (9.3 %), and α -copaene (8.2 %). The essential oil has significant acetylcholinesterase inhibitory activity (IC₅₀ value of 31.33 \pm 1.03 μ g/mL)²². The sample of essential oil analysed from Burkina Faso consists mainly of limonene (25.52 %), α -humulene (16.05 %), β -elemene (5.03 %), germacrene D (4.45 %) and β -pinene (4.02 %)²³.

The objective of the present paper is to report, for the first time, the chemical composition and antimicrobial activity of essential oils hydrodistilled from the leaves of *V. patula* and *G. maderaspatana* growing in Vietnam. This is in continuation of extensive research aimed at the characterization of the volatile constituents and biological potentials of the poorly studied species of Vietnamese flora²⁴⁻²⁶.

Material and methods

The leaves of *V. patula* and *G. maderaspatana*
Large quantities of the leaves of both *V. patula* and *G. maderaspatana* were collected from Hanh

Dich Commune, Pu Hoat Nature Reserve (19°44'32"N; 104°48'10"E), Nghe An Province, Vietnam, on August 2020, at an elevation of 816 m. Both samples were identified and authenticated by Dr. Le Thi Huong, School of Science Education, Vinh University, Vietnam. Moreover, voucher specimens LTH 873 and LTH 874, respectively were deposited in the plant specimen room, Vinh University, Vietnam.

Hydrodistillation process to obtain essential oils

The collected samples of the leaves of *V. patula* and *G. maderaspatana* were made clean through separation from dust and other particles. Thereafter, samples were separately chopped in a grinder to obtained 2 kg of each sample. 2.0 kg of the fresh chopped leaves of each plant was subjected to separate hydrodistillation inside a Clevenger-type apparatus according to established specifications as described in previous studies²⁴⁻²⁶. The chopped leaves sample was carefully introduced into a clean and dry 5 L flask. Distilled water was added into the flask until it covered the surface of the sample completely. The procedure was maintained at normal pressure over a period of 3 h. The collected essential oils of *V. patula* and *G. maderaspatana* were dried with anhydrous sodium sulfate and drained into an amber-colored vial for storage at 4°C until analysis. All measurements were performed in triplicate.

Instrumental analysis of the chemical constituents of the essential oils

Gas chromatographic analysis (GC) was performed on Agilent GC 7890A attached to FID detector of Agilent Technologies, USA, and fitted with HP-5MS chromatographic column with a length of 60 m, inner diameter (ID) = 0.25 mm, and film thickness of 0.25 µm. Helium was the carrier gas with a flow rate of 1.0 mL/min. The inlet temperature was 250°C, and the oven temperature was program from 60°C to 240°C at a rate of 4°C/min, with a 2 min hold. The split ratio was 100:1, and the volume of diluted (10 % *n*-hexane solution) essential oils injected into the column was 1.0 µL. The interface temperature was 270°C while the detector temperature was 260°C. Quantification was done using the

calibration curves generated from the analyses of representative standard compounds from each class.

An Agilent GC 7890A chromatograph equipped with a capillary of molten silica HP-5MS (60 m × 0.25 mm, film thickness of 0.25 µm) and coupled with a mass spectrometer (HP 5973 MSD) was used for GC/MS analysis of the essential oils. Similar conditions as described were used for GC analysis, with Helium (1 mL/min) as the carrier gas. The MS conditions were as the follows- ionization voltage of 70 eV; emission current of 40 mA; acquisition and a scan range of 35–450 amu with a sampling rate of 1.0 scan/s.

The identification of constituents of essential oils from the GC/MS spectral of *V. patula* and *G. maderaspatana* was performed based on a comparison of retention indices (RI Exp.) with reference to a homologous series of *n*-alkanes (C₄-C₄₀), under identical experimental conditions. In some cases, co-injection with known compounds under the same GC conditions was employed. The mass spectral (MS) fragmentation patterns were checked with those of other essential oils of known composition in literature²⁷ as described recently²⁴⁻²⁶.

