Journal of Essential Oil Bearing Plants

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/teop20

Chemical Compositions of Essential Oils and Antimicrobial Activities of Asteraceae: The Leaves of *Blumea lacera* (Burm. f.) DC., *Tridax procumbens* (L.) L., and *Ageratum houstonianum* Mill., from Vietnam

Tran Minh Hoi, Nguyen Huy Hung, Le Thi Huong, Dang Viet Hau, Dang Thi Hong Duyen, William N. Setzer & Isiaka Ajani Ogunwande

To cite this article: Tran Minh Hoi, Nguyen Huy Hung, Le Thi Huong, Dang Viet Hau, Dang Thi Hong Duyen, William N. Setzer & Isiaka Ajani Ogunwande (2023) Chemical Compositions of Essential Oils and Antimicrobial Activities of Asteraceae: The Leaves of *Blumea lacera* (Burm. f.) DC., *Tridax procumbens* (L.) L., and *Ageratum houstonianum* Mill., from Vietnam, Journal of Essential Oil Bearing Plants, 26:1, 130-142, DOI: 10.1080/0972060X.2023.2165169

To link to this article: https://doi.org/10.1080/0972060X.2023.2165169

Published online: 26 Jan 2023.

Journal of Essential Oil Bearing Plants

Taylor & Francis
Taylor & Francis Group

https://www.tandfonline.com/journals/teop20 ISSN Print: 0972-060X; ISSN Online: 0976-5026

Article

Chemical Compositions of Essential Oils and Antimicrobial Activities of Asteraceae: The Leaves of *Blumea lacera* (Burm. f.) DC., *Tridax procumbens* (L.) L., and *Ageratum houstonianum* Mill., from Vietnam

Tran Minh Hoi [1,2], Nguyen Huy Hung [3], Le Thi Huong [4]*, Dang Viet Hau [5], Dang Thi Hong Duyen [5], William N. Setzer [6,7], Isiaka Ajani Ogunwande [8]*

[1] Department of Plant Resources, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology

[2] Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18-Hoang Quoc Viet, Cau Giay, Hanoi, 10072, Vietnam

[3] Center for Advanced Chemistry, Institute of Research and Development, Duy Tan University, 03 Quang Trung, Da Nang, Vietnam

[4] School of Natural Science Education, Vinh University, 182 Le Duan, Vinh City, Nghe An Province 4300, Vietnam

[5] Center for Research and Technology Transfer, Vietnam Academy of Science and Technology, Ha Noi; 100000;

[6] Aromatic Plant Research Center, 230 N 1200 E, Suite 100, Lehi, UT 84043, USA

[7] Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA

[8] Foresight Institute of Research and Translation, Ibadan, Nigeria

* *Corresponding Authors: lehuong223@gmail.com (Le Thi Huong)*
isiakaogunwande@gmail.com (Isiaka Ajani Ogunwande)

Received 23 October 2022; Received in revised form 14 December 2022; Accepted 17 December 2022

Abstract: The paper reports the chemical compositions and antimicrobial activity of essential oils from *Blumea lacera* (Burm. f.) DC., *Tridax procumbens* (L.) L., and *Ageratum houstonianum* Mill., of Asteraceae family grown in Vietnam. The essential oils were isolated by hydrodisitllation and analysed by GC and GC/MS. The Minimum inhibitory concentration (MIC) and Median inhibitory concentration (IC_{50}) values were measured by the microdilution broth susceptibility assay. The main compounds of *B. lacera* were germacrene D (25.5%), 2,5-dimethoxy-*p*-cymene (20.6%), β-caryophyllene (17.9%), thymol methyl ether (6.3%). *T. procumbens* afforded essential oil whose major constituent was β-pinene (13.4%), while germacrene D (21.3%), α-pinene (19.0%), and β-caryophyllene (8.9%) constitute the bulk of *A. houstenianum* essential oil. The leaf essential oils of *A. houstenianum* collected in August (MIC, 8.0 µg/mL) and *B. lacera* (MIC, 16.0 µg/mL) were the most active against *Enterococcus faecalis* ATCC 29212, with corresponding IC_{50} values of 4.00 µg/mL and 5.67 µg/mL, respectively. The essential oil of *T. procumbens* exhibited the most potent activity towards *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 14579, with MIC value of 32.0 µg/mL and IC_{50} values of 12.45

DOI: 10.1080/0972060X.2023.2165169

µg/mL and 7.89 µg/mL, respectively. Also, both *A. houstenianum* essential oils collected in August and April displayed antibacterial action against *Pseudomonas aeruginosa* ATCC 27853 with MIC values of of 64.0 µg/mL ($IC_{50,}$ 21.45 µg/mL) and 32.0 µg/mL and ($IC_{50,}$ 15.22 µg/mL). Both *B. lacera* and *T. procumbens* showed anti-candidal property with MIC value of 16.0 µg/mL, as well as IC_{50} values of 5.69 µg/mL and 8.66 µg/mL, respectively.

Keywords: Asteraceae, essential oil, terpenes, antibacterial activity, anti-candidal activity.

