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RESEARCH ARTICLE

Research on chemical constituents, anti-bacterial and anti-cancer effects of components isolated from *Zingiber officinale* Roscoe from Vietnam

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Abstract

Ginger, a commonly used spice and medicinal herb, is an abundant source of bioactive compounds. However, the utilization of ginger in the pharmaceutical industry is still moderate and not commensurate with the potential of the Vietnamese horticulture industry, mainly due to the lack of information about the quality of input materials. In this study, we compared the volatile compounds of gingers collected from 13 provinces of Vietnam using GC/MS and GC-FID analysis to provide a basis for selecting and standardizing input materials. Furthermore, ginger essential oil from Ben Tre province of Vietnam exhibited significant antibacterial activity particularly against Gram-positive bacteria, including *S. aureus* and *S. epidermidis,* with inhibition zones of 30.00 ± 1.41 and 24.67 ± 3.30 mm, respectively. However, no significant inhibition was observed against Gram-negative bacteria, *P. aeruginosa* and *E. coli*. We also isolated 5 non-volatile compounds from ginger extract, namely 6-shogaol (1), quercetin (2), rutin (3), β-sitosterol (4), and β-sitosterol-3-O-β-D-glucopyranoside (5). Among these, compounds 1–3 displayed cytotoxicity against Hep3B, SK-LU-1, MCF-7, SK-LU-1, SW480, and HepG2 tumour cell lines, with IC_{50} values ranging between 62.7 ± 2.1 and 97.6 ± 1.1 µM, using Ellipticine as a positive control. Compounds 4 and 5 showed cytotoxicity against Hep3B and HepG2 tumor cells, with the IC_{50} values ranging between 21.5 ± 5.1 and 46.9 ± 3.7 µM but did not exhibit any significant cytotoxicity against SW480 and SK-LU-1 cells. Compound 4 also demonstrated middling cytotoxicity against the MCF7 cell lines, with an IC_{50} value of 43.6 ± 5.1 µM. These findings suggest further applications of Vietnamese ginger for the treatment of infectious and cancer-related diseases.

Keywords

Zingiber officinale; 6-shogaol; antimicrobial activities; anti-cancer activities; cytotoxic

Introduction

Zingiber officinale Roscoe, commonly known as ginger, belongs to the Zingeberaceae family (1, 2). This plant is widely used as a spice in East and Southeast Asian culinary cultures and is also valued for its medicinal properties, offering various health benefits (3). Ginger has been traditionally

utilized to alleviate gastrointestinal complications, nausea, common colds, kidney stones, heart disease, and fever (4, 5). Moreover, some research indicates that ginger extracts possess antimicrobial, anti-inflammatory, and anticancer properties, making it a potential source of bioactive compounds for treating infectious and cancer-related diseases (6-8). At the molecular level, ginger contains numerous compounds with biological effects, both volatile and non-volatile. Monoterpenes (such as pinene, camphene, eucalyptol, curcumene, etc.) are characterized by isoprene units or isoprene linked with cyclopropane, cyclohexane, cyclobutane, and have been shown to exhibit antipancreatitis, neuroprotective, anxiolytic, and gastroprotective when used as essential oil or flavour (9, 10). Phenolic compounds belong to the non-volatile compounds found in ginger extract. They are characterized by the presence of hydroxyl groups and are well-known for their antioxidant activity. Among these compounds, shogaols are one of the main bioactive compounds of ginger. They are produced through the conversion of gingerols at a suitable temperature and time during extraction, which has been shown to suppress the growth of cancer cells and exhibit antinflammatory properties. Furthermore, shogaols have shown potential effects against COVID-19 (11-13). β-sitosterol belongs to phytosterols group and is found in numerous plants. It has been shown in many studies to possess anticancer, inflammatory properties, as well as a protective effect against NAFLD and respiratory diseases. Additionally, it has shown hepatoprotective properties, among others (14).

Recently, ginger and its derivatives have shown great potential and have been used as functional foods due to their ability to reduce the risk of many diseases, as demonstrated at the molecular level *in vitro* and *in vivo*. There is a growing body of evidence supporting the health benefits of ginger. However, utilization of ginger in the pharmaceutical and food industries is still moderate and not commensurate with its potential, mainly due to a lack of information on the quality of input material, especially for gingers grown in different regions of Vietnam.

Additionally, ginger is one of the strategic crops cultivated for centuries in Vietnam and contributed 5 million US dollars to Vietnamese economy in 2018 (15). According to Van et al. (2019), ecological conditions can affect the chemical composition of crops, leading to variations in raw material quality (16). In order to establish a comprehensive database for the application and selection of ginger in the pharmaceutical and food industries, we conducted a study. This study involved investigating the volatile compounds of ginger from 13 locations in Vietnam and examining the anti-bacterial activity of essential oil, as well as the anti-cancer effect of five non-volatile compounds isolated from ginger.

Materials and Methods

Reagents, chemicals, and plant materials

Methanol (MeOH), and distilled water used for TLC were supplied by Fisher Scientific Korea Ltd. (Korea). Analytical grade MeOH, n-hexane, ethanol (EtOH), n-hexane and ethyl acetate (EtOAc) were provided by Chemsol, Vietnam. TLC was supplied by Merck (Germany).

Specimens of ginger (*Zingiber officinale*) were collected from 13 locations in western provinces of Vietnam, including Vinh Long, Bac Lieu, Tien Giang, An Giang, Long An, Can Tho, Ben Tre, Hau Giang, Tra Vinh, Dong Thap, Kien Giang and Soc Trang in December 2021. The specimens were identified by Dr. Nguyen Ngoc Tuan – Institute of Food and Biotechnology, Industrial University of Ho Chi Minh City. Voucher specimens have been deposited in the Department of Pharmaceutical Biochemistry, Institute of Applied Materials Science, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam.