Antimicrobial screening

Microorganisms

The microorganisms used in this study consist of Gram-positive bacteria, *Enterococcus faecalis* ATCC 299212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC14579; Gram-negative bacteria, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* ATCC 13076, and the yeast, *Candida albicans* ATCC 10231. The strains were obtained from the laboratory stock of the Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, Hanoi, Vietnam. The Mueller-Hinton Agar (MHA) and Sabouraud Agar (SA) were used as testing media respectively for bacteria and fungi.

Screening of the essential oils for antimicrobial activity

The Minimum inhibitory concentration (MIC) and median inhibitory concentration (IC₅₀) values were

measured by the microdilution broth susceptibility assay as described previously. The choice of investigated concentrations was based on previous reports on similar reports where essential oils are active within specific concentration ranges²⁴⁻²⁶. A 2-fold dilution range was used for the experiment. Stock solutions of the essential oils were prepared in 1 % dimethylsulfoxide. Dilution series (2-fold) were prepared from 16,384 to 2 µg/mL (2¹⁴, 2¹³, 2¹², 2¹¹, 2¹⁰, 2⁹, 2⁷, 2⁵, 2³, and 2¹ µg/mL) in sterile distilled water in micro-test tubes from where they were transferred to 96-well microtiter plates. Bacteria grown in double-strength Mueller-Hinton broth or double-strength tryptic soy broth and fungi grown in double-strength Sabouraud dextrose broth were standardized to 5 × 10⁵ and 1 × 10³ CFU/mL, respectively. The last row, containing only the serial dilutions of samples without microorganisms, was used as a negative control. Streptomycin was used as the antibacterial standard while nystatin and cycloheximide were used as antifungal standards.

The test was based on the assessment of growth through turbidimetry (use of optical density as a measure of growth)²⁴⁻²⁶. The cultures of tested microorganisms grown overnight are diluted and read on a spectrophotometer at 600 nm in comparison with McFarland reagents (Barium Sulphate) to obtain the microbial load as standardized culture. After incubation at 37°C for 24 h, the MIC values were determined to be the lowest concentration of essential oils which completely inhibited the growth of microorganisms.

The IC₅₀ values were determined by the percentage of microorganisms that inhibited growth based on the turbidity measurement data of EPOCH2C spectrophotometer (BioTeK Instruments, Inc Highland Park Winooski, VT, USA) and Rawdata computer software (Brussels, Belgium) according to the following equations:

$$\% \text{ inhibition} = \frac{\text{ODcontrol}(+) \times \text{ODtest agent} - \text{ODcontrol}(-)}{\text{ODcontrol}(+) \times \text{ODcontrol}(-)} \times 100$$

$$\text{IC}_{50} = \frac{\text{High}_{\text{conc}} - (\text{High inh}\% - 50\%) \times (\text{High Conc} - \text{Low Conc})}{(\text{High inh}\% - \text{Low inh}\%)}$$

Where OD is the optical density, control (+) is the cells in medium without the antimicrobial agent,

test agent corresponds to a known concentration of the antimicrobial agent, control (-) is the culture medium without essential oils, High Conc/Low Conc is the concentration of test agent at high concentration/low concentration, and High Inh%/Low Inh% is the % inhibition at high concentration/% inhibition at low concentration.

Statistical analysis

All results of chemical composition and antimicrobial experiments were repeated three times and are expressed as mean ± standard deviation (SD).

Results and discussion

Chemical composition of V. patula essential oil

The leaves of *V. patula* were collected and processed before extraction of the essential oil. The hydrodistillation of the leaves produced light-yellow colored essential oil. The yield of essential oil was 1.73 g ± 0.01 (v/w), based on a dry weight basis. Table 1 presents the compounds as identified by GC/MS. The composition of the essential was dominated by the sesquiterpene class of compounds in the proportion of sesquiterpene hydrocarbons (55.0 %) and oxygenated sesquiterpenes (21.6 %). Monoterpene hydrocarbon was identified in the proportion of 13.7 % while the oxygenated counterparts were few (0.3 %). The main constituents of *V. patula* were the sesquiterpenes namely β-caryophyllene (28.5 %), caryophyllene oxide (16.6 %), α-copaene (9.0 %), and α-humulene (7.1 %). β-Pinene (6.9 %) was the only monoterpene compound identified in sizeable amount. In addition, humulene oxide II (2.1 %), α-pinene (1.4 %), isocumene (1.3 %), γ-elemene (1.3 %), and β-selinene (1.1 %) were the other compounds present in amount >1 %.

