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Chemical composition and antimicrobial activity of essential oils of *Amomum glabrum* S.Q.Tong (Zingiberaceae) from Vietnam

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Abstract

Background: Vietnam is a tropical country blessed with many plants. The majority of these floras have not been exploited for their chemical constituents and biological potential. In an attempt to source for natural products to combat microbial infections, plant products can act in this regard. The aim of this paper was to report the chemical composition and antimicrobial activity of essential oil from *Amomum glabrum* growing in Vietnam.

Methods: The leaves and rhizomes of *Amomum glabrum* S.Q.Tong (Zingiberaceae) were collected from Pù Hoạt Nature Reserve, 18°17′15″N, 105°21′39″E, north-central Vietnam, at an elevation of 124 m in October 2018. The fresh samples were subjected to hydrodistillation process using Clevenger-apparatus to obtain essential oils. The essential oils were subjected to gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS). The antimicrobial activity was determined by microdilution broth susceptibility assay. All experiments were done in triplicate analyses.

Results: The major compounds in the leaf essential oil were β-pinene (62.2%) and α-pinene (13.1%) while the rhizome oil comprised mainly of β-pinene (53.7%), α-pinene (10.1%) and fenchyl acetate (11.3%). The antimicrobial study showed that the leaf essential oil was active against *Enterococcus faecalis* ATCC 299212, *Bacillus cereus* ATCC 14579 and *Candida albican*s ATCC 10231 with minimum inhibitory concentration (MIC) values of 4.23 µg/mL, 67.98 µg/mL and 1.56 µg/mL respectively, while MIC values of 18.67 µg/mL, 9.78 µg/mL and 10.23 µg/mL respectively were shown by the rhizome essential oil towards the same microorganisms. Both essential oils inhibited the growth of *Staphylococcus aureus* ATCC 25923 with the MIC value of 5.67 µg/mL.

Conclusion: This study, therefore, concludes that the essential oils from the leaves and rhizomes of *A. glabrum* possess antimicrobial effect which may be due to the compounds present therein. This report, the first of its kind, indicates the potential of *A. glabrum* essential oils as sources of antimicrobial principle.

Keywords: *Amomum glabrum;* essential oil composition; α-pinene; β-pinene; fenchyl acetate; antimicrobial activity

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Background

In recent times, the chemical constituents and biological activities of essential oils from some *Amomum* plants [1-4] and other poorly studied species of Vietnamese flora [5,6] were published. *Amomum* is a genus of plants native to China, the Indian subcontinent, Southeast Asia, New Guinea, and Queensland [7]. *Amomum* plants from Vietnam and other parts of the world have been described as sources of biologically active components [3,4]. .*Amomum glabrum* S.Q. Tong (family Zingiberaceae) is known as Sa nhân nhẵn in Vietnam and distributed within Nghe An Province (Pù Hoạt Nature Reserve), Ha Tinh Province (Vu Quang National Park). *A. glabrum* is a plant which grows up to 0.8-1.5 m tall. The leaf sheaths are green glabrous while the bracts are reddish in colour and subovate in shape. Flowering occurs in May [8,9]. The plant has been used in ethnomedicine for the treatment of inflammation, malaria and microbial infections [8].

Our previous investigations into the volatile constituents of *Amomum* plants grown in Vietnam have produced results which have been published [1-4]. The main class of compounds in the leaves of *A. longiligulare* [1], *A. maximum* [10], and *A. aculeatum* [11] were monoterpene hydrocarbons and sesquiterpene hydrocarbons. The essential oil of the stem of *A. longiligulare* [1] and *A. gagnepainii* [3] were dominated by sesquiterpene hydrocarbons and oxygenated counterparts. Monoterpene hydrocarbons occurred in higher quantity in all parts of *A. repoense* [3], the rhizome of *A. rubidium* [4] and the leaf of *A. villosum* [12]. Oxygenated monoterpenes and sesquiterpene compounds constituted the bulk of essential oil from the leaves and fruits of *A. muricarpum* [10]. However, fatty acids were identified in quantity in the root of *A. longiligulare* [1]. In addition, the larvicidal [4] and antimicrobial [13, 14] activities of essential oils from other species of *Amomum* have been reported.

