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Research Article

Rhizome essential oil of *Alpinia pinnanensis* from Vietnam: Chemical composition and *in vitro* evaluation of antimicrobial and cytotoxic activities

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Abstract

Alpinia pinnanensis T.L.Wu & S.J.Chen, a species in the Zingiberaceae family, was identified and described from Phu Tho Province, Vietnam. In this study, the essential oil (EO) was extracted from *A. pinnanensis* rhizomes using hydrodistillation, and its phytochemical constituent was analyzed by gas chromatography-mass spectrometry (GC-MS). The main ingredients identified were *β*-myrcene (18.72%), farnesol (12.17%), *β*-linalool (11.91%), and 1,8-cineole (8.82%). The EO exhibited weak antimicrobial activity against *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Candida albicans* with minimum inhibitory concentration (MIC) values ranging from 128 to 256 µg/mL, comparable to positive controls, streptomycin and cycloheximide. Additionally, the EO exhibited cytotoxic effects against HeLa (human cervical carcinoma) and HepG2 (human hepatocellular carcinoma) cell lines, with IC50 values of 8.50 ± 0.29 µg/mL and 7.84 ± 0.15 µg/mL, respectively. This is the first study to report on the phytochemical composition, antimicrobial, and cytotoxic effects of the EO from *A. pinnanensis* rhizomes.

Keywords

Alpinia pinnanensis, essential oil, GC-MS, antimicrobial activity, cytotoxicity

Introduction

Alpinia Roxb. is the largest genus in the Zingiberaceae family, comprising around 500 species (Youn et al. 2024). This genus is primarily distributed across tropical and subtropical regions of Asia and Oceania (Van et al. 2021). *Alpinia* species are perennial herbaceous plants that typically grow to a height of 2–4 meters, although some species can reach up to 12 meters (Smith 1990; Van et al. 2021). Several species within this genus are of considerable ethnomedicinal and culinary importance in countries such as China, India, Japan, and Vietnam (Zhang et al. 2016; Van et al. 2021).

Traditionally, different parts of *Alpinia* plants have been utilized in the treatment of gastrointestinal ailments, including indigestion, abdominal pain, nausea, and intestinal parasitic infections (Itokawa et al. 1985; Roy and Swargiary 2009). Phytochemical analyses have identified a wide range of bioactive metabolites in *Alpinia* species, including terpenoids, phenylpropanoids, diarylheptanoids, flavonoids, and lignans (Zhang et al. 2016). Notably, *Alpinia* is also recognized for its aromatic properties, with various plant parts like fruits, seeds, leaves, rhizomes, roots, shoots, stems, pseudostems, inflorescences, flowers, and petals producing essential oils (EOs) (Van et al. 2021). These EOs are predominantly composed of oxygenated monoterpenes, monoterpene hydrocarbons, and oxygenated sesquiterpenes (Dũng et al. 1994; Hung et al. 2018).

Many of such compounds have been demonstrated to possess significant bioactivities, including anticancer (Chun et al. 1999; Samarghandian et al. 2014), anti-ulcerogenic (Al- Yahya et al. 1990), antimicrobial (Niyomkam et al. 2010; Rao et al. 2010), hypoglycemic (Rajasekar et al. 2014), anti-nausea (Shin et al. 2002; Yang et al. 2002), cardioprotective (Chang et al. 2013), neuroprotective (Li et al. 2013; Shi et al. 2015), and anxiolytic activities (De Sousa et al. 2015).

Alpinia pinnanensis T.L.Wu & S.J.Chen, a herbaceous plant reaching approximately 1.5 meters in height, features lanceolate leaves with distinctive golden trichomes (Huong et al. 2017). Previous phytochemical investigations of this species have led to the identification of diarylheptanoids, flavonoids, and triterpenoids (Giang et al. 2005). EOs have also been extracted from various parts of *A. pinnanensis*, including its leaves, stems, root bark, and fruits (Huong et al. 2017). However, the chemical composition of the EO derived specifically from the rhizomes of *A. pinnanensis* has not been reported. This study was therefore conducted to analyze the chemical constituents of EO from *A. pinnanensis* rhizomes and to assess its *in vitro* antimicrobial and cytotoxic activities.

Materials and methods

Plant materials

The fresh rhizomes of *A. pinnanensis* were collected from Kiet Son commune (21°15'31.5"N, 104°56'12.6"E), Tan Son district, Phu Tho province, Vietnam, in May 2023. The plant was identified by Assoc. Prof. Dr. Nguyen Hoang Tuan (Faculty of Pharmacognosy and Traditional Medicine, Hanoi University of Pharmacy, Vietnam), and a voucher specimen (AP-0523) was deposited at the Laboratory of the Department of Chemistry, Vinh University, Nghe An Province, Vietnam.