Introduction

The Asteraceae family is the largest family of flora in the word. There are about 1550 genera and about 23.000 species [1]. In Vietnam there are about 126 genera and 379 species [2]. Many species are used as medicine, for essential oils, for ornamental [2,3]. *Blumea lacera* (Burm. f.) DC., is an erect, annual plant with branched stems 18 - 100 cm tall. The plant is harvested from the wild for local use - mainly as a medicine but also for food and as a source of essential oil [4]. In Vietnam, leaves are fragrant, used as a vegetable, cure boils, stop bleeding, and hemorrhage [3]. *Ageratum houstonianum* Mil., is a usually erect, and often much-branched, annual plant growing 30 - 100 cm tall. The plant is sometimes harvested from the wild for local medicinal use. Very ornamental, it is often grown in gardens [1]. *Tridax procumbens* (L.) L., is best known as a widespread weed and pest plant. It is native to the tropical Americas, but it has been introduced to tropical, subtropical, and mild temperate regions worldwide [2]. The whole tree is use to cure swelling, and as remedies in antiseptic, cooling, cough, rheumatism [3]. Currently (Table 1), there have been some studies on essential oils and biological activities of *B. lacera* [5-9], *T. procumbens* [10-15], and *A. houstenianum* [16-22].

The studied essential oils exhibited diverse chemical compositional pattern. For example, previously essential from the plant, *B. lacer,* studied in Vietnam contained β-caryophyllene (8.3 - 12.0%), thymolhydroquinon-dimethylether (6.6 - 11.9%) and caryophyllene oxide (11.9 - 21.7%) [7]. However, 2,5-dimethoxy-p-cymene (28.7 - 0.4%), β-caryophyllene (25.5 - 0.5%), carvotanacetone (24.5 - 0.4%), chrysanthenone (21.9 - 9.8%) and 2,6-dimethyl phenol (11.4 - 1.8%) were the main compounds of the leaf essential oil of *B. lacera* analysed from India [9]. With respect to the previous analysis of essential oils of *T. procumbens* [10-15] from other parts of the world, apart from ubiquitous terpenes, non-terpenoid compounds such as dibutyl phthalate [10], 1,2-cyclooctanediol [11], hexanal [11], 4-heptenal [11] and 1,3,6-octatriene [14] were also identified in larger proportions in the essential oils. The essential oils of *A. houstenianum* from all parts of the world presented a compositional pattern dominated by precocene I, β-caryophyllene and precocene II [16-22]. There seems to be homogeneity in the compositions of essential oils of *A. houstenianum* reported so far in the literature.

The aim of the present communication was to report the chemical constituents and the antimicrobial activity of the essential oils hydrodistilled from the leaves of *B. lacera*, *T. procumbens*, and *A. houstenianum* harvested in Vietnam. This is in continuation of the ongoing analysis of volatile compounds and antimicrobial potentials of Vietnamese plants [23-27].

Materials and methods

Plants collection

All the plant samples were collected from Pù Hoạt Nature Reserve (GPS: 19°35′19″N 104°43′7″ E) at an elevation of 870 m. The samples of *B. lacera* and *A. houstonianum* (AH2) were collected in April 2020, while *T. procumbens* and another sample of *A. houstonianum* (AH1) were collected in August 2020. The samples were identified by Dr. Le Thi Huong and voucher specimens, LTH 758, 875, 878 and 756, respectively, have been deposited in the plant specimen room, Vinh University. In each case, the fresh leaves were chopped and 2.0 kg was subjected to hydrodistillation using a Clevenger-type apparatus as described previously [23-27].

Analysis of the oils

Gas chromatographic (GC) analysis was performed on an Agilent Technologies HP 7890A Plus Gas chromatograph equipped with an FID and fitted with HP-5ms column (30 m

Table 1. Previously analysed essential oil constituents of the studied plants

Plant part	Origin	Main constituents	Biological activity	References
Blumea lacera				
Leaves	Nigeria	Thymoquinol dimethyl ether, β-caryophyllene, α-humulene and E-β-farnesene[a]	-	5
Aerial parts	Nepal	(Z)-Lachnophyllum ester (25.5%), (Z)-lachnophyllic acid (17.0%), germacrene D (11.0%), (E)-β-farnesene (10.1%), bicyclogermacrene (5.2%)	cytotoxic activity against MDA-MD-231, MCF-7, and 5637 human tumor cells, anti-bacterial, antifungal activity.	6
Aerial parts	Vietnam	β-Caryophyllene (8.3%-12.0%), thymolhydroquinon-dimethylether (6.6%-11.9%) and caryophyllene oxide (11.9%-21.7%)	-	7
Leaves	Thailand	4-Terpineol and α-terpinen-7-al	Antibacterial against *Bacillus subtilis* and *Staphylococcus aureus*	8
Leaves	India	2,5-Dimethoxy-p-cymene (28.7–0.4%), β-caryophyllene (25.5–0.5%), carvotanacetone (24.5–0.4%), chrysanthenone (21.9–9.8%) and 2,6-dimethyl phenol (11.4–1.8%)	-	9
Tridax procumbens				
Leaves	India	Dibutyl phthalate (19.29%), *trans*-(α)-caryophyllene (9.55%),	IC_{50} value of MCF-7 cell line was 96.6 µg/ml	10
Leaves	India	1,2-Cyclooctanediol (11.49%), hexanal (5.34%), 4-heptenal (4.92%)	Fumigant activity against *Sitophilus zeamais*	11
Leaves	Brazil	Thymol (48.22%), γ-terpinene (15.93%), and *o*-cymene (10.27%)	Larvicidal, antioxidant, cytotoxicity on using *Artemia salina*	12
Flowers	India	(Z)-Falcarinol (25.9%), α-selinene (15.3%), limonene (8.3%)	Antimicrobial	13

table 1. (continued).