The authors are not aware of any information on the volatile and non-volatile constituents as well as biological activities of *A. glabrum* and thus aroused our interest in the present study. The aim of the present study was to examine the chemical constituents and antimicrobial activity of essential oils from the leaves and rhizomes of *Amomum glabrum* S.Q. Tong growing in Vietnam. The present report was in continuation of our extensive study on the biological activities of essential oils from Vietnamese plants [13-16].

Methods

Reagents

All chemicals and materials used in the experiment were pure products of Sigma-Aldrich (San Louis, MI, USA) distributor. They include, Mueller-Hinton Agar, Sabouraud Agar, Dimethylsulfoxide, Streptomycin, Nystatin and Cycloheximide.

Collection of the leaves and rhizomes of A. glabrum

The leaves and rhizomes of *A. glabrum* were collected from plants wild-growing in Pù Hoạt Nature Reserve, (GPS: 18°17′15″N, 105°21′39″E), north-central Vietnam, at an elevation of 124 m, in October 2018. The plant samples were identified by Dr. Dai, D.N. and voucher a specimen (NTC 754) was deposited in the plant specimen room, Faculty Agriculture, Forestry and Fishery, Nghe An, College of Economics. In each case, the fresh leaves and rhizomes were chopped to obtain an approximate weight of 2.0 kg which was subjected to hydrodistillation using a Clevenger-type apparatus.

Hydrodistillation of the samples

To obtain essential oils, the leaves and rhizomes of *A. glabrum* were grinded to reduce the surface area for easy volatilization of the oil. For the experiment, 2000 g of each sample of the leaves and rhizomes of *A. glabrum* was subjected to hydrodistillation separately according to specification [17], using a Clevenger-type apparatus as described in previous studies [13-16]. The samples were carefully and separately packed inside 5 L flask to which distilled water was added and ensured that the samples were completely covered. The time used for distillation was 3 h and at atmospheric pressure. The essential oils were stored in weighed sample bottles and kept refrigerated (4 ^{o}C) until the time of chemical and biological analyses. The hydrodistillation process was done in triplicates.

Analysis of the constituents of the essential oils

Gas chromatographic (GC) analysis was performed on an Agilent Technologies HP 7890A Plus Gas chromatograph equipped with a Flame Ionization Detector (FID) and fitted with HP-5MS column (30 m × 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; 99.9999% purity), injector temperature (PTV: programmable temperature vaporization) 250 ^{o}C, detector temperature 260 ^{o}C, column temperature programmed from 60 ^{o}C (2 min hold) to 220 ^{o}C (10 min hold) at 4 ^{o}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. The temperature of the injection port was 220 ^{o}C, while MS interface was MS Workstation 6.2

An Agilent Technologies (Santa Clara, California, USA) HP 7890A Plus Chromatograph fitted with a fused silica capillary HP-5ms 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; 99.9999% purity) as a carrier gas. The mass spectrum (MS) conditions were as follows: ionization voltage 70 eV; emission current 40 mA; acquisition scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s.

Identification of essential oil components

The constituents of the essential oils were identified from the GC/MS spectral obtained. This was made possible by comparison of their retention indices (RI) with homologous series of *n*-alkanes. For few of the constituents, the method of co-injection with known compounds that were run with the same GC conditions was employed. The mass fragmentation patterns and calculated retention indices of each compound were checked and compared with known essential oil compositions available in the databases [18-20].

