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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE LEAF ESSENTIAL OIL OF *Vernonia solanifolia*

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While working on a guide to new plants in Pu Hot Nature Reserve, Nghe An Province and adjacent areas, *Vernonia solanifolia* Benth. was collected that does not match any of the previously known *Vernonia* plants. The lack of information on the phytochemical constituents and biological studies on *V. solanifolia* led to this study. This article, reported for the first time the results of the investigation into the chemical constituents and antimicrobial activity of essential oil from the leaves of *V. solanifolia* grown in Vietnam. This is part of the on-going extensive research aimed at the characterization of the volatile constituents and antimicrobial activity of the poorly described species of Vietnamese flora [1–6].

The compositions and biological activities of essential oils from *Vernonia* plants grown in Vietnam [7, 8] and other parts of the world [9–11] have been reported. The essential oils were characterized by large contents of terpene compounds. The main constituents of *V. patula* leaf oil from Vietnam were β-caryophyllene, caryophyllene oxide, α-copaene, and α-humulene [7], whereas the leaf oil of *V. amygdalina* contained caryophyllene oxide and β-caryophyllene [8]. Moreover, caryophyllene oxide and humulene epoxide II predominate in the stem oil of *V. amygdalina* from Vietnam [8]. In previous studies, β-caryophyllene and germacrene D were the abundant compounds of *V. chalybaea* from Brazil [9], consistent with data obtained for *V. migeodii* from Nigeria [10]. However, the compositional pattern of essential oil from the leaves of *V. amygdalina* analyzed from Nigeria was dominated by α-muurolol [11]. In addition, the leaf essential oil of *V. patula* displayed antimicrobial activity toward *Enterococcus faecalis* ATCC299212 with a minimum inhibitory concentration (MIC) value of 8.67 μg/mL and anticandidal property against *Candida albicans* ATCC10231 with a MIC value of 15.99 μg/mL [7]. The antifungal and antioxidant activities of essential oils of *V. chalybaea* from Brazil were reported [9].

The leaves of *V. solanifolia* were collected from Hanh Dich Commune, Pu Hoat Nature Reserve, Nghe An Province (GPS: 19°44′32″N; 104°48′10″E) in August 2020, at an elevation of 816 m. The leaf sample was identified by Dr. L. T. Huong. A voucher specimen, LTH 776, was deposited at the Botany Museum, Nghe An College of Economics, Vietnam, for future reference. Two kilograms of a fresh leaf sample were prepared and pulverized for essential oil isolation. Essential oil was obtained by hydrodistillation in a Clevenger-type distillation unit designed according to an established specification [12], as described in previous studies [1–6].

The instrumental analysis of the essential oil was achieved using gas chromatography (GC), which was performed on an Agilent Technologies HP 7890 Plus gas chromatograph equipped with a flame ionization detector (FID) and fitted with an HP-5MS column (30 m × 0.25 mm, film thickness 0.25 μm; Agilent Technology). The analytical conditions were as described previously [1–6]. An Agilent Technologies HP 7890N Plus Chromatograph fitted with 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 this gas chromatography-mass spectrometry (GC-MS) experiment, under the same conditions as those used for the GC analysis as described above.

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TABLE 1. Constituents of Essential Oils from the Leaves of *Vernonia solanifolia*

Compound[a]	RI[b]	Composition, %	Compound[a]	RI[b]	Composition, %
α-Thujene	930	0.6	*cis*-β-Elemene	1403	0.2
α-Pinene	939	4.0	Cyperene	1418	0.1
Sabinene	980	49.2	β-Caryophyllene	1438	7.8
β-Pinene	984	1.0	α-Humulene	1471	0.9
Myrcene	992	1.6	9-*epi*-(*E*)-caryophyllene	1478	0.9
α-Phellandrene	1010	0.2	γ-Muurolene	1490	0.2
α-Terpinene	1022	1.1	Germacrene D	1499	3.2
o-Cymene	1030	0.4	(*E*,*E*)-α-Farnesene	1513	0.3
Limonene	1034	0.4	α-Muurolene	1514	0.4
β-Phellandrene	1036	1.6	γ-Cadinene	1532	0.3
(*Z*)-β-Ocimene	1038	0.3	Eugenol acetate	1533	0.1
(*E*)-β-Ocimene	1049	1.1	δ-Cadinene	1537	10.9
γ-Terpinene	1063	1.2	Scapanol	1594	0.1
cis-Terpinene hydrate	1074	0.3	*epi*-α-Muurolol	1661	0.1
Terpinolene	1094	0.4	Total		92.7
trans-Terpinene hydrate	1106	0.1	Monoterpene hydrocarbons		62.9
Geijerene	1151	0.2	Oxygenated monoterpenes		2.2
Terpinen-4-ol	1187	1.8	Sesquiterpene hydrocarbons		26.7
α-Copaene	1389	1.3	Oxygenated sesquiterpenes		0.9
β-Cubebene	1402	0.2			

[a]Elution order on HP-5MS column; [b]Retention indices on HP-5MS column.