Extraction of ginger essential oils by steam distillation:

Ginger essential oils were extracted using the steam distillation method. After harvesting, the gingers were naturally dried until their moisture content was reduced by 50%. The dried gingers were then cut into 1-2 cm pieces and placed in a flask connected to a Clevenger essential oil distillation system. Steam distillation was conducted using a ratio of 1:6 (g:mL) of ginger to water, and the process lasted for 2 hours. The obtained essential oil was collected and treated with anhydrous sodium sulphate (Na_2SO_4) to remove any remaining water. This ensures that the essential oil is anhydrous and free from moisture. The steam distillation method allows for the efficient extraction of ginger essential oils and can provide valuable samples for further analysis and testing. It is essential to follow proper safety protocols and guidelines during the extraction process.

Determination of chemical compositions of essential oils of gingers:

The chemical compositions of ginger essential oils were determined using GC-MS and GC-FID analysis. The GC-MS system used was an HP7890A model GC (Agilent Technologies, Santa Clara, CA, USA) connected with an HP5975C MS detector for vaporized chromatogram operation. The stationary phase separation was achieved using an HP5 MS column (60 m × 0.25 mm, film thickness 0.25 µm) (Agilent Technologies, US). The emission current was set at 40 mA, the electron impact ionization voltage was 70 eV, and the acquisitions scan mass range was 35-450 amu. Helium was used as carrier gas for GC program. The temperature of injector was set at 250°C, and the injection volume was 1 µL with a split ratio of 100:1 for the samples. The temperature operation started at 60°C and elevated to 240°C with speed of increasing 4°C/min. For GC -FID analysis, the operating program was implemented with analogous running conditions as GC-MS operating program.

The percentage of composition of the ginger essential oil was measured based on the peak area of GC-FID. Comparing the retention time with mass spectra with W09N08, HPCH1607 mass spectral libraries, and the data of NIST Chemistry WebBook used for identifying the constituents obtained in each samples.

Determination of antibacterial activity of essential oil:

Four bacterial strains were used to determine antibacterial activity: *Staphylococcus epidermidis* (ATCC 12228), *Pseudomonas aeruginosa* (ATCC 15442), *Staphylococcus aureus* (ATCC 23235), and *Escherichia coli* (ATCC 11229). All of these bacterial strains were preserved in the Biotechnology Laboratory of Saigon Hi-Tech Park in Ho Chi Minh City, Vietnam.

The agar-well diffusion method was used for determining the antibacterial activity using Muller Hinton Agar nutrient media (17). Briefly, each agar plate was inoculated with 100 µL of 10^5 CFU/ml of each bacterial suspension; wells of 8 mm in diameter were subsequently cut into agar plates using sterile metal cylinders. Ginger essential oils were dissolved in dimethyl sulfoxide (DMSO) at a range of different concentrations, including 1, 10, 50, 100%, and then were added into each well with the amount of 100 µL. Penicillin was used as positive control for both *S. epidermidis*, and *S. aureus*, while ampicillin and gentamicin were used as positive controls for *P. aeruginosa* and *E. coli*, respectively. All the plates were then kept at 37°C for 18-24 hours. DMSO was used as a negative control for all tests. The plates were then incubated at 37°C for 18-24 hours. Inhibition zones were observed and measured using a calliper. Each experiment was independently conducted in triplicate.

Extraction and isolation of non-volatile compounds from ginger:

Ginger (10 kg) was dried at 60˚C, powdered, and extracted by soaking with MeOH (50 L) at room temperature for three days. The resulting ginger extract was then evaporated at low pressure at 50˚C to obtain the crude extract (1024 g). The crude extract was further subjected to extraction with increasing polarity solvents, namely, n-hexane, ethyl acetate, and butanol, to obtain the respective fractions: n-hexane fraction (122 g), EtOAc fraction (271 g), and BuOH fraction (145 g), respectively.

The EtOAc extract was subsequently separated using column chromatography (CC) with the solvent system acetone- n-hexane (0:100; 1:50; 1:30; 1:20; 1:15; 1:5; 1:2; 1:1), obtaining 8 major fractions (EA1 → EA8). The EA1 fraction was further carried out by CC with the solvent system acetone-n-hexane (1:15 → 1:10) to obtain 7 fractions. EA 1.1 fraction was performed on CC with the solvent system acetone-n-hexane (1:15) yielding compound 4 (172 mg). EA1.3 fraction was carried out on the CC of the solvent system acetone-n-hexane (1:10 → 1:4) and yielded compound 1 (35 mg). The EA1.4 fraction was chromatographed on a silica gel column with the solvent combining MeOH - $CHCl_3$ (1:20) to obtain compound 2 (83 mg).

The n-butanol extract was separated on a silica gel CC with the eluent system MeOH - $CHCl_3$ (1:30 → 1:5) to obtain 10 fractions (B1 → B10). The fraction B5 was further purified on CC with the solvent system MeOH - $CHCl_3$ (1:10 → 1:5) and yielded compound 3 (34 mg). Similarly, the B6 fraction was subjected to CC with the solvent system MeOH - $CHCl_3$ (1:7), leading to the isolation of compound 5 (112 mg).

The structures of these compounds were clarified and identified by comparing them with spectral data of and reference spectra. The detection of TLC zones was performed by exposing the TLC plates to UV light at 254 nm or 365 nm or by using a solution of H_2SO_4/EtOH.