Preparation of essential oil

The rhizomes (350 g) of *A. pinnanensis* were hydro-distilled for 3 h (beginning from the water boiling point) using a Clevenger-type apparatus, according to the Vietnamese Pharmacopoeia (Committee of Vietnamese Pharmacopoeia 2017). Then, the obtained EO was removed from all water traces with Na_2SO_4 and stored in sealed glass vials at 4 °C before ananlysis.

Gas chromatography-mass spectrometry (GC-MS) analysis

The phytochemical component of the EO extracted from *A. pinnanensis* was analyzed using GC-MS. The analysis was conducted on an Agilent GC-7980 system coupled with an Agilent MS 5977C mass spectrometer operating in electron ionization (EI) mode. Separation was achieved using an HP-5MS UI column (30 m × 0.25 mm i.d. × 0.25 µm film thickness; Agilent Technologies). Helium was employed as the carrier gas at a flow rate of 1.0 mL/min. The injection volume was 1 µL with a split ratio of 20:1. The oven temperature program started at 60 °C (held for 3 minute), increased at a rate of 3 °C/min to 180 °C, then rised to 240 °C at a rate of 5 °C/min, and was held at this final temperature for 5 minutes. The quadrupole temperature was 150 °C, and ionization energy was 70 eV. Mass spectra were acquired in the range of 50–550 amu with a scan rate of 2.0 scans/second. Identification of individual components was achieved by comparing the acquired mass spectra with those in the NIST17 library, followed by confirmation through comparison of retention indices relative to a homologous series of n-alkanes. Quantification of the constituents was based on the relative percentage of peak areas.

Assessment of antimicrobial assay

The antimicrobial property of the EO extracted from *A. pinnanensis* rhizomes was evaluated against Gram-positive bacterial strains (*Enterococcus faecalis* ATCC 299212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579), Gram-negative bacterial strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* ATCC 13076), and a pathogenic yeast (*Candida albicans* ATCC 10231). All microbial strains were purchased from the National Institute for Food Control (Hanoi, Vietnam). The antibacterial and antifungal activities of the EO were measured using Andrews's method (Andrews 2001). Stock solutions of the EO were prepared in 1% DMSO. Briefly, the bacterial and yeast suspensions were adjusted to a concentration of approximately 2×10^5 CFU/mL. A 50 µL aliquot of each microbial suspension was inoculated into Luria-Bertani medium containing different concentrations of the EO (256 µg/mL, 128 µg/mL, 64 µg/mL, 32 µg/mL, 16 µg/mL, 8 µg/mL, 4 µg/mL, and 2 µg/mL), as well as EO-free control solutions. The mixtures were incubated at 37 °C for 24 hours. The antimicrobial activities of the EO were determined by the Minimum Inhibitory Concentration (MIC), defined as the lowest concentration of the EO that completely inhibited microbial growth after 24 hours of incubation. Streptomycin was used as a positive control for bacterial strains, and cycloheximide was used for the yeast strain. All experiments were conducted in triplicate.

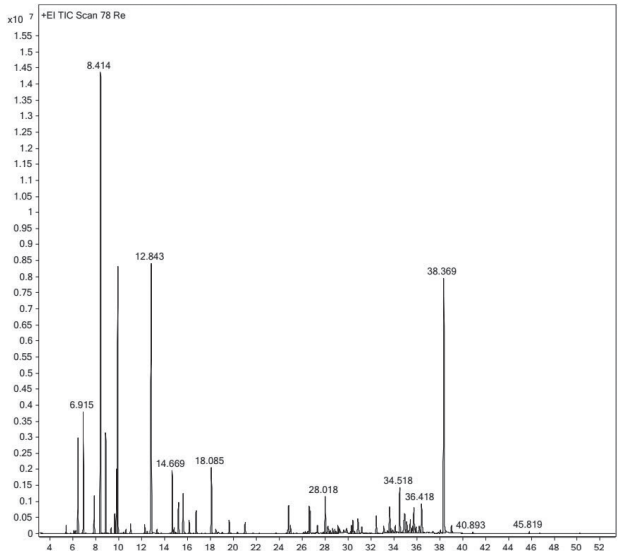

Figure 1. The GC chromatogram of *A. pinnanensis* rhizome essential oil.