Plant part	Origin	Main constituents	Biological activity	References
Tridax procumbens				
Leaves	India	1,3,6-Octatriene (20.90%), α-pinene (10.84%), β-pinene, phellandrene (9.64%) and sabinene (6.98%)	Anti-Microbial and Anti-Inflammatory activity	14
Leaves	Ivory Coast	p-Cymene (2.2%-11.3%), β-caryophyllene (1.5%-6.0%), β-selilene (1.8%-10.0%), elemol (0=16.0%),	-	15
Ageratum houstonianum				
Leaves and flowers	Egypt	Precocene I (22.4-34.6 %), β-caryophyllene (63.9-13.7 %) and precocene II (10.8-29.6 %)	Antibacterial angainst *Bacillus subtilis* and *Staphylococcus aureus* and *in vitro* cytotoxicity against colon (HCT-116) carcinoma cell line	16
Leaves	India	Precocene I and precocene II	Trypanocidal activity on *Trypanosoma evansi*	17
Leaves	India	Precocene I (23.34%), precocene II (43.99%) and β-caryophyllene (9.18%)		18
Leaves	China	Precocene II (62.68%), precocene I (13.21%), and β-caryophyllene (7.92%)	Toxicity against booklice, *Liposcelis bostrychophila* Badonnel	19
Leaves	Nigeria	Precocene II (42.16%), precoene I (18.65%), (*E*)-caryphyllene (15.56%)	Insecticidal	20
Leaves	Cameroon	Precocene I (32%) and precocene II (24%)	-	21
Leaves	Leaves	Precocene II (52.64%), precoene I (22.45%), (*E*)-caryphyllene (9.66%)	Antibacterial against *Micrococcus lutea* and *Rhodococcus rhodochrous*	22

- No information; [a]Quantitative data not known

× 0.25 mm, film thickness 0.25 µm, Agilent Technologies, (Santa Clara, California, USA). The analytical conditions were: carrier gas H_2 (1 mL/min), injector temperature (PTV: programmable temperature vaporization) 250°C, detector temperature 260°C, column temperature programmed from 60°C (2 min hold) to 220°C (10 min hold) at 4°C/min. Samples were injected using a split mode with a split ratio of 10:1. The volume injected was 1.0 µL. Inlet pressure was 6.1 kPa. Quantification in the GC was done by external standard method using calibration curves generated by running GC analysis of representative compounds as described previously [23-27].

An Agilent Technologies (Santa Clara, California, USA) HP 7890A Plus Chromatograph fitted with a fused silica capillary HP-5 MS column (30 m × 0.25 mm, film thickness 0.25 µm) and interfaced with a mass spectrometer HP 5973 MSD was used for the GC/MS analysis, under the same conditions as those used for GC analysis. The conditions were the same as described above with He (1 mL/min) as carrier gas. The MS conditions were as follows: ionization voltage 70 eV; emission current 40 mA; acquisitions scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s.

The method used for the identification of the compounds of essential oils involves the comparison of retention indices (RI) from GC with reference to a homologous series of *n*-alkanes (C_8 - C_{40}), under identical experimental conditions; using of co-injection with known compounds under the identical GC conditions for some compounds; and checking the mass spectral (MS) fragmentation patterns with available known composition in literature [28] as described recently.

Antimicrobial activity assays

The microorganisms used in the study of the antimicrobial activity of the essential oils consist of three strains of Gram-positive bacteria, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579, three strains of Gram-negative bacteria, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* ATCC 13076, and one strain of yeast, *Candida albicans* ATCC 10231. The strains were obtained from the laboratory stock of 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 (IC_{50}) values were measured by the microdilution broth susceptibility assay as described previously [23-27]. The choice of investigated concentrations was based on previous reports on similar reports where essential oils have been found to be active within specific concentration ranges [23-27]. 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 (2^{14}, 2^{13}, 2^{12}, 2^{11}, 2^{10}, 2^9, 2^7, 2^5, 2^3 and 2^1 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 5×10^5 and 1×10^3 CFU/mL, respectively. The last row of the micro-test tubes containing only the serial dilutions of samples without microorganisms was used as a positive (no growth) control. Sterile distilled water and medium served as a negative (no antimicrobial agent) 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). The cultures of tested microorganisms grown overnight are diluted and read on 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 IC_{50} 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:

$$\% \text{ Inhibition} = \frac{OD_{control}\,(+) - Od_{test}\ \text{agent}}{OD_{control}\,(+) - OD_{control}\,(-)}\ x\ 100$$

$$IC_{50} = High_{conc} - \frac{(High_{inh\%} - 50\%)\ x\ (High_{conc} - Low_{conc})}{(High_{inh\%} - Low_{inh\%})}$$

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

The essential oil composition of B. lacera

The obtained yield of the studied essential oil of *B. lacera* was 0.32% (w/w) and yellow coloured. From the analysed data (Table 2), the total contents was 97.5%, with sesquiterpene hydrocarbons (59.8%) and oxygenated monoterpenes (30.8%) in abundant. Monoterpene hydrocarbons (2.3%) and oxygenated sesquiterpenes (3.8%) were present in much lower amount. The amount of non-terpenes was 0.8%. The major compounds of the essential oil were germacrene D (25.5%), 2,5-dimethoxy-*p*-cymene (20.6%), and β-caryophyllene (17.9%). There are sizeable quantity of thymol methyl ether (6.3%), γ-curcumene (4.6%), and α-humulene (4.5%). A comparative analysis of this study and previous studies indicated some quantitative and qualitative variations. Both germacrene D and β-caryophyllene, two of the major compounds in this essential oil, were also identified in large quantities from samples analysed in Nigeria [5], Nepal [6], Vietnam [7] and India [9]. Also, 2,5-dimethoxy-p-cymene, present in higher amount in the present analysed oil sample was also the main compound of Indian [9] sample. However, thymoquinol dimethyl ether the main constituent of sample from Nigeria [5], (*Z*)-lachnophyllum ester and (*Z*)-lachnophyllic acid, the compounds occurring in higher amount in Nepal sample [6], thymolhydroquinon-dimethylether previously identified in sample of *B. lacera* from Vietnam [7], 4-terpineol and α-terpinen-7-al confirmed in Thailand essential oil [8], as well as carvotanacetone, chrysanthenone and 2,6-dimethyl phenol identified previously in Indian [9] sample, were conspicuously absent in the present investigated oil sample. Moreover, the content of caryophyllene oxide in this sample (1.1%) was lower when compared with amount present in a sample of *B. lacera* previously analysed in Vietnam (11.9%-21.7%) [7].