Microorganisms

The microorganisms (non-resistance) used in the evaluation of the antimicrobial activity of the essential oils were three strains of Gram-positive bacteria, *Enterococcus faecalis* (ATCC299212), *Staphylococcus aureus* (ATCC25923), *Bacillus cereus*

(ATCC14579), three strains of Gram-negative bacteria, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC27853), *Salmonella enterica* (ATCC13076) and one strain of yeast, *Candida albicans* (ATCC 10231). Testing media included Mueller-Hinton Agar (MHA) used for bacteria and Sabouraud Agar (SA) used for fungi. The strains were obtained from the laboratory stock of Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, Hanoi, Vietnam.

Antimicrobial activity assay

The Minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were measured by the microdilution broth susceptibility assay [13-16, 21]. The choice of investigated concentrations was based on our previous reports on similar investigations where essential oils have been found to be active within specific concentration range [13-16]. A 2-fold dilution range was used for the experiment. Stock solutions of the oil 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, nystatin and cycloheximide were used as antifungal standards. Steptomycin is used to treat or prevent infections that are proven or strongly suspected to be caused by a number of gram-positive and gram-negative bacteria including tuberculosis, *Mycobacterium,* endocarditis etc [22]. Cycloheximide is a naturally occurring fungicide [23]. Nystatin is an antifungal that works by stopping the growth of fungus of the mouth or intestines. It has been used to treat *Candida* infections [24].

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:

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

$$IC_{50} = \text{High}_{conc} - \frac{(\text{Highinh\%} - 50\%) \times (\text{HighConc} - \text{Low Conc})}{(\text{Highinh\%} - \text{Lowinh\%})}$$

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

Statistical analysis (ANOVA) of the differences between mean values obtained for experimental groups were calculated as a mean of standard deviation (SD, ±) of three independent measurements using Microsoft excel program 2003.

Results

Yields and colour of the essential oils

The percentage yields of the essential oil were obtained as 0.18% ± 0.01 (v/w, leaf) and 0.21% ± 0.01 (v/w, rhizome). The obtained essential oils were yellow-coloured with sweet and strong odour.

Chemical constituents of the essential oils

Thirty-four different compounds (Table 1) which represents 92.3% of the volatile contents were identified from the GC/MS spectra of the leaf oil of *A. glabrum*. Fig. 1 and Fig. 2 represents the GC chromatogram of the leaves and rhizomes essential oils of *A. glabrum,* respectively. The class of compounds occurring in higher amounts in the leaf oil of *A. glabrum* was monoterpene hydrocarbons (80.4%). The main constituents of the leaf essential oil were β-pinene (62.2%) and α-pinene (13.1%). Except for bulnesol (2.1%), limonene (1.9%) and pentadecanal (1.0%), all other compounds were identified in an amount less than 1%. On the other hand, thirty-five compounds accounting for 93.6% of the oil contents were identified in the rhizome oil. Monoterpene hydrocarbons (60.4%) and oxygenated monoterpene (15.4%) were the main classes of compounds occurring in the oil. In all, sesquiterpenes accounted for 12.8% of the oil contents. The significant constituents of the rhizome oil were β-pinene (43.7%), fenchyl acetate (11.3%) and α-pinene (10.0%). Other compounds identified in sizeable proportions include camphene (3.8%), limonene (3.3%), bulnesol (2.4%), γ-bicyclohomofarnesal and aristolochene (2.0%).

Antimicrobial study of the essential oils

The results of the antimicrobial assay are shown in Table 2. Both essential oils exhibited varying degree of antimicrobial activity against the tested microorganisms. The leaf essential oil inhibited the growth of *E. faecalis* ATCC29912, *S. aureus* ATCC2592, *B. cereus* ATCC14579 and *C. albicans* ATCC10231 with MIC values of 8.0 µg/mL, 16.0 µg/mL, 128.0 µg/mL and 8.0 µg/mL, respectively. However, the MIC values of the rhizome essential oil towards the same microorganisms were 64.0 µg/mL, 16.0 µg/mL, 32.0 µg/mL and 32.0 µg/mL, respectively.