Nuclear magnetic resonance spectrum (NMR) measurements were carried out on Bruker 500 MHz and 125 MHz instruments at the Institute of Chemical Technology in Hanoi, Vietnam.

Determination of anti-cancer effect of non-volatile compounds:

The SW480 (human colon adenocarcinoma), HepG2 (human hepatocellular carcinoma), A549 (human lung adenocarcinoma epithelial), Hep3B (human hepatocellular carcinoma), and MCF-7 (human breast adenocarcinoma) tumor cell lines were provided by the American Type Culture Collection (ATCC). Whole tumor cell lines were sustained in RPMI (GibcoBRL, NY, USA), adding penicillin (100 units/mL) streptomycin (100 µg/mL) and 10% fetal bovine serum (FBS) kept at 37°C in a modified humidified atmosphere containing 5% CO_2. The cell lines were kept at the Laboratory of Experimental Biology, Institute of Natural Compounds Chemistry, Vietnam Academy of Science and Technology.

A Modified MTT assay (11-15) was used for measuring cytotoxic activity. MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide has been evaluated by the US National Cancer Institute (NCI) as a standard and effective method for rapid screening of cancer patients. The principle of the method is to indirectly determine the activity of the reagent through the ability of cells to inhibit the NAD(P)H-dependent oxidoreductase enzyme. This enzyme catalyzes the reduction of MTT tetrazolium dye to an insoluble purple formazan, which can reflect the relative number of growing cells when measured at λ = 540/720 nm.

The cytotoxicity of ginger compounds on five human tumour cell lines (Hep3B, SK-LU-1, MCF-7, SK-LU-1, SW480, HepG2) was investigated. The cells were cultured at 37˚C with 5% CO_2 in a suitable medium: DMEM (Dulbecco's Modified Eagle Medium), EMEM (Eagle's Minimum Essential Medium, Sigma-Aldrich, USA), or RPMI 1640 (Thermo Fisher, Waltham, Germany), supplemented with 2mM L-glutamine, antibiotics (Penicillin + Streptomycin sulphate) and 5-10% calf serum. For the experiments, a 96-well microplate containing 1.5 x 10^5 cells/well was incubated with a purified substance in a concentration range between 50 µg/mL and 1µg/mL (µM). Ellipticine combined with DMSO was used as a positive (+) standard. Each experiment with a difference in concentration range was tested in triplicate. Dimethyl sulfoxide (DMSO, Sigma-Aldrich) was used for dissolved formazan crystalline. The wavelength λ = 540/720 nm in Infinite F50 instrument (Tecan, Männedorf, Switzerland) measured the ability to inhibit compounds in each cell line.

The ability of inhibition for tumor cell proliferation at each concentration was calculated and compared with the positive control according to:

Cell inhibition rate (%) = (1-(OD_{sample}/ $OD_{control\ (-)}$)) Í 100%

To determine the concentration that resulted in 50% inhibition of cell life (IC50), Table Curve AISN Software (Jandel Scientific, San Rafael, CA) was employed for calculations. The IC50 represents the concentration at which cell growth is inhibited by 50%.

Statistical analysis:

The standard deviation (SD) and mean of all the results were determined. One-way analysis of variance (ANOVA) was used for calculating statistically. The results were statistically significant if confidence interval was taken 95% and $p < 0.05$.

Results and Discussion

Determinination of chemical components of the essential oils of Zingiber officinale

The GC-MS analysis method was used for determining the components of the essential oils of ginger (*Z. officinale*). Identification of these components was based on retention time (RT) and comparison with standard compounds in the mass spectrum library of the NIST 14 Chemistry WebBook. The major components and their percentages in the essential oils were summarized in Table 1. The essential oils of ginger were found to contain terpenoids, including both mono- and sesquiterpenes. Among the monoterpenes presented, over 30 different compounds were identified, such as α-pinene (ranging from 0 to 6.71%), camphene (7.06 to 25.41%), β-myrcene (0 to 2.32%), and eucalyptol (8.27 to 33.16%), among others. The essential oils also contained major sesquiterpenes, such as α-curcumene, (-)-zingiberene, β-bisabolene, and sesquiphellandrene, which are commonly found in ginger and turmeric essential oils. Notably, the composition and content of ginger essential oil varied among different provinces. For instance, Tien Giang, Bac Lieu, Soc Trang, An Giang, and Long An ginger essential oils were found to have approximately 20-30 compounds, while Hau Giang, Can Tho, Dong Thap, Tra Vinh, and Ben Tre ginger essential oils had over 30-45 chemical compounds, respectively, indicating a higher complexity in their composition. Particularly, the essential oil from Vinh Long was found to have only six compounds, including 9-octadecenoic acid, methyl ester (42.15%), camphene (9.35%), and eucalyptol (17.57%), among others. This difference in the chemical composition of ginger essential oils is attributed to the climatic conditions and geographical origins of ginger plants (18).