TABLE 2. Antimicrobial Activity of the Leaf Essential Oil of *Vernonia solanifolia*

Microorganism	MIC, μg/mL	IC_{50}, μg/mL
Enterococcus faecalis ATCC299212	32.20 ± 0.10	64.0 ± 0.00
Staphylococcus aureus ATCC25923	33.56 ± 0.50	64.0 ± 0.00
Bacillus cereus ATCC14579	32.76 ± 0.10	64.0± 0.00
Pseudomonas aeruginosa ATCC27853	15.68 ± 0.50	32.0 ± 0.10
*Candida albican*s ATCC10231	16.33 ± 0.10	32.0 ± 0.00

Escherichia coli ATCC25922 and *Salmonella enterica* ATCC13076 – no activity.

The MS conditions were as follows: ionization voltage 70 eV; emission current 40 mA; acquisitions scan mass range of 35–350 amu and at a sampling rate of 1.0 scan/s. The identification of constituents from the GC-MS spectra of *V. solanifolia* was performed based on retention indices (RIs) determined with reference to a homologous series of *n*-alkanes (C_4–C_{40}), under identical experimental conditions. The mass spectral fragmentation patterns were checked with those of other essential oils of known composition [13].

The antimicrobial activity was measured by the determination of MIC and median inhibitory concentration (IC_{50}) values. This was achieved using the microdilution broth susceptibility assay as described previously [1–6]. Stock solutions of the oil were prepared in dimethylsulfoxide (DMSO). Dilution series (2^{14}, 2^{13}, 2^{12}, 2^{11}, 2^{10}, 2^9, 2^7, 2^5, 2^3, and 2^1 μg/mL) were prepared in sterile distilled water inside the micro-test tubes from where they were transferred separately to 96-well microtiter plates. Bacteria grown in double-strength Mueller–Hinton broth or double-strength tryptic soy broth, and fungi sustained in double-strength Sabouraud dextrose broth, were standardized to 5×10^5 and 1×10^3 CFU/mL, respectively. DMSO was used as a negative control. Streptomycin was used as the antibacterial standard for Gram-positive bacteria, where nystatin and cycloheximide were used as Gram-negative and antifungal standards, respectively. All experiments were performed in triplicate. After incubation at 37°C for 24 h, the MIC values were determined as the lowest concentration of essential oils of *V. solanifolia*, which completely inhibited the growth of the microorganisms. The IC_{50} values were determined by the percentage of

microorganism inhibited growth based on the turbidity measurement data of an EPOCH2C spectrophotometer (BioTeK Instruments, Highland Park, Winooski, VT, USA) and Rawdata computer software (Belgium).

The average yield of the essential oil was 0.35% (v/w, ± 0.01). The essential oil was colored light yellow. Thirty-four compounds, which accounted for 91.1% of the total content, were identified in the essential oil. The classes of compounds occurring in higher amounts were monoterpene hydrocarbons (62.9%) and sesquiterpene hydrocarbons (26.7%), as seen in Table 1. The oxygenated terpenes were identified in much lower amounts of 2.2% and 0.9% for oxygenated monoterpenes and oxygenated sesquiterpenes respectively. The main constituents of the leaf oil were sabinene (49.2%), δ-cadinene (10.9%), β-caryophyllene (7.8%), α-pinene (4.0%), and germacrene D (3.2%). It could be seen that monoterpenes and sesquiterpenes were the main constituents of the essential oil of *V. solanifolia*, which is consistent with data reported for other *Vernonia* oil samples analyzed from Vietnam [7, 8] and other parts of the world [9–11]. Interestingly, these terpene compounds differed from one another and from species to species. On a chemotaxonomic scale [11], the leaf essential oil of *V. solanifolia* can be classified into a group with an abundance of monoterpene hydrocarbons.

Essential oil from the leaf of *V. solanifolia* displayed antimicrobial activity toward four of the tested microorganisms, and anticandidal activity, with the MIC values < 50 µg/mL (Table 2). The essential oil exhibited antimicrobial activity against *Pseudomonas aeruginosa* ATCC27853 with an MIC value of 15.68 µg/mL, and anticandidal action toward *Candida albican*s ATCC10231 with an MIC value of 16.33 µg/mL). These values are twice as active than MIC values of 32.20 µg/mL, 33.56 µg/mL, and 32.76 µg/mL recorded against *Enterococcus faecalis* ATCC299212, *Staphylococcus aureus* ATCC25923, and *Bacillus cereus* ATCC14579 respectively. The MIC and IC_{50} values provided evidence that the leaf oil of *V. solanifola* displayed potent antimicrobial and anticandidal activities against *P. aeruginosa*, *C. albicans*, *E. faecalis*, *S. aureus*, and *B. cereus.* Recent findings indicated that substances with MIC values ≤ 100 µg/mL were considered to have good antimicrobial activity [14]. Thus, the essential oil of *V. solanifolia* should be considered a promising antimicrobial agent because the essential oil displayed activity at MIC < 50 µg/mL. However, the leaf oil exhibited no antimicrobial action against *Escherichia coli* ATCC25922 and *Salmonella enterica* ATCC13076. Streptomycin, the standard antimicrobial agent for Gram-positive bacteria, displayed antimicrobial activity with MIC values within the range 0.28 µg/mL to 3.20 µg/mL. In addition, nystatin, used as a standard antimicrobial agent for Gram-negative bacteria, had an MIC value of 8.0 µg/mL, with cycloheximide, an anticandidal agent, showing activity at an MIC of 3.20 µg/mL.