The potency of the essential oils against the tested microorganisms defined by IC_{50} is shown in Table 2. In addition, the leaf essential oil exhibited activity towards *E. faecalis* ATCC29912, *S. aureus* ATCC2592, *B. cereus* ATCC14579, *E. coli* ATCC25922 and *C. albicans* ATCC10231, with inhibitory concentrations (IC_{50}) of 4.23 µg/mL, 5.67 µg/mL, 67.98 µg/mL and 1.56 µg/mL, respectively. On the other hand, IC_{50} values of 18.67 µg/mL, 5.67 µg/mL, 9.78 µg/mL and 10.23 µg/mL, were displayed by the rhizome essential oil against *E. faecalis* ATCC29912, *S. aureus* ATCC2592, *B. cereus* ATCC14579 and *C. albicans*

ATCC10231, respectively. Streptomycin displayed antimicrobial activity with MIC values in the range of 0.5 μg/mL -1.6 μg/mL, while the MIC values in the range of 1.2 μg/mL -3.7 μg/mL were obtained for Cycloheximide. Nystatin on the other hand exhibited MIC values in the range of 2.8 μg/mL -4.3 μg/mL.

Discussion

This report was the first of its kind aimed at the evaluation of the chemical constituents and antimicrobial potentials of essential oils from the leaves and rhizomes of *A. glabrum*. The seemingly low yields of the essential oils falls within the data obtained for essential oils of some *Amomum* plants from Vietnam. *A. rubidium* rhizome [4] was obtained in a yield of 0.13%, 0.22% for *A. aculeatum* [11], 0.20% and 0.25% respectively for *A. gagnepainii* and *A. repoense* [3], as well as 0.30% and 0.25% for the leaf and roots of *A. villosum* [12]. In addition, the leaves and stems essential oils of *A. rubisum* were obtained in yields of 0.22% and 0.15%, respectively [14].

The leaf essential oil contained higher amount of monoterpene hydrocarbons (80.4% vs. 60.4%) than the rhizome essential oil which in turn had larger percentage of the oxygen-containing monoterpenes (2.3% vs. 15.4%). The quantities of the sesquiterpenes in both essential oils were much lower than their monoterpene counterparts. Although, α-pinene and β-pinene were the main constituents of both essential oils, the leaf essential oil had higher quantity of the latter while the rhizome essential oil contained larger amount of the former. However, fenchyl acetate, a significant compound of the rhizome essential oil was not identified in the leaf essential oil. The abundance of terpene compounds in the essential oils of *A. glabrumm* was in agreement with previous findings on the essential oil compositions of other *Amomum* plants [1-4, 10-11]. The abundance of monoterpene hydrocarbon compounds confers qualitative similarity with the chemical components identified in leaf essential oil of *A. villosum* [12] and *A. repoense* [3]. The high contents of β-pinene and α-pinene makes the essential oils similar to the leaf oils of *A. repoense* [13], *A. maximum* [10], *A. muricarpum* [10], and *A. villosum* [12].

Information obtained from data on the previous analysis of the chemical constituents of essential oils some *Amomum* plants growing all over the world [25-27] revealed that monoterpene and sesquiterpene compounds predominate. However, the identities of these compounds differ from one another. From the foregoing, essential oils of *Amomum* species so far analysed all over the world exhibited chemical variability. The amount and the composition of the bioactive substances may vary among the same or different plant species, and according to different factors such as the extraction methods, the geographic and the growing conditions, the harvest time [28]. This may be responsible for the observed variations in the chemical constituents of *Amomum* essential oil samples.