Antibacterial activity of essential oil

The essential oil of ginger collected from Ben Tre province, Vietnam, was used for determining the antibacterial activity (Table 2 and Figure 3). The growth of Gram-positive bacteria including *S. epidermidis* and *S. aureus* was inhibited by essential oil, whereas no antibacterial activity against *P. aeruginosa* and *E. coli* was observed. *S. aureus* was seen to have more sensitivity to ginger oil extract than *S. epidermidis* since the inhibition zone diameter of about 20 mm was measured when applying 10 and 50 % ginger oil concentration, respectively, to these bacteria. The inhibition ability of ginger compounds on Gram-positive bacteria could be related to several mechanisms, including interference with energy

Table 1. Major components of essential oils of *Zingiber officinale* in 13 provinces in Vietnam

RT	Compounds	% Area												
		An Giang	Bac Lieu	Ben Tre	Ca Mau	Can Tho	Dong Thap	Hau Giang	Kien Giang	Long An	Soc Trang	Tien Giang	Tra Vinh	Vinh Long
4.193	α - Pinene	4.71	3.8	6.71	2.27	3	2.75	1.49	3.84	4.35	4.08	5.17	3.55	0
4.499	Camphene	15.85	11.62	25.41	8.32	9.65	11.88	7.06	10.58	17.37	14.2	22.33	12.24	9.35
5.474	β- Myrcene	-	2.32	2.04	1.35	1.89	-	1.11	-	1.28	-	2.05	1.86	0
6.45	Eucalyptol	16.16	18.53	25.69	18.68	11.79	8.27	13.78	23.9	23.62	15.11	33.16	12.77	17.57
10.163	endo-Borneol	1.92	-	1.89	-	-	-	0.33	-	3.45	-	4.46	-	-
12.322	Carveol	12.61	0.42	-	17.95	-	5.09	-	-	-	-	-	-	-
12.331	1,3,8-p-Menthatriene	-	15.91	-	-	-	3.36	-	-	7.95	9.16	-	-	-
12.771	1,3,7-Octatriene, 3,7-dimethyl-	2.13	2.94	-	-	-	-	-	-	15.68	-	-	-	-
13.156	2,6-Octadienal, 3,7-dimethyl-, (E)	1.96	-	8.25	27.9	10.49	-	13.64	1.79	-	-	7.15	-	-
13.166	Citral	17.8	24.13	-	3.08	-	-	-	15.23	-	-	0.35	10.21	-
18.807	α - Curcumene	1.65	1.62	1.96	2.12	7.92	3.74	7.77	3.45	1.94	2.81	2.04	6.58	-
19.124	(-)-Zingiberene	-	-	6.22	-	11.87	-	8.77	8.65	-	-	5.35	14.3	-
19.147	α - Bergamotene	6.72	-	0.28	-	-	13.4	-	-	8.08	-	-	-	-
19.483	β - Bisabolene	2.63	-	2.2	2.15	6.56	4.66	5.99	4.13	2.37	-	1.97	0.53	-
19.861	Sesquiphellandrene	2.39	-	2.53	2.34	6.35	-	5.73	3.35	3.2	-	-	6.65	-

Table 2. Antibacterial activity of ginger essential oil

Ginger essential oil concentration (%)	*Diameter of Inhibition zone (mm)*			
	S. aureus	*S. epidermidis*	*E. coli*	*P. aeruginosa*
100	24.67 ±3.30	30.00 ±1.41	0	0
50	24.33 ±0.94	20.33 ±0.47	0	0
10	20.33 ±0.74	16.33 ±1.25	0	0
1	0	0	0	0