Since this is the first report on the volatile of *V. patula*, the present data could not be compared with other analysed samples of the same species. However, it is well known that terpene compounds, as identified in the present study, predominates in the essential oils of the family *Vernonia* described from Vietnam and other parts of the world. In previous studies, β-caryophyllene and germacrene D were the main compound of *V. chalybaea* from Brazil²⁸, consistent with data obtained for *V. migeodii* from Nigeria²⁹. *Vernonia amygdalina*

leaf essential oil from Nigeria was dominated by α -muurolol³⁰, while the leaf sample from Vietnam contained caryophyllene oxide and β -caryophyllene, with caryophyllene oxide and humulene epoxide II predominates in the stem oil³¹. On chemotaxonomic scale³⁰, the leaf essential oil of *V. patula* can be classified with an abundance of sesquiterpene hydrocarbons.

Chemical composition of *G. maderaspatana* leaf essential oil

The essential oil obtained from leaves of *G. maderaspatana* was light-yellow colored in a

yield of $2.11 \text{ g} \pm 0.01$ (v/w), based on a dry weight basis. The constituents present in the essential oil were presented in Table 1. The classes of compounds identified in the essential oils were the monoterpene hydrocarbons (42.3 %) and sesquiterpene hydrocarbons (48.4 %). The oxygen-containing terpene compounds were less common in the proportion of 3.2 % (oxygenated monoterpenes) and 4.5 % (oxygenated sesquiterpenes). The mixture of monoterpenes and sesquiterpenes hydrocarbons represented by myrcene (27.7 %), α -humulene (19.7 %), and germacrene D (15.8 %) were the main compounds of essential oil.

Table 1. Essential oil composition (%) of the leaves of *Vernonia patula* and *Grangea maderaspatana*

No.	Compounds ^a	RI (Exp.)	RI (Lit.)	Concentration (%) ^b	
				<i>V. patula</i>	<i>G. maderaspatana</i>
1	α -Pinene	940	932	1.4	0.8
2	Sabinene	979	968	0.3	1.4
3	β -Pinene	985	978	6.9	5.9
4	Myrcene	992	988	-	27.7
5	α -Phellandrene	1011	1008	-	1.4
6	α -Terpinene	1022	1016	-	0.1
7	o-Cymene	1030	1028	0.1	-
8	Limonene	1034	1032	0.3	3.3
9	β -Phellandrene	1035	1034	1.7	1.5
10	<i>trans</i> -Sabinol	1148	1150	0.3	-
11	Dehydroedulane	1305	1300	0.2	-
12	Silphin-1-ene	1360	1360	0.2	-
13	Cycolsativene	1382	1380	0.8	-
14	Terpinolene	1094	1088	-	0.2
15	Linalool	1101	1100	-	0.4
16	<i>cis</i> -p-Menth-2-en-1-ol	1128	1126	-	1.2
17	<i>cis</i> -Piperitol	1203	1204	-	0.2
18	<i>trans</i> -Piperitol	1214	1214	-	0.4
19	Silphiperfol-5-ene	1358	1360	-	0.2
20	Neryl acetate	1365	1368	-	0.1
21	Geranyl acetate	1384	1383	-	0.7
22	α -Copaene	1390	1387	9.0	-
23	Maaliene	1397	1397	0.2	-
24	β -Bourbonene	1399	1398	0.4	-
25	<i>cis</i> - β -Elemene	1408	1407	0.9	3.4
26	Isocumene	1403	1405	1.3	-
27	Cyperene	1417	1415	0.3	-
28	α -Gurjumene	1426	1424	0.4	-
29	β -Isocumene	1427	1427	0.4	-

table 1. (continued).