The analysed essential oil composition of T. procumbens

The hydrodistilled essential oil of *T. procumbens* was obtained in a yield of 0.28% (w/w). The essential oil was yellow coloured. From the analysed GC/MS spectra, oxygenated monoterpene (17.7%), sesquiterpene hydrocarbons (10.7%), oxygenated sesquiterpenes (32.2%) and diterpenes (9.4%) were identified in abundant (Table 2). The non-terpenes were present in 0.3%, as well as monoterpene hydrocarbons (4.6%). The main constituents of the essential oil were β-pinene (13.4%), phytol (7.2%), *trans*-calamine-10-ol (6.0%), *neo*-intermedeol (5.2%), and β-acoradiene (4.2%). From Table 1, it could be seen that β-pinene, *trans*-calamine-10-ol and phytol present in sizeable content in this study were not

Table 2. Compounds identified in the studied essential oils from Vietnam

No.	RT (min)	Compounds	RI_{cal}	RI_{db}	*B. lacera*	*T. procumbens*	AH1	AH2
1	9.07	Santolina triene	907	906	1.3	-	-	-
2	10.23	α-Thujene	930	924	-	-	0.9	0.6
3	10.17	α-Pinene	940	932	0.4	2.2	19.0	8.9
4	10.62	Camphene	955	946	-	0.7	0.6	0.4
5	11.33	Sabinene	979	969	-	-	1.0	1.1
6	11.50	β-Pinene	985	974	-	13.4	0.6	0.5
7	11.73	Myrcene	992	988	-	0.6	6.0	5.1
8	12.34	α-Phellandrene	1011	1002	-	-	0.5	0.5
9	12.49	α-Terpinene	1022	1024	-	-	0.7	0.7
10	13.08	*p*-Cymene	1030	1024	0.1	0.2	1.4	1.0
11	13.15	Limonene	1034	1024	0.2	0.6	5.8	4.7
12	13.21	β-Phellandrene	1036	1025	-	-	-	0.2
13	13.25	1,8-Cineole	1037	1026	0.4	-	-	-
14	13.28	(*Z*)-β-Ocimene	1037	1032	-	-	0.2	0.1
15	13.66	(*E*)-β-Ocimene	1048	1044	-		2.7	2.9
16	14.15	γ-Terpinene	1063	1054	-	-	6.5	5.5
17	15.21	Terpinolene	1094	1086	-	-	4.5	4.3
18	15.79	δ-2-Carene	1106	1102	-	-	0.3	-
19	15.85	(*E*)-4,8-Dimethylnona-1,3,7-triene	1118	1117	0.5	-	-	-
20	16.04	1,3,8-*p*-Menthatriene	1126	1119	0.3	-	-	-
21	16.37	*trans*-Sabinol	1148	1137	-	0.8	-	-
22	16.97	Geijerene	1151	1138	-	-	-	0.4
23	17.31	Pinocarvone	1172	1160	-	0.6	-	-
24	18.92	α-Terpineol	1197	1186	-	0.4	-	-
25	19.88	Myrtenol	1204	1194	-	0.2	-	-
26	20.13	Myrtenal	1206	1195	-	0.2	-	-
27	21.11	*endo*-Fenchyl acetate	1227	1218	-	1.1	-	-
28	21.39	Thymol methyl ether	1240	1232	6.3	-	-	-
29	22.49	*iso*-Bornyl acetate	1293	1283	-	-	0.8	0.7
30	22.54	Bornyl acetate	1294	1287	-	0.3	-	-
31	22.61	Pregeijerene	1305	1285		-	-	0.2
32	22.87	*cis*-Pinocarvyl acetate	1320	1311	-	0.4	-	-
33	23.11	Methyl acetate	1332	1330	-	0.3	-	-
34	23.96	δ-Elemene	1348	1335	1.0	-	1.0	1.3
35	24.48	Eugenol	1367	1356	-	-	-	0.8
36	25.31	α-Copaene	1390	1374	0.3	-	-	0.2
37	25.73	β-Cubebene	1402	1387	0.6	-	-	0.6
38	25.91	*trans*-β-Elemene	1408	1389	0.5	0.8	-	0.9
39	26.27	Petasitene	1412	1405	-	-	0.1	0.1
40	26.46	*trans*-α-Bergamotene	1428	1425	1.0	-	-	-
41	26.59	2,5-Dimethoxy-*p*-cymene	1429	1418	20.6	0.3	-	-
42	26.65	α-Santalene	1431	1416	-	-	1.2	-
43	26.83	β-Caryophyllene	1437	1417	17.9	0.4	8.9	12.9
44	27.09	β-Gurjunene	1444	1431	-	-	0.2	0.2
45	27.23	α-Guaiene	1451	1437	0.2	-	-	-
46	27.78	*epi*-β-Santalene	1459	1445	-	-	0.2	-
47	27.82	(*Z*)-β-Farnesene	1461	1440	0.4	-	-	0.2

table 2. (continued).