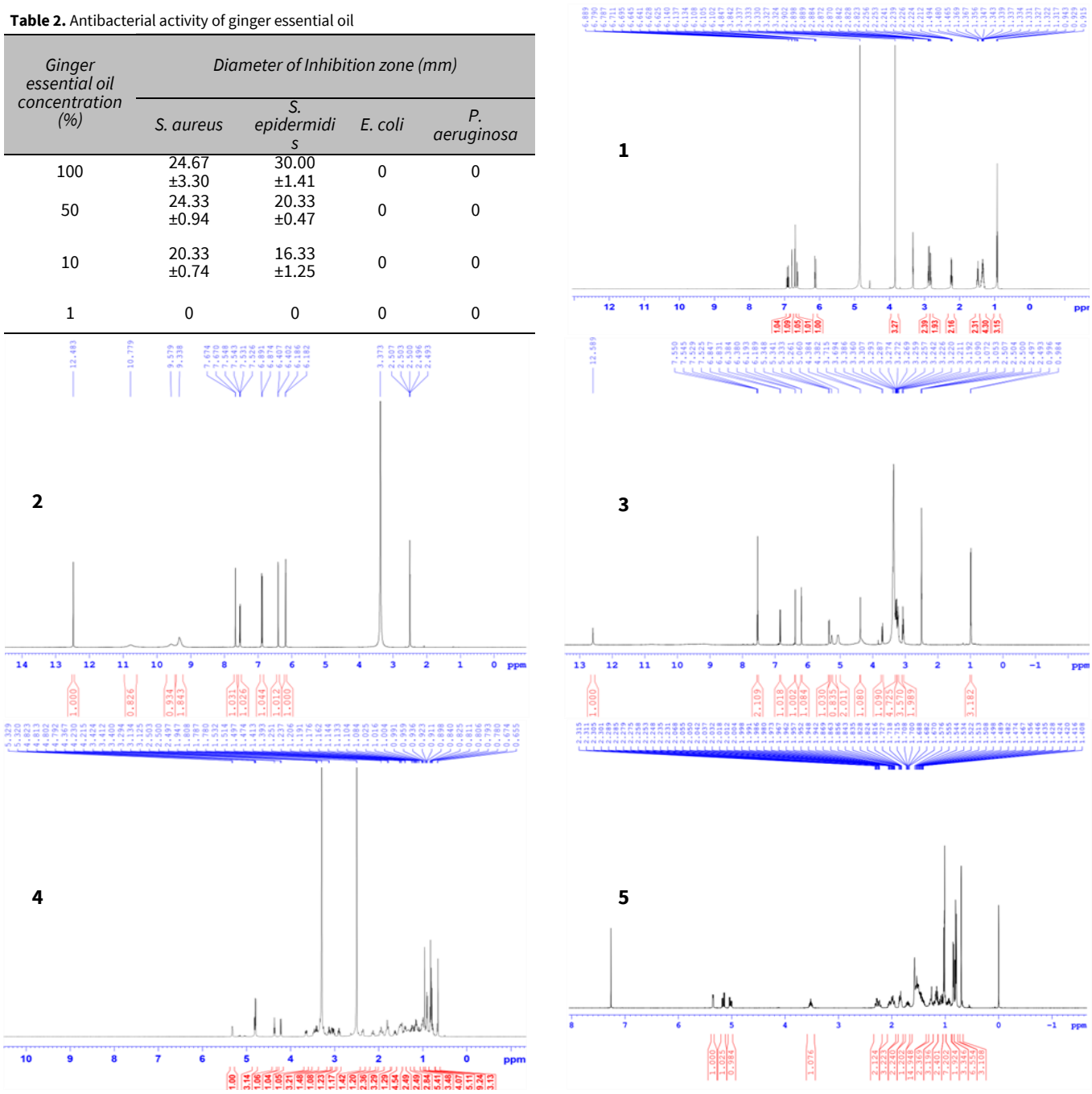


Figure 1. ^{1}H-NMR spectra of compounds 1-5

metabolism, disruption of cell membrane-related proteins, and interference with DNA metabolism. These mechanisms can collectively lead to the disruption of the bacterial cell membrane (19). The minimum inhibition concentration (MIC) of ginger essential oil for both of *S. aureus* and *S. epidermidis* was 4.17 ± 1.18%.

The presence of antibacterial properties related to monoterpenoid compounds in ginger essential oil provides a good explanation for its antibacterial effect. The data from this research were in line with other work conducted with samples from Thailand, in which ginger essential oil showed antibacterial activity to Gram-positive (+) bacteria including *B. subtilis* and *S. aureus*, but not against Gram-negative (-) as *E. coli* (20). However, the ginger extraction from Saudi Arabia and China was dried and dissolved in DMSO to 10 µL to restrain the growth of both Gram-positive bacteria (9-13 mm inhibition zone diameter) and Gram-negative bacteria (7-10 mm inhibition zone diameter). Both Saudi and Chinese ginger essential oils exhibited an 8 mm and 10 mm zone of inhibition against *E. coli*, respectively (19).

Wang et al. (2020) showed that the inhibition zone diameter of ginger essential oil against *S. aureus* and *E. coli* was 12.3 and 17.2 mm, respectively, with a minimum inhibitory concentration of 1mg/ml (21). Another study using disc diffusion reported that the essential oil of ginger grown in Mexico inhibited the growth of *S. epidermidis* and *S. aureus* with MIC values of 0.5 mg/mL and 0.25 mg/mL, respectively (22). It might be that ginger grown in different geographical areas exhibits different antibacterial activity. Further investigation of ginger essential oil collected from various areas against bacteria should be considered.

Determination of the structures of non-volatile compounds isolated from Vietnamese ginger