Assessment of cytotoxicity assay

The cytotoxic properties of EO extracted from *A. pinnanensis* rhizomes were assessed against HeLa (human cervical carcinoma) and HepG2 (human hepatocellular carcinoma) cell lines using the Sulforhodamine B (SRB) assay, as previously described (Diep et al. 2023). In brief, the cells were seeded in 96-well plates and treated with EOs dissolved in 10% dimethyl sulfoxide (DMSO) at various concentrations. Following incubation, cells were fixed with trichloroacetic acid, stained with SRB, and the optical density (OD) was measured at 540 nm using a microplate reader. Ellipticine served as the positive control.

The percentage of cell growth inhibition was calculated using the formula:

$$(\%)\ \textbf{inhibition} = 100\% - [(\mathbf{OD}_{\text{sample}} - \mathbf{OD}_{\text{day 0}})/(\mathbf{OD}_{\text{blank control}} - \mathbf{OD}_{\text{day 0}})] \times 100$$

Experiments were performed in triplicate to ensure reliability. IC_{50} values (the concentration of the EO required to inhibit 50% of cell growth) were determined using TableCurve 2Dv4 software.

Results and discussion

The phytochemical constituent of the rhizome EO was analyzed using GC-MS, identifying a total of 55 volatile components, which accounted for 96.13% of the total oil content (Fig. 1, Table 1). The primary chemical classes in the rhizome oil included monoterpene hydrocarbons (33.02%), oxygenated monoterpenes (31.36%), and oxygenated sesquiterpenes (23.13%). The major constituents (≥ 5%) were *β*-myrcene (18.72%), farnesol (12.17%), *β*-linalool (11.91%), and 1,8-cineole (8.82%). Additionally, several prominent compounds (≥ 2%) were identified, including camphene (3.46%), α-phellandrene (3.09%), *α*-pinene (2.63%), fenchyl acetate (2.53%), camphor (2.39%), and *α*-limonene (2.22%).

Comparatively, EOs derived from other parts of *A. pinnanensis* exhibited distinct chemical profiles. The leaf oil was dominated by 1,8-cineole (20.5%), 4-phenyl-2-butanol (19.5%), and *α*-phellandrene (10.8%). The stem oil primarily contained 1,8-cineole (10.0%), *β*-elemene (8.7%), *α*-gurjunene (7.6%), *β*-pinene (7.3%), and (E,E)-farnesol (7.2%). The root oil featured (E,E)-farnesol (8.4%), *α*-gurjunene (6.2%), camphene (5.6%), fenchyl acetate (5.4%), linalool (4.6%), and *β*-pinene (4.6%). In contrast, the fruit oil was characterized by *α*-cadinol (18.1%) and *β*-caryophyllene (11.4%), along with (E,E)-farnesol (6.3%), *β*-pinene (6.1%), *β*-elemene (5.6%), and *α*-pinene (5.1%) (Huong et al. 2017). This analysis highlights the diversity of chemical compositions in EOs from different parts of *A. pinnanensis*, with each exhibiting a unique profile of major and minor constituents.

The oil sample was then evaluated for its antimicrobial activities against several bacterial and fungal strains using the broth microdilution method, with streptomycin and cycloheximide as positive controls (Table 2). For bacterial strains, the EO demonstrated variable inhibitory effects. No antimicrobial activity was observed against *E. faecalis*, *S. aureus*, *E. coli*, and *S. enterica*, as evidenced by the lack of inhibition at the tested concentrations. In contrast, *B. cereus* showed a MIC of 128 μg/mL, indicating moderate antibacterial activity. *P. aeruginosa* exhibited MIC values of 256 μg/mL, suggesting relatively weak antibacterial effects of the EO. Regarding antifungal activity, the EO exhibited weak inhibition of *C. albicans*, with a MIC of 256 μg/mL, indicating a limited antifungal potential. The relatively low antimicrobial activity of EO from *A. pinnanensis* rhizomes can be attributed to its chemical composition. Previous studies have indicated that EOs rich in aldehydes or phenols exhibit the strongest antibacterial activity, followed by those containing terpene alcohols (Bassolé and Juliani 2012; Hamad et al. 2016). In contrast, EOs containing ketones, esters, or acetates generally display weaker antimicrobial effects, while oils primarily composed of terpene hydrocarbons are often inactive (Kalemba and Kunicka 2003; Bassolé and Juliani 2012; Hamad et al. 2016; Lis et al. 2017). The EO from *A. pinnanensis* rhizomes contains a high proportion of terpene hydrocarbons and terpene alcohols, both of which exhibit relatively low antimicrobial activity. This explains the observed weak antibacterial effects of the oil against the tested bacterial strains.