No.	RT (min)	Compounds	RI_{cal}	RI_{db}	*B. lacera*	*T. procumbens*	AH1	AH2
48	27.91	α-Humulene	1472	1452	4.5	0.6	3.7	2.1
49	28.14	9-*epi*-(*E*)-Caryophyllene	1479	1464	-	-	-	0.2
50	28.32	β-Acoradiene	1480	1469	-	4.2	-	-
51	28.41	γ-Curcumene	1489	1481	4.6	-	-	-
52	28.49	γ-Muurolene	1490	1478	-	0.6	0.8	1.0
53	28.56	Neryl isobutanoate	1492	1490	2.5	-	-	-
54	28.60	Dodecanol	1493	1469	-	0.3	-	-
55	28.62	*epi*-Zonarene	1494	1501	-	-	0.3	-
56	28.75	Germacrene D	1497	1484	25.5	-	21.3	28.1
57	28.80	Aristolochene	1500	1487	-	0.5	-	-
58	28.90	α-Zingiberene	1504	1493	1.2	-	-	-
59	28.95	β-Selinene	1505	1489	-	0.8	-	-
60	29.03	α-Chamigrene	1506	1476	-	-	0.5	0.2
61	29.17	α-Selinene	1511	1498	-	2.4	-	-
62	29.19	(*E*,*E*)-α-Farnesene	1512	1505	0.5	-	-	0.5
63	29.20	α-Muurolene	1513	1500	-	0.2	-	-
64	29.23	Bicyclogermacrene	1514	1500	0.4	-	5.7	7.0
65	29.31	β-Bisabolene	1518	1505	0.2	-	-	0.1
66	29.38	δ-Amorphene	1521	1511	0.3	-	-	-
67	29.46	γ-Cadinene	1530	1513	-	0.2	0.2	0.3
68	29.80	Eugenol acetate	1533	1521	-	-	-	0.6
69	29.91	δ-Cadinene	1537	1522	0.4	-	0.3	0.6
70	30.95	(*E*)-Nerolidol	1571	1561	0.3	0.7	-	-
71	31.14	Germacrene B	1577	1559	1.3	-	-	0.2
72	31.52	Scapanol	1594	1593	-	-	-	0.3
73	31.74	Spathulenol	1595	1577	-	1.0	0.3	0.4
74	31.94	Caryophyllene oxide	1605	1582	1.1	3.8	0.2	0.4
75	32.12	Guaiol	1615	1600	0.3	-	-	-
76	32.96	*epi*-Cedrol	1624	1618	-	0.6	-	-
77	33.01	Zingiberenol	1626	1616	0.3	-	-	-
78	33.06	Humulene epoxide II	1632	1608	0.2	3.8	-	-
79	33.33	α-Acorenol	1643	1632	0.2	-	-	-
80	33.59	1,2-Diacetoxy-4-allybenzene	1652	1638	0.3	-	-	-
81	33.68	*epi*-α-Cadinol	1656	1638	-	0.5	0.6	1.3
82	33.70	*epi*-α-Muurolol	1658	1640	-	0.5	-	-
83	33.72	α-Muurolol	1662	1644	-	0.3	-	-
84	33.87	β-Eudesmol	1673	1649	0.9	-	-	-
85	33.90	α-Cadinol	1675	1652	-	2.6	0.5	0.6
86	33.99	*ar*-Turmerone	1677	1668	0.2	-	-	-
87	34.12	*cis*-Calamene-10-ol	1678	1660	-	3.1	-	-
88	35.04	*neo*-Phytadiene	1680	1830	-	1.9	-	-
89	35.57	Anhydroencecalinol	1685	1677	-	-	0.5	-
90	35.61	14-Hydroxy-9-*epi*-(*E*)-caryophyllene	1687	1668	-	0.4	-	-
91	35.82	*trans*-Calamine-10-ol	1688	1668	-	6.0	-	-
92	36.19	*neo*-Intermedeol	1689	1665	-	5.2	-	-
93	37.23	α-Bisabolol	1700	1685	0.3	-	-	-
94	49.233	1-Phenylhepta-1,3,5-triyne	1743	1719	-	-	-	0.1
95	41.39	6,10,14-Trimethylpentadecan-2-one	1847	1838	-	3.7	-	-

table 2. (continued).

No.	RT (min)	Compounds	RI_{cal}	RI_{db}	*B. lacera*	*T. procumbens*	AH1	AH2
96	44.09	*iso*-Phytol	1950	1949	-	0.3	-	-
97	45.38	Phytol	2117	2109	-	7.2	-	-
		Total			97.5	74.4	98.0	99.0
Monoterpene hydrocarbons (Sr. No. 1-12, 14-18, 20)					2.3	17.7	50.7	36.9
Oxygenated monoterpenoids (Sr. No. 13, 21-33)					30.8	4.6	0.8	0.9
Sesquiterpene hydrocarbons (Sr. No. 34-36,40,42-52,55-67,69,71)					59.8	10.7	44.4	56.7
Oxygenated sesquiterpenoids (Sr. No. 53,70,72-79,81-87,89, 90-92, 95)					3.8	32.2	2.1	3.0
Phenylpropanoids (Sr. No. 35, 68)					-	-	-	1.4
Diterpenes (Sr. No. 88, 96, 97)					-	9.4	-	-
Others (Sr. No.19, 41, 54, 80, 94)					0.8	0.3	-	0.1