Compound 1 is a yellow syrup with a pungent taste. The ^{1}H

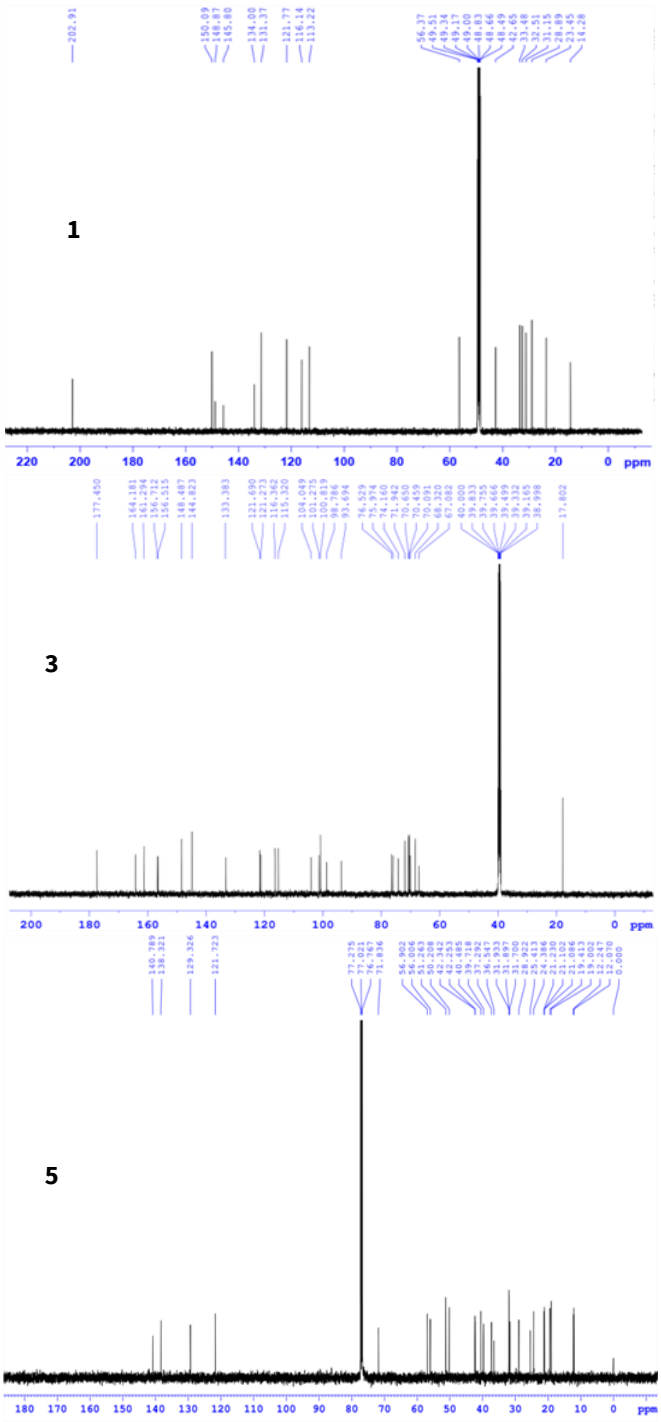


Figure 2. ^{13}C-NMR spectra of compounds 1-5

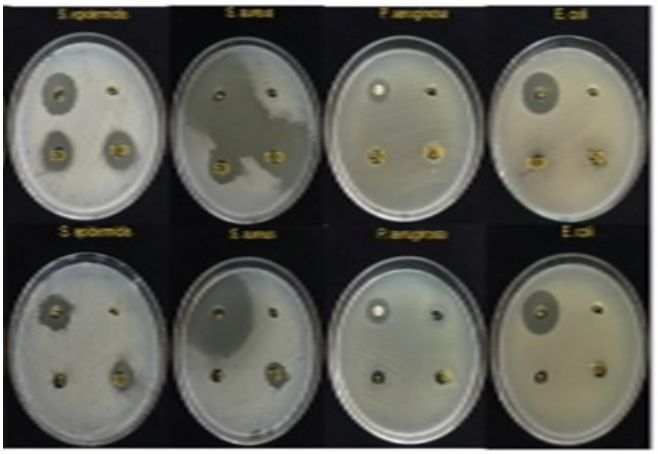


Figure 3. Inhibition zone induced by Ginger essential oil (Ben Tre) on tested bacterial strains including *S. epidermidis*; *S. aureus*; *P. aeruginosa*; and *E. coli.*

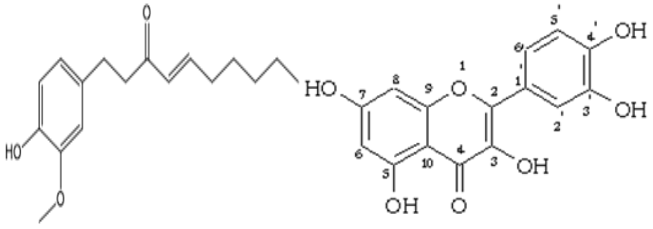
-NMR spectrum revealed the presence of three aryl protons, two olefinic protons, one methoxy group (δ_H 3.87), and a doublet for the H-4 proton (δ_H 6, 09). These groups indicate that the molecule contains both phenyl and alkyl groups. The HSQC and ^{13}C-NMR spectra indicate the presence of unsaturated alkyl groups containing 10 carbons. The positions of the three quaternary carbons in the aryl group were clarified. In addition, a carbonyl group is attached to the alkyl group at (δ_C 200.4). In the HMBC correlation, two olefinic protons at δ_H 6.09 (δ_C 130.9) and δ_H 6.11 (δ_C 148.5) correlated to each other and the ketone carbon at (δ_C 200.4). Along with other data being summarized (23), the molecular structure was elucidated. The NMR results revealed that the isolated compound is (E)-1-(4-hydroxy-3-methoxyphenyl)-dec-4-en-3-one, commonly known as 6-shogaol.

Compound 2 is a yellow crystal, needle-shaped, with a melting point of 313-314 °C. The UV spectrum indicates that the compound 2 belongs to the flavone group. The ESI mass spectrum (positive) of compound 2 gives a pseudo-molecular ion peak at m/z 303 $(M+H)^+$, corresponding to the molecular formula $C_{15}H_{10}O_7$. The ^{1}H-NMR spectrum shows a doublet signal at δ 6.38 and 6.16 ppm (1H, J = 1.8 Hz), which is characteristic of the two protons C-8 and C-6 of the A ring. In addition, there are 3 interactive signals at and δ 6.85 ppm (1H, d, J = 8.8 Hz) are assigned to H-2', H -6' and H-5'. The DEPT and ^{13}C-NMR spectrum of compound 2 show signals of 27 carbons, including 15 carbons in the flavone framework. Comparing the obtained data with the previous study (24), it can be

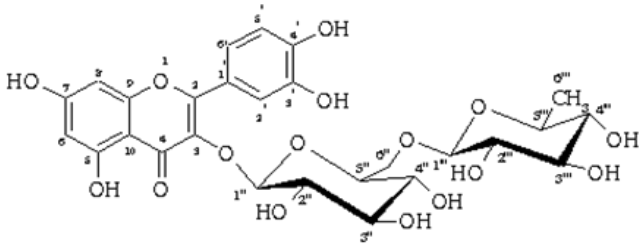
concluded that compound 2 is quercetin, which has also been isolated and found in many plants and has many valuable activities, typically antioxidant and vascular wall strengthening.