The cytotoxic effects of the EO of *A. pinnanensis* rhizomes on HepG2 and HeLa cancer cell lines were evaluated. The results in Table 3 demonstrate that this EO exhibited activity on all evaluated cell lines. Specifically, the IC_{50} values of the EO of *A. pinnanensis* rhizomes for HepG2 and HeLa cell lines were 7.84 ± 0.15 and 8.50 ± 0.29 μg/mL, respectively. The cytotoxic effects of this EO are likely attributed to its major components. Previous studies have shown that *β*-myrcene inhibits the invasion of metastatic MDA-MB-231 human breast cancer cells by suppressing TNFα-mediated NF-*κ*B activation, achieved through the inhibition of IKK, which subsequently downregulates MMP-9 expression (Lee et al. 2015). Furthermore, *β*-myrcene demonstrated significant antiproliferative activity against A549 lung cancer cells by activating apoptosis via mitochondria-mediated cell death signaling and inducing oxidative stress (Bai and Tang 2020).

Table 1. Chemical compositions of essential oil distilled from *A. pinnanensis* rhizomes.

No.	RT (min)	Compounds	RI (cal.)	RI (lit.)	Concentration (%)
1	5.399	2-Heptanol	900	901	0.21
2	6.085	Tricyclene	925	925	0.09
3	6.229	α-Thujene	930	929	0.07
4	6.446	α-Pinene	937	937	2.63
5	6.915	Camphene	952	952	3.46
6	7.859	β-Pinene	979	979	1.14
7	**8.414**	**β-Myrcene**	**993**	**991**	**18.72**
8	8.866	α-Phellandrene	1005	1005	3.09
9	9.336	α-Terpinene	1018	1017	0.20
10	9.645	p-Cymene	1027	1025	0.64
11	9.811	α-Limonene	1031	1030	2.22
12	**9.914**	**1,8-Cineole**	**1034**	**1032**	**8.82**
13	10.617	(E)-β-Ocimene	1052	1049	0.13
14	11.035	γ-Terpinene	1062	1060	0.32
15	12.271	Terpinolene	1089	1088	0.31
16	12.483	2-Nonanone	1093	1092	0.08
17	**12.843**	**β-Linalool**	**1101**	**1099**	**11.91**
18	13.335	Fenchol	1114	1113	0.15
19	14.669	Camphor	1046	1045	2.39
20	15.212	Isoborneol	1158	1157	1.20
21	15.613	Borneol	1167	1166	1.63
22	16.151	Terpinen-4-ol	1178	1177	0.51
23	16.751	α-Terpineol	1190	1189	0.87
24	18.085	Fenchyl acetate	1221	1223	2.53
25	18.462	Neryl alcohol	1230	1228	0.25
26	19.635	Linalyl acetate	1257	1257	0.57
27	21.026	Isobornyl acetate	1287	1286	0.53
28	24.819	α-Copaene	1376	1376	1.28
29	24.991	Daucene	1380	1381	0.36
30	26.622	β-Caryophyllene	1418	1419	1.10
31	26.685	α-Santalene	1420	1420	0.68
32	27.337	α-Bergamotene	1436	1435	0.27
33	28.018	α-Caryophyllene	1453	1454	1.52
34	28.247	(E)-β-Famesene	1459	1457	0.53
35	28.670	4,5-di-epi-aristolochene	1469	1469	0.19
36	29.134	γ-Himachalene	1480	1477	0.30
37	29.878	β-Dihydroagarofuran	1497	1496	0.24
38	30.307	β-Bisabolene	1508	1509	0.35
39	30.433	β-Curcumene	1511	1514	0.63
40	30.868	δ-Cadinene	1523	1524	0.87
41	31.205	(E)-γ-Bisabolene	1532	1533	0.25
42	32.470	Nerolidol	1565	1564	0.69
43	33.122	β-Caryophyllene epoxide	1581	1581	0.28
44	33.631	Carotol	1594	1594	1.15
45	34.123	Humulene epoxide 2	1607	1606	0.31
46	34.518	epi-Cedrol	1618	1618	1.98
47	34.953	γ-Eudesmole	1630	1631	1.56
48	35.251	Isospathulenol	1638	1638	0.10
49	35.434	Selina-3,11-dien-6α-ol	1643	1642	0.77
50	35.531	δ-Cadinol	1646	1645	0.24
51	35.646	β-Eudesmol	1649	1649	0.31
52	35.749	Isoamyl geranate	1652	1650	1.34
53	36.418	Epi-β-bisabolol	1670	1670	1.67
54	**38.369**	**Farnesol**	**1724**	**1722**	**12.17**
55	39.039	Farnesal	1743	1740	0.32
		Monoterpene hydrocarbons (No. 2–11, 13–15)			33.02
		Oxygenated monoterpenes (No. 12, 17–27)			31.36
		Sesquiterpene hydrocarbons (No. 28–36, 38–41)			8.33
		Oxygenated sesquiterpenes (No. 37, 42–55)			23.13
		Others (No. 1, 16)			0.29
		Total			**96.13**