RI_{cal} = Retention index determined with respect to a homologous series of *n*-alkanes on an HP-5ms column; RI_{db} = Retention index from the databases; AH 1 = *A. houstonianum* 1; AH 2 = *A. houstonianum* 2; - = not identified

reported previously as dominant compounds of *T. procumbens* [10-15]. Several compounds such as dibutyl phthalate [10], 1,2-cyclooctanediol [11], thymol [12], *γ*-terpinene [12], *o*-cymene [12], as well as (Z)-falcarinol [13], α-selinene [13], 1,3,6-octatriene [14], β-phellandrene [14], and elemol [15], that are characteristics of previously analysed essential oils of *T. procumbens*, were not identified in the present investigated oil sample.

The identified components of the leaf essential oil of A. houstonianum

The calculated yield of the essential oil of *A. houstonianum* was 0.41% (w/w, sample AH1) and 0.38% (w/w, sample AH2). Both essential oils were light-yellow coloured. Sample AH1 (collected in August 2022) and sample AH2 (harvested in April 2022) consists mainly of monoterpene hydrocarbons (50.7% and 36.9%, respectively) and sesquiterpene hydrocarbons (44.4% and 56.7%, respectively). Both samples (Table 2) have lower quantities of oxygenated monoterpenes (0.8% vs. 0.9%) and oxygenated sesquiterpenes (2.1% vs. 3.0%). The April sample has 1.4% of phenylpropanoids. The main constituents of the essential oils were germacrene D (21.3% and 28.1%), α-pinene (19.0% and 8.9%), β-caryophyllene (8.9% and 12.9%), bicyclogermacrene (5.7% and 7.0%), γ-terpinene (6.5% and 5.5%), myrcene (6.0% and 5.1%) and limonene (5.8% and 4.7%). Previously investigates essential oil samples of *A. houstonianum* comprised mainly of either precocene I and precocene II [17,21,22], and at times along with β-caryophyllene [16,18-20]. However, neither precocene I nor precocene II could be identified in this studied essential oil samples of *A. houstonianum* from Vietnam. This is an unusual compositional pattern of essential oil of *A. houstonianum.* β-Caryophyllene as previously identified in the essential oil [16-,22], was also identified in the investigated oil sample. This is the first report on the essential oil of *A. houstonianum* from Vietnam.

The observed antimicrobial activity of the essential oils

From Table 3, all the studied essential oils were inactive towards *S. enterica* ATCC 13076, while only *Tridax procumbens* showed moderate activity against *E. coli* ATCC 25922 with MIC value of 128.0 µg/mL, and IC_{50} value of 45.56 µg/mL. However, only *B. lacera* essential oil did not show any activity to *P. aeruginosa* ATCC 27853. Overall, all the essential oils showed moderate activity to the studied microorganisms. The leaf essential oil *B. lacera* displayed antibacterial activity to *E. faecalis* ATCC 29212, *S. aurues*

Table 3. The antimicrobial activity of the studied essential oils of from Vietnam

Microorganisms	MIC (µg/mL)[a]				IC_{50} (µg/mL)[a]			
	B. l	***T. p***	***A.h* 1**	***A.h* 2**	***B. l***	***T. p***	***A.h* 1**	***A.h* 2**
Enterococcus faecalis ATCC 29212	16.0	128.0	8.0	128.0	5.67	65.33	4.00	64.33
Staphylococcus aureus ATCC 25923	64.0	32.0	64.0	64.0	18.98	12.45	19.78	32.68
Bacillus cereus ATCC 14579	64.0	32.0	64.0	64.0	29.67	7.89	18.78	34.22
Escherichia coli ATCC 25922	-	128.0	-	-	nt	45.67	nt	nt
Pseudomonas aeruginosa ATCC 27853	-	128.0	64.0	32.0	nt	45.56	21.45	15.22
Salmonella enterica ATCC 13076	-	-	-	-	nt	nt	nt	nt
*Candida albican*s ATCC 10231	16.0	16.0	64.0	128.0	5.69	8.66	33.45	65.66

[a] Mean value of three replicate assays
SD standard deviations are insignificant
- no activity; nt, Not tested
B.l, Blumea lacera; *T.p, Tridax procumbens*
A.h1; Ageratum houstonianum collected in August
A.h2, Ageratum houstonianum collected in April

ATCC 25923 and *B. cereus* ATCC 14579, with MIC values of 16.0, 64.0 and 64.0 µg/mL, respectively. The corresponding IC_{50} values were 5.67, 18.98 and 29.67 µg/mL, respectively. In addition, the essential oil inhibited the growth of *C. albicans* ATCC 10231 with MIC value of 16.0 µg/mL, and IC_{50} value of 5.69 µg/mL. The observed antimicrobial activity of the studied *B. lacera* essential oil is in conformity with previous report which described the analysed essential oil from Thailand to be active against strains of both *B. subtilis* and *S. aureus* [8].

The leaf essential oil of *T. procumbens* exhibited the most potent activity towards *S. aureus* ATCC 25923 and *B. cereus* ATCC 14579, with MIC value of 32.0 µg/mL and IC_{50} values of 12.45 µg/mL and 7.89 µg/mL, respectively. In addition, the oil exhibited similar activity like *B. lavera* against equal *C. albicans* ATCC 10231 with MIC value of 16.0 µg/mL. Moreover, the essential oil also showed similar activity pattern to *E. faecalis* ATCC 29212, *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, with MIC value of 128.0 µg/mL. The IC_{50} values were observed at 65.33, 45.67 and 45.56 µg/mL, respectively. The essential oils of *T. procumbens* from India was reported to show some antimicrobial property [13,14].