No.	Compounds ^a	RI (Exp.)	RI (Lit.)	Concentration (%) ^b	
				<i>V. patula</i>	<i>G. maderaspatana</i>
30	β-Caryophyllene	1437	1433	28.5	3.3
31	γ-Elemene	1445	1443	1.3	-
32	Aromadendrene	1453	1449	0.1	-
33	Sesquisabinene A	1456	1452	0.5	-
34	α-Humulene	1472	1465	7.1	19.7
35	9- <i>epi</i> -(<i>E</i>)-Caryophyllene	1478	1480	0.3	-
36	γ-Muurolene	1490	1485	0.9	0.5
37	<i>trans</i> -β-Bergamotene	1496	1494	0.3	-
38	Germacrene D	1497	1495	0.3	15.8
39	β-Selinene	1505	1498	1.1	1.1
40	(<i>E,E</i>)-α-Farnesene	1511	1505	-	0.8
41	α-Muurolene	1513	1514	-	0.9
42	α-Bulnesene	1521	1526	0.2	-
43	γ-Cadinene	1530	1528	0.6	0.8
44	δ-Cadinene	1537	1530	0.6	2.1
45	α-Calacorene	1560	1554	0.3	-
46	(<i>E</i>)-Nerolidol	1571	1560	-	0.6
47	Palustrol	1589	1587	0.2	-
48	Germacrene D-4-ol	1592	1590	-	0.7
49	Caryophyllene oxide	1605	1601	16.6	-
50	Geranyl isovalerate	1610	1606	-	0.1
51	Viridiflorol	1612	1610	-	0.1
52	Humulene oxide I	1621	1623	0.3	-
53	Humulene oxide II	1632	1630	2.1	0.4
54	10- <i>epi</i> -γ-Eudesmol	1640	1644	-	0.3
55	<i>epi</i> -α-Cadinol	1656	1658	0.8	0.2
56	<i>epi</i> -α-Muurolol	1658	1660	-	0.2
57	Porosadienol	1667	1666	0.3	-
58	α-Cadinol	1675	1672	0.4	0.7
59	<i>cis</i> -Calamene-10-ol	1678	1680	0.6	-
60	<i>trans</i> -Calamine-10-ol	1688	1684	0.3	-
61	<i>neo</i> -Intermedeol	1689	1685	-	1.0
62	Neophytadiene	1690	1693	-	0.2
	Total			90.6	98.4
	Monoterpene hydrocarbons (Sr. No. 1-9, 11-14)			13.7	42.3
	Monoterpene oxygenated (Sr. No. 10, 15-18, 20, 21)			0.3	3.2
	Sesquiterpene hydrocarbons (Sr. No. 19, 22-45)			55.0	48.4
	Sesquiterpenes oxygenated (Sr. No. 46-62)			21.6	4.5

^aElution order on HP-5MS column

RI (Exp.) Retention indices on HP-5MS column

RI (Lit.) Literature retention indices

^bStandard deviation were insignificant and excluded from the Table to avoid congestion

(-) Not identified

There were significant amount of β -pinene (5.9 %), *cis*- β -elemene (3.4 %), limonene (3.3 %), β -caryophyllene (3.3 %) and δ -cadinene (2.1 %). It should be noted that the content of α -humulene, β -caryophyllene, and α -copaene were lower than previously reported for the sample analysed from India²². On the other hand, the high contents of myrcene and germacrene D in this study also confer a difference between this study and samples of essential oils reported previously^{15, 22,23}. Moreover, γ -gurjunene, terpinyl acetate and hinesol previously reported for other analysed oil¹⁵ were not identified in the present investigated oil sample. Also, limonene the main compound of essential oil from Burkina Faso²³ occurred in a lower quantity in the present study. A noteworthy observation was α -humulene featured prominently in the compositions of *G. maderaspatana* essential oils collected from India^{15,22}, Burkina Faso²³, and this study, which may be of chemotaxonomic interest.