Compound 3 is yellow crystals with a melting point of 214 – 215˚C. The UV spectrum of compound 3 is also specific for compounds belonging to the flavone group. The ESI (positive) mass spectrum of compound 3 gives the ion peak m/z at 611 $[M+H]^+$ corresponding to the molecular formula $C_{27}H_{30}O_{16}$. The DEPT and ^{13}C-NMR spectrum of compound 3 showed corresponding signals including 27 carbons, of which 15 carbons belong to the flavone group, and 12 carbons belong to glucose and rhamnose. The ^{1}H-NMR spectrum shows a doublet signal at δ 6.38 and δ6.19 ppm (1H, J=1.8 Hz), characterizing two protons belonging to C-8 and C-6 of the A-ring. Simultaneously, the ^{1}H-NMR spectrum also shows that three signals interact with each other at δ 7.55 (2H, dd, J = 8.5, 2.0 Hz) and δ 6.85 ppm (1H, d, J = 8.8 Hz) are assigned for H-2', H-6' and H-5'. The presence of signals of two anomeric protons at δ 5.34 (d, J = 7.0 Hz) and 4.38 (brs) indicates that the molecule has a disaccharide. Signals of rutinose include glucose (δ 100.1, 70.5, 70.7, 71.9, 68.3 and 17.8), (δ 101.3, 74.2, 76.5, 70.1, 76.5 and 67.1) and rhamnose (δ 103.0, 74.1, 72.2, 72.1, 70.2, and 18.1) are assigned by ^{13}C-NMR spectrum. Comparing the obtained data with the previous study, (25), it can be concluded that compound 3 is a flavonoid named rutin (quercetin-3-O-α-L-rhamnopyranosyl-(1→6)-β-D glucopyranosite).

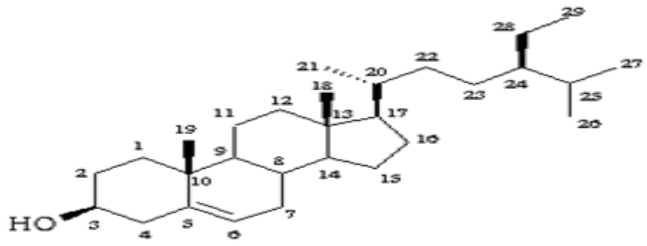
Compound 4 is colorless crystals, needle-shaped with a melting point of 135-136˚C. The EI-MS spectrum gives a molecular ion peak at m/z 414 $(M)^+$, corresponding to formula $C_{29}H_{50}O$. The ^{1}H-NMR spectrum of compound 4 shows a signal of 6 methyl groups at d 0.68 (3H, s, 18-CH_3), 0.81 (3H, d, J = 6.5 Hz, 27-CH_3), 0.83 (3H, d, J = 6.5 Hz, H-26), 0.84 (3H, d, J = 7.0 Hz, 29-CH_3), 0.92 (3H, d, J = 6.2 Hz, 21-CH_3), 1.01 (3H, s, 19-CH_3). The ^{13}C-NMR spectrum of compound 5 has 29 signals showing that there are 29 carbon atoms including 7 carbons in CH groups, 12 carbons in CH_2 groups, 6 carbons in CH_3 groups, and 4 quaternary carbons. The EI-MS and NMR spectra of compound 4 are completely consistent with β-sitosterol (26), which exists very commonly in many natural plant species. Compound 5 is an amorphous solid, colorless with a melting point of 282 - 283˚C. On the EI-MS spectrum, the molecular ion peak at m/z 396 (M-$C_6H_{12}O_6$) is shown. The ^{13}C-NMR spectrum shows 35 signals of carbon atoms, of which 7 are bonded to oxygen (in the range of 61.2 to 100.9 ppm). There are 2 signals at 140.6 and 121.3 ppm belonging to an olefin bond. The ^{1}H-NMR spectrum shows that the anomeric proton (H-1') of the sugar fraction appears as a doublet at 5.03 ppm, J = 7.0 Hz and C-1' at d 100.9 ppm, respectively. Data of ^{13}C - and ^{1}H-NMR spectra show that this is the structure of a glucoside compound with the formula $C_{35}H_{60}O_6$. In addition, the presence of the 396 m/z fragment (M-$C_6H_{12}O_6$) on the EI-MS spectrum also confirmed that a hexoses molecule was severed from the sitosterol glucoside molecule. These data allow confirming that the compound named as β-sitosterol-3-O-β-D-glucopyranoside (12).



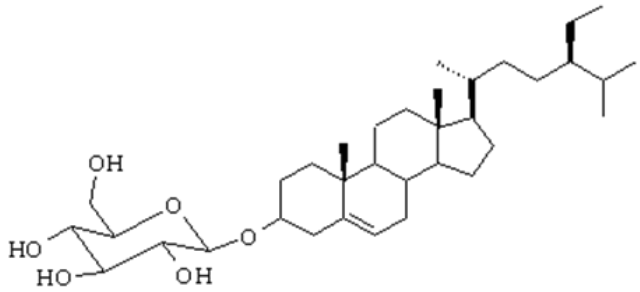
(**1**) 6-shogaol. (**2**) Quercetin



(**3**) Rutin



(**4**) b-sitosterol



(**5**) b-sitosterol-3-O-b-D-glucopyranoside

Anticancer effect of 5 non-volatile compounds isolated from Vietnamese ginger

Five types of human cancer cell lines including Hep3B, SK-LU-1, MCF-7, SK-LU-1, SW480, and HepG2 were used for examining anticancer activity *in vitro*. These purified compounds were tested for cytotoxicity as previously described. The bioactivity data is presented in Table 3. The cytotoxicity against compounds 1-3 had a range between 62.7 ± 2.1 and 97.6 ± 1.1 µM, showing weak inhibition for all five cancer cell lines. The positive control, Ellipticine, had IC_{50} values between 0.4 ± 0.1 and 0.5 ± 0.1 µM. Compounds 4 and 5 showed moderate cytotoxicity against HepG2 and Hep3B with IC_{50} values between 21.5 ± 5.1 and 46.9 ± 3.7 µM, but both of these compounds did not display significant cytotoxic activity for SK-LU-1 and SW480 cell lines within the experimental range, with IC_{50} values > 100 µM. Additionally, compound 4 also showed moderate cytotoxicity against MCF7, having an IC_{50} value of 43.6 ± 5.1 µM as shown in Table 3.