RT (min): Retention time (min).
RI (cal.): Retention indices calculated from retention times in relation to those of a series of C_7-C_{30} n-alkanes.
RI (lit.): Retention indices taken from the literature.

At a concentration of 50 nM, *β*-myrcene also inhibited HeLa cell proliferation by increasing the cell doubling time and significantly altering cell motility, which suggests cell cycle arrest and reduced invasion capacity (Pincigher et al. 2023). Similarly, farnesol has been recognized for its anti-neoplastic effects in various cancers, including prostate, breast, lung,

Table 2. Antimicrobial activity of the essential oil of the *A. pinnanensis* rhizomes.

Microorganisms	MIC (µg/mL)		
	EO	Streptomycin	Cycloheximide
E. faecalis ATCC 299212	-	256	NT
S. aureus ATCC 25923	-	128	NT
B. cereus ATCC 14579	128	128	NT
E. coli ATCC 25922	-	32	NT
P. aeruginosa ATCC 27853	256	256	NT
S. enterica ATCC 13076	-	128	NT
C. albicans ATCC 10231	256	NT	32

Note: EO: Essential oil; "-": No activity; "NT": Not tested.

Table 3. Cytotoxic activity of the essential oil from *A. pinnanensis* rhizomes.

Samples	Half-maximal inhibitory concentration (IC_{50}, µg/mL)	
	HepG2	HeLa
EO	7.84 ± 0.15	8.50 ± 0.29
Ellipticine	0.33 ± 0.03	0.35 ± 0.02

EO: Essential oil; HeLa: Human cervical adenocarcinoma cells; HepG2: Human hepatocellular carcinoma; Ellipticine: Positive control; Data are shown as mean ± standard deviation (n = 3).

and pancreatic cancers and multiple myeloma, by inhibiting cell proliferation *in vitro* and suppressing tumor growth *in vivo* (Jung et al. 2018). *β*-linalool, another major component of *A. pinnanensis* rhizome EO, also exhibited cytotoxic effects in human breast cancer cells (MCF-7, MDA-MB-231, T-47D, A549) (Chang and Shen 2014; Rodenak-Kladniew et al. 2020; Elbe et al. 2022), human hematopoietic malignancies (Kasumi-1, HL-60, Molt-4, H-9), lymphoma (Raji) (Gu et al. 2010), human oral cancer cells (OECM 1) (Pan and Zhang 2019), human colon cancer cells (SW 620) (Chang and Shen 2014), and human hepatocellular carcinoma (HepG2) (Chang and Shen 2014). Lastly, 1,8-cineole exhibited antiproliferative effects on human colon cancer cell lines (HCT116 and RKO) (Murata et al. 2013) and human breast cancer cells (A549) (Rodenak-Kladniew et al. 2020).

Conclusion

This study presents the first comprehensive analysis of the chemical composition, antimicrobial properties, and cytotoxic activity of EO extracted from the rhizomes of *Alpinia pinnanensis* collected in Phu Tho Province, Vietnam. The chemical profile of the EO was extensively characterized, revealing weak antimicrobial activity. However, the oil exhibited notable cytotoxic effects against HeLa and HepG2 cancer cell lines. These results provide valuable insights into the potential therapeutic applications of *A. pinnanensis* EO.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

The authors declared that no experiments on animals were performed for the present study.

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Author contributions

Conceptualization, N.T.T., T.V.C., L.D.G., H.T-T., and V.T.N.; methodology, V.T.N., N.T.V. and L.D.G..; software, H.T-T and T.V.C; investigation, N.T.T., L.D.G., H.T-T., L.T.G.A., and N.T.V., writingoriginal-draft preparation, V.T.N., N.T.T., H.T-T.; writingreview and editing N.T.T., H.T-T., and V.T.N.; visualization, N.T.T., H.T-T., and L.T.G.A.; supervision, H.T-T and V.T.N.

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Data availability

All of the data that support the findings of this study are available in the main text.

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