Antimicrobial activity of the essential oils

Both essential oils displayed antimicrobial activity against the tested gram-positive and gram-negative bacteria with the minimum inhibitory concentrations (MIC) less than 50.0 $\mu\text{g/mL}$ (8.67-24.56 $\mu\text{g/mL}$). The leaf essential oil of *V. patula* was the most potent towards *Enterococcus faecalis* (ATCC 299212) with MIC value of 8.67 $\mu\text{g/mL}$ (Table 2). The essential oils also showed anti-candidal properties with MIC values of 15.99 $\mu\text{g/mL}$ (*V. patula*) and 65.67 $\mu\text{g/mL}$ (*G. maderaspatana*). Overall, *V. patula* essential oil exhibited potential antimicrobial activity greater than *G. maderaspatana* oil. However, both essential oils did not inhibit the growth of *Escherichia coli* ATCC 25922 and *Salmonella enterica* ATCC 13076. The antimicrobial and anticandidal actions of *G. maderaspatana* are in agreement with a previous report¹⁵. The observed antimicrobial result of *V. patula* and *G. maderaspatana* essential oils was in agreement with previous information that essential oils from Vietnam and other parts of the world selectively inhibited the growth of different microorganisms^{15,24-26}. Accordingly³², substances with MIC values ≤ 100 $\mu\text{g/mL}$ were considered to be of good antimicrobial activity,

Table 2. Antimicrobial activity of *V. patula* and *G. maderaspatana* leaves essential oils

Sample	Gram (+)		Gram (-)		Yeast		
	<i>Enterococcus faecalis</i> ATCC 299212	<i>Staphylococcus aureus</i> ATCC 25923	<i>Bacillus cereus</i> ATCC 14579	<i>Escherichia coli</i> ATCC 25922		<i>Pseudomonas aeruginosa</i> ATCC 27853	<i>Salmonella enterica</i> ATCC 13076
<i>V. patula</i>	16.0	64.0	32.0	-	64.0	-	32.0
<i>G. maderaspatana</i>	32.0	64.0	64.0	-	64.0	-	128.0
<i>V. patula</i>	8.67	19.34	15.67	-	23.45	-	15.99
<i>G. maderaspatana</i>	15.67	23.46	21.45	-	24.56	-	65.67

IC₅₀ ($\mu\text{g/mL}$)^a

MIC ($\mu\text{g/mL}$)^a

^aStandard deviation in the range $\pm 0.00-0.01$

- No activity

while MIC values from 500 - 100 µg/mL are considered as moderate activity. In addition, MIC values from 1000 - 500 µg/mL are said to be of weak activity while MIC value above 1000 µg/mL is considered inactive. Thus, essential oils from leaves of *V. patula* and *G. maderaspatana* possessed good activity against the tested microorganisms except for *E. coli* ATCC 25922 and *S. enterica* ATCC 13076. It is believed that the constituents present in the studied essential oils might have influenced the observed antimicrobial activity of *G. maderaspatana* against microorganisms¹⁵. The chemical components and antimicrobial activity of *V. patula* leaf essential oils were being reported for the first time.

Previous studies have shown that the biological activities of essential oils from different species of plants are dependent of the major compounds of abundance. In some other cases, synergies between the major and some minor constituents have also enhanced the activity of natural products including essential oils^{24-26,33}. These compounds exhibit activity by firstly destroying the microbial cytoplasmic wall to enhance permeability and passage of large protons and ions³⁴. Nevertheless, the antibacterial effect can be sum up as cumulative actions of several compounds and not to a specific compound³⁴. For example, α-humulene has shown antibacterial activity against *B. cereus* and *S. aureus*³⁵. Also, β-caryophyllene demonstrated selective antibacterial activity against *S. aureus*³⁶ and antifungal effect²⁸. Further, due to the complexity of the composition

of the essential oils, it is also difficult to explain the mechanism of action of these blends but is important to underline that the wide variety of composition is a positive factor that may limit the development of resistance which is otherwise very common for synthetic drug³⁷. In light of this, essential oils may represent a valid alternative to avoid the multidrug resistance of many pathogens, or they could be used in combination with antimicrobials to improve their effectiveness against different infectious diseases.

Conclusions

In summary, this study provides information on the chemical composition and antimicrobial activity of essential oils from the leaves of *V. patula* and *G. maderaspatana*. The major components of *V. patula* were β-caryophyllene, caryophyllene oxide, α-copaene, and α-humulene, while *G. maderaspatana* consists mainly of myrcene, α-humulene, and germacrene D. Both essential oils exhibited good antimicrobial and anti-candidal activity with the MIC value < 50.0 µg/mL. The chemical components and antimicrobial activity of the *V. patula* leaf essential oil was reported for the first time and open up a possibility for further development of antimicrobial herbs.

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