Table 3. Cytotoxic activity of compounds **1**–**5** from the *Zingiber officinale*

Compound	SK-LU-1	HepG2	Hep3B	SW480	MCF7
	IC_{50} (μM)[a]	IC_{50} (μM)[a]	IC_{50} (μM)[a]	IC_{50} (μM)[a]	IC_{50} (μM)[a]
1	62.7 ± 2.1	62.7 ± 2.4	64.3 ± 1.7	94.7 ± 1.8	84.3± 1.8
2	97.6 ± 1.1	92.7 ± 1.8	95.2 ± 1.8	75.3 ± 2.8	85.6± 0.8
3	82.4 ± 1,1	81.5 ± 1,8	84.7 ± 1,8	94.2 ± 2.8	86.2± 0.8
4	>100	21.5 ± 5.1	21.7 ± 2.8	>100	43.6± 5.1
5	>100	46.9 ± 3.7	35.2 ± 3.4	>100	>100
Ellipticine[b]	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5± 0.1	0.4 ± 0.1

[a] Concentration necessary for 50 % inhibition (IC_{50}). Results are presented as means ± SD (n = 3). *** p < 0.001 compared with the control value. [b] Ellipticine was used as a positive control.

The anti-cancer effects of the 5 non-volatile compounds exert through a variety of mechanisms with different manners and target cells. According to (27) compound 1**,** 6-shogaol, down-regulates notch targeting genes, resulting in the inhibition and apoptosis of autophagy in breast tumor cells. Additionally, 6-shogaol directly regulates the Akt1/2 pathway and induces apoptosis in lung tumor cells (28). In liver cancer cells, 6-shogaol induces endoplasmic reticulum stress and apoptosis via the PERK/eIF2α axis (29). Moreover, 6-shogaol has been reported to reduce tumor cell propagation and induce tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-mediated cell death in liver tumor cells (30). In blood cancer, 6-shogaol is related to the mechanisms of reduced expression of the antiapoptotic proteins B-cell lymphoma (Bcl)-2, and in lung cancer, it is related to triggering the BCL2/BAX-mediated apoptosis pathway (31, 32). On the other hand, compound 4, β-sitosterol, may trigger apoptosis in lung cancer cells via altertion of Trx/Trx1 reductase to induce ROS mediated mitochondrial dysregulation and p53 activation (29). Quercetin (compound 2), one of the well-known dietary flavonoids, exert its anti-cancer effect aganist colon cancer via surpression of NF-κB pathway (33). Rutin (compound 3) is a bioflavonoid that has been demonstrated as a safe anticancer agent relate to signalling pathways such as Wnt/β-catenin, p53-independent pathway, PI3K/Akt, JAK/STAT, MAPK, p53, apoptosis as well as NF-κB signalling pathways (34). The differences in mechanism of action of each compounds may account for their variation in anti-cancer effects. Taken together, these findings suggest the application of these compound and ginger in the pharmaceutical industry to cure cancer-related diseases.

Conclusion

In this study, we identified and compared volatile compounds of essential oils extracted from ginger collected from 13 locations in Vietnam to provide a basis for selection and standardization of raw materials from different geographic origins. Among them, ginger was collected from the western provinces of vietnam, including Vinh Long, Bac Lieu, Ben Tre, Tien Giang, An Giang, Long An, Can Tho, Kien Giang, Dong Thap, Tra Vinh, Soc Trang, and Hau Giang province. Vietnam zinger is a high potential source for application in the pharmaceutical industry with a diversity of volatile and nonvolatile compounds (5 compounds, including 6-shogaol (1), quercetin (2), rutin (3),β-sitosterol (4), andβ-sitosterol-3-O-β-glucopyranoside (5). Furthermore, the essential oil of ginger collected from these western provinces of Vietnam, exhibited strong antibacterial activity against pathogenic strains, such as *S. aureus* and *S. epidermidis*. Note that ginger is a promising source for the extraction of anti-cancer compounds. Compounds 1–3 isolated from ginger displayed inhibition against five tumor cell lines (Hep3B, SK-LU-1, MCF-7, SK-LU -1, SW480, and HepG2) with the IC50 values ranging between 62.7 ± 2.1 and 97.6 ± 1.1 μM. Compounds 4 and 5 showed mild cytotoxicity against HepG2 and Hep3B tumor cell lines, with the IC50 values ranging between 21.5 ± 5.1 and 46.9 ± 3.7 μM but did not exhibit significant cytotoxic activity against SW480 and SK-LU-1 cells. Compound 4 also demonstrated moderate cytotoxicity against MCF7 cell lines, with an IC50 value of 43.6 ± 5.1 μM. Therefore, *Zingiber officinale* contains many compounds with anticancer activity. In conclusion, this study provides scientific evidence supporting the potential application and pharmacological value of Vietnamese ginger in the treatment of infectious and cancer-related diseases.

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Authors' contributions

NTN, NNT, KATN conceived of the study and participated in its design and coordination. PCK and GBT participated in study design and coordination. TVNL, CKN, NTTN, NAL, TLT extracted the volatile and non-volatile compounds from ginger. NNT, NNT, KATN carried out anti-bacterial and anticancer assays. NTN and KATN performed the statistical analysis. NNT, KATN, NNT, PCK, and GBT

interpreted the data and drafted the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: GBT is one of members of the editorial board of Plant Science Today Journal. Therefore, she excluded herself from the manuscript revision process and decision.

Ethical issues: None

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