The leaf essential oils of *A. houstenianum* collected in August (MIC, 8.0 µg/mL and IC_{50} value, 4.00 µg/mL) was the most active against *E. faecalis* ATCC 299212, when compared with sample collected in April 2020. However, both essential oils exhibited equal antimicrobial activity against *S. aurues* ATCC 25923 and *B. cereus* ATCC 14579, with MIC value of 64.0 respectively. While the August sample exhibited activity against *P. aeruginosa* ATCC 27853 with MIC value of 64.0 µg/mL, the essential oil from sample collected in April displayed much stronger activity with MIC value of 32.0 µg/mL. Previously, essential oil from the leaves and flowers of *A. houstonianum* has shown activities against *B. subtilis* and *S. aureus* [16].

Expectedly, the studied essential do not possess the same chemical compositions. The

studied plants did not belong to the same genus and neither are they from the same family of plants. It is well known that the differences in the environmental and ecological conditions at the site of the collection of the plants would also have influence on the chemical constituents and biological potential of each of the studied plant samples. Other factors such as the age of the plant, time of collection and handling procedures would contribute greatly to the observed variations and differences in chemical contents and antimicrobial activity when compared with samples analyzed from other parts of the world.

Conclusion

In the present study, several qualitative and quantitative variations were observed between the compositional and antimicrobial activity of essential oils when compared with data from previously analysed oil samples from various parts of the world. The main compounds of *B. lacera* were germacrene D (25.5%), 2,5-dimethoxy-*p*-cymene (20.6%), and β-caryophyllene (17.9%), while *T. procumbens* comprised mainly of β-pinene (13.4%), with germacrene D (21.3% and 28.1%), α-pinene (19.0% and 8.9%), β-caryophyllene (8.9% and 12.9%) constituting the bulk of *A. houstenianum* essential oil. The leaf essential oils of *A. houstenianum* collected in August (MIC, 8.0 µg/mL) and *B. lacera* (MIC, 16.0 µg/mL) were the most active against *E. faecalis* ATCC 29212, while the essential oil of *T. procumbens* exhibited the most potent activity towards *S. aureus* ATCC 25923 and *Bacillus cereus* ATCC 14579, with MIC value of 32.0 µg/mL. Both *B. lacera* and *T. procumbens* showed anti-candidal property with MIC value of 16.0 µg/mL.

Competing interests

The authors declare that no competing interest exists.

Acknowledgments

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number: 106.03-2019.315

References

1. **Wu, Z.Y., Raven, P.H. and Hong, D.Y., eds., (2011).** In: Flora of China Volume 20-21 (Asteraceae). Science Press (Beijing) & Missouri Botanical Garden Press (St. Louis), USA.
2. **Bien, L.K. (2007).** Flora of Vietnam, Vol. 7. Asteraceae, Science and Technics Publishing House (Vietnamese)
3. **Chi, V.V. (2012).** Medicinal Dictionary, Medicinal Publishing House, Hanoi, Vietnam, pp. 896.
4. **Aban J. Randeria, (1960).** The Composite Genus Blumea, A Taxonomic Revision, Blumea. 10: 176 - 317
5. **Laakso, I., Seppänen-Laakso, T., Hiltunen, R. and Ekundayo, O. (1989).** Composition of the essential oil of *Blumea lacera* DC. (Asteraceae) leaves from Nigeria. Flav. Fragr. J. 4: 73-75.
6. **Satyal, P., Chhetri, B.K., Dosoky, N.S., Shrestha, S., Poudel, A. and Setzer, W.N. (2015).** Chemical composition of *Blumea lacera* essential oil from Nepal. Biological activities of the essential oil and (*Z*)-lachnophyllum ester. Nat. Prod. Commun. 10: 1749-1750.
7. **Hac, L.V. and Muoi, T.T. (2013).** Essential oils of *Blumea lacera* (Burm. F) DC. (Asteraceae) produced from aerial parts of plants grown in Central of Vietnam. J. Essent. Oil Bearing Plants. 16(1): 36-40.
8. **Trikunakornwong, A., Nongluksna, S., De-Eknamkul, W. and Nijsiri, R. (1998).** Chemical composition and antimicrobial activity of essential oils from some Thai Blumea species. In: Proceedings of the Twenty-four Congress on Science and Technology of Thailand, Faculty of Science, Chulalongkorn University, Bangkok Press, Thailand, pp. 310-311.
9. **Joshi, R.K., Pai, S.R., Narajan, H. and Vetrivel, U. (2022).** Identification of potentially bioactive compounds from *Blumea lacera* essential oil by gas chromatography-mass spectroscopy and molecular docking studies for targeting inflammatory bowel disease. Nat. Prod. Res. (in press).