To our knowledge, this is the first report on the antimicrobial activity of the essential oil of *V. solanifolia*, and the results in this study are comparable with data on the antimicrobial potential of other *Vernonia* essential oils reported in the literature. For example, *V. patula* leaf oil from Vietnam like *V. solanifolia* inhibited the growth of some of the tested microorganisms, except for *E. coli* and *S. enterica* [7]. *V. chalybaea* could only inhibit *Trichophyton rubrum* but not *C. albicans* [9]. The antimicrobial activities of the essential oil of *V. solanifolia* can be related to its main compounds or some synergy between the major and minor compounds. These compounds exhibit activity by first destroying the microbial cytoplasmic wall to enhance permeability and the passage of large protons and ions [15]. Nevertheless, the antibacterial effect can be summed up as the cumulative actions of several compounds and not to a specific compound [15]. Further, owing to the complexity of the composition of the essential oils, it is also difficult to explain the mechanism of action of these blends, but it is important to underline that the wide variety of compositions is a positive factor that may limit the development of resistance, which is otherwise very common for synthetic drugs [16]. The major constituents of the essential oil of *V. solanifolia* have shown antimicrobial activity against *E. faecalis*, *S. aureus*, *B. cereus*, and *C. albicans* [1–6, 9, 17], and likely account for the observed antimicrobial activity. In light of this, essential oils may represent a valid alternative to avoiding the multidrug resistance of many pathogens, or they could be used in combination with antimicrobials to improve their effectiveness against different infectious diseases.

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REFERENCES

1. L. T. Huong, L. N. Sam, B. D. Thach, D. N. Dai, and I. A. Ogunwande, *Chem. Nat. Compd.*, **57**, 574 (2021).
2. L. T. Huong, L. D. Linh, and I. A. Ogunwande, *J. Essent. Oil Bearing Plants*, **24**, 201 (2021).
3. L. T. Huong, N. T. Viet, L. Y. Sam, C. N. Giang, N. H. Hung, D. N. Dai, and I. A. Ogunwande, *Bol. Latinoam. Caribe Plantas Med. Aroma*., **20**, 81 (2021).
4. B. B. Thinh, R. V. Doudkin, L. D. Chac, H. V. Chinh, N. T. M. Hong, and I. A. Ogunwande, *J. Essent. Oil Bearing Plants*, **24**, 461 (2021).
5. L. N. Sam, L. T. Huong, P. N. Minh, B. T. Vinh, D. N. Dai, W. N. Setzer, and I. A. Ogunwande, *J. Essent. Oil Bearing Plants*, **23**, 803 (2020).
6. N. T. Chung, L. T. Huong, and I. A. Ogunwande, *Rec. Nat. Prod*., **14**, 372 (2020).
7. T. M. Hoi, N. T. Chung, L. T. Huong, and I. A. Ogunwande, *J. Essent. Oil Bearing Plants*, **24**, 500 (2021).
8. T. D. Ngoc and M. V. T. Ha, *Am. J. Essent. Oil Nat. Prod*., **9**, 39 (2021).
9. A. C. N. Sobrinho, S. M. de Morais, E. B. de Souza, M. R. S. Albuquerque, H. S. dos Santos, C. S. de Paula Cavalcante, H. A. de Sousa, and R. O. dos Santos Fontenelle, *Braz. Arch. Biol. Technol*., **63**, e20190177 (2020).
10. A. O. Ogunbinu, S. O. Okeniyi, I. A. Ogunwande, G. Flamini, and P. L. Cioni, *J. Essent. Oil Res*., **21**, 246 (2009).
11. A. O. Ogunbinu, I. A. Ogunwande, G. Flamini, P. L. Cioni, and S. O. Okeniyi, *Nat. Prod. Commun*., **4**, 421 (2009).
12. *Vietnamese Pharmacopoeia*, Medical Publishing House, Hanoi, Vietnam, 2009.
13. National Institute of Science and Technology, Chemistry Web Book Data, Data from NIST Standard Reference Database 69 (2011).
14. F. B. Holetz, G. L. Pessini, N. R. Sanches, D. A. G. Cortez, C. V. Nakamura, and B. P. Dias-Filho, *Mem. Inst. Oswaldo Cruz*., **97**, 1027 (2002).
15. M. Ghavam, M. L. Manca, M. Manconi, and G. Bacchetta, *Sci. Rep*., **10**, 15647 (2020).
16. N. Salem, S. Kefi, O. Tabben, A. Ayed, S. Jallouli, and N. Feres, *Ind. Crops Prod*., **124**, 115 (2018).
17. G. Lang and G. Buchbaeur, *Flavour Frag. J*., **27**, 13 (2012).