10. **Poonkodi Kathirvel**, **V Jayapriya**, **K Sujitha**, **R Jeevitha, Megha, P.U (2017).** *In-vitro* Anticancer Activity and Essential oil Composition of *Tridax procumbens* (L.). Am. J. Pharm Tech. Res. 7: 1-7
11. **Ruchuon, W. and Kaewduangta, W. (2022).** Fumigant activity of *Tridax procumbens* (Asterales: Asteraceae) essential oil against *Sitophilus zeamais* (Coleoptera: iCurculionidae) and its effects on Thai rice seed germination. J. Entomol. Sci. 57: 561-572.
12. **Brandão, L.B., Santos, L.L., Martins, R.L., Rodrigues, A.B.L., de Rabelo, E.M., Galardo, A.K.R., and da S. de Almeida, S.S.M. (2012).** Larvicidal evaluation against *Aedes aegypti* and antioxidant and cytotoxic potential of the essential oil of *Tridax procumbens* L. leaves. The Scient. World. 2021: Article ID 2172919.
13. **Koshi, R.K. and Badakar, V. (2012).** Chemical composition and *in vitro* antimicrobial activity of the essential oil of the flowers of *Tridax procumbens*. Nat. Prod. Commun. 7: 941-942.
14. **Manjamalai, A., Valavil, S. and Berlin Grace, V.M. (2012).** Evaluation of essential oil of *Tridax procumbens* L. for antimicrobial and anti-inflammatory activity. Int. J. Pharm. Pharmaceut. Sci. 4: 356-363.
15. **Coulibaly, A., Soro, Y., Siaka, S., Nea, F. and Tonzibo, Z.F. (2020).** Chemical variability of essential oil of *Tridax procumbens* Linn (Asteraceae) from five cities of Cote Divoire. Int. J. Biol. Chem. Sci. 14: 1843-1852.
16. **El Hadidy, D., El Sayed, A.M., El Tantawy, M. and El Alfy, T. (2019).** Phytochemical analysis and biological activities of essential oils of the leaves and flowers of *Ageratum houstonianum* Mill. cultivated in Egypt. J. Essent. Oil Bearing Plants. 22(5): 1241-125.
17. **Shaba, P., Pandey, N.N., Rao, J.R., Dey, S., Mandal, B.D., Kurade N.P.. Singh, R.K., Bhanuprakash, V. and Chaudary, P. (2020).** Trypanocidal activity of purified precocene I by reverse-phase High Performance Liquid Chromatography from essential oil of *Ageratum houstonianum* aerial parts. Am. J. Pharmacol. Pharmacother.7: 1-5.
18. **Chandra, S., Shahi, A.K., Dutt, P. and Tava, A. (1996).** Essential oil composition of *Ageratum houstonianum* Mill. from Jammu region of India. J. Essent. Oil Res. 8: 129-134.
19. **Lu, X.N., Liu, X.C., Liu, Q.Z. and Liu, Z.L. (2014).** Isolation of insecticidal constituents from the essential oil of *Ageratum houstonianum* Mill. against *Liposcelis bostrychophila* Badonnel. J. Chem. 2014: Article ID 645687.
20. **Olonisakin A., Dolma, S.K., Verma, P.K., Singh, B. and Reddy, S.G.E. (2019).** Volatile, nonvolatile composition and biological activities of *Ageratum houstonianum* Mill. against diamondback moth, *Plutella xylostella* (L.) and aphid, *Aphis craccivora* Koch. Indian J. Experiment. Biol. 57: 908-915.
21. **Menut, C., Lamaty, G., Zollo, P.H.A., Kuiate, J.R. and Bessiere, J.M. (1993).** Aromatic plants of tropical Central Africa-part X: chemical composition of the essential oils of *Ageratum houstonianum* Mill. and *Ageratum conyzoides* L. from Cameroon. Flav. Fragr. J. 8: 1-4.
22. **Kurade, N.P., Jaitak, V., Kaul, V.K. and Sharma, O.P. (2010).** Chemical composition and antibacterial activity of essential oils of *Lantana camara*, *Ageratum houstonianum* and *Eupatorium adenophorum.* Pharm. Biol. 48: 539-544.
23. **Huong, L.T., Huong, T.T., Dai, D.N. and Ogunwande, I.A. (2022).** Chemical compositions, mosquito larvicidal and antimicrobial activities of essential oils of *Hedychium stenopetalum* Lodd. and *Hedychium villosum* Wall. of Zingiberaceae family from Vietnam. J. Essent. Oil Bearing Plants. 25(4): 924-938.
24. **Huong, T.H., Linh, L.D., Dai, D.N., and Ogunwande, I.A. (2022).** Essential oil compo-sitions and antimicrobial activity of the leaves and rhizomes of *Alpinia calcicola* Q. B. Nguyen & M.F. Newman from Vietnam. Chem. Nat. Prod. 58: 939-942.
25. **Huong, L.T., Hoi, T.N., Chung, N.T., Binh, T.D., Son, D.H., and Ogunwande,**

I.A. (2022). The leaf essential oil of *Vernonia solanifola* Benth*:* composition and antimicrobial activity. Chem. Nat. Prod. 58: 943-946.

26. **Thinh, B.B., Thanh, V.Q., Thin, D.B. and Ogunwande, I.A. (2022).** Chemical compositions and antimicrobial activity of the leaf essential oils obtained from the leaves and stems of *Schisandra perulata* Gagnep. J. Essent. Oil Bearing Plants. 25(4): 773-778.
27. **Huong, L.T., Chung, N.T., Chau, D.T.M., Dai, D.N., and Ogunwande, I.A. (2022)**. Annonaceae essential oils: antimicrobial and compositions of the leaves of *Uvaria hamiltonii* Hook. f. & Thoms. and *Fissistigma kwangsiensis* Tsiang & P. T. Li. Rec. Nat. Prod. 16(4): 387-392.
28. **National Institute of Science and Technology (2018).** NIST Chemistry Webbook. Data from NIST Standard Reference Database 69.