From Table 2, it can be deduced that the leaf essential oil exhibited stronger antimicrobial activity towards *E. faecalis* ATCC29912 and *C. albicans* ATCC10231 than the rhizome essential oil, depicted by lower MIC and IC_{50} values. However, the rhizome essential oil showed much greater activity against *B. cereus* ATCC14579 than the leaf essential oil. Both essential oils displayed similar antimicrobial activity and inhibitory patterns against *S. aureus* ATCC2592. The MIC was to determine the minimum concentration that prevented the growth of test microbes, however IC_{50} is that concentration that achieved 50% growth inhibition. Ordinarily, IC_{50} is used to test the relative potency of tested products when comparative study is conducted. In the present work, it may be useful in drug formulation for some applications including the use of essential oils as component of antimicrobial packaging. *Enterococcus faecalis* is a gram-positive bacterium associated with complicated infections such as abdominal, skin, urinary tracts, postsurgical wound infections, endocarditis etc; *S. aureus* is known to be a bacterium that causes pains, burns, sore throats, and pus infections on the skin and internal organs including infectious endocarditis; *B. cereus* is nonpathogenic but it can contaminate food; while *C. albicans* causes baby thrush in children and gynecological diseases. These assay results can be the basis to open new broader research of the antimicrobial activity of essential oils from *A. glabrum*.

It could be observed that essential oils from the leaves and rhizomes of *A. glabrum* only displayed antimicrobial activity towards the gram-positive pathogens and yeast. Hence it may be concluded that the essential oils of *A. glabrum* are Gram-positive specific. It is probable therefore, to suggest that the studied essential oils may be acting to antagonize the various mechanisms via which the microorganisms exert effect. Therefore, the observed antimicrobial activities of the leaves and rhizomes essential oils of *A. glabrum* are in consistent with the ethnomedicinal usage of the plant for the treatment of microbial infections [8]. The results of MIC obtained in this study confirmed known observations of Gram-positive bacteria being more susceptible to growth inhibition by plant essential oils than Gram-negative bacteria. These differences could be attributed in part to the great complexity of the double membrane-containing cell envelope in Gram-negative bacteria compared to the single membrane structure of the Gram-positive ones [29]. These differences may also be attributed to the presence of the lipopolysaccharides in the outer membrane of the Gram-negative bacteria, which provides a hydrophilic surface and functions as a permeability barrier for many plant extracts, antibiotics, detergents, and lipophilic compounds [30, 31]. However, the ability of essential oils to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control is the most likely reason for its lethal action [32]. It is believed that essential oils can coagulate the cytoplasm and damage lipids and proteins [31].

The observed antimicrobial result of *A. glabrum* essential oil was in agreement with previous information that *Amomum* essential oils from Vietnam and other parts of the world selectively inhibited the growth of different microorganisms. For example, the rhizome essential oil of *A. rubidium* from Vietnam displayed inhibitory action against *Escherichia coli* ATCC25922 and *Fusarium oxysporum* ATCC48112 with MIC value of 50 μg/mL [13]. In addition, the leaf and stem essential oils of *A. rubidium* from Vietnam showed stronger inhibition of *Pseudomonas aeruginosa* ATCC25923 with MIC of 25 μg/mL and 50 μg/mL respectively, while both essential oils inhibited the growth of *F. oxysporum* ATCC48112 with MIC 50 μg/mL [14]. The results in this study are comparable with data obtained on the antimicrobial actions of other *Amomum* essential oil reported in the literature from other parts of the world. The leaf and fruit essential oils of *A. subulatum* showed good results against *Bacillus pumilus, Staphylococcus. epidermidis, P. aeruginosa* and *S. cerevisiae* [33, 34]. The rind essential oil of *A. subulatum* was only active against *Aspergillus niger* [27]. The essential oil from *A. uliginosum* fruit displayed antibacterial activity against *E. coli* and *S. aureus* [35]. The essential oil of *A. tsao-ko* exerted the strongest bactericidal activity against *S. aureus* [26, 36]. There is a report describing the

antibacterial activity of essential oil from the fruits of *A. kravanh* against some tested microorganisms [37].

Essential oil constituents were previously reported to inhibit significantly the growth and cell viability of potential infections by broad-spectrum microorganisms. The antimicrobial activities of the essential oil *of A. glabrum* can be related to its main compounds or a synergy between the major and some minor constituents. The antimicrobial activity of the essential oil from the leaves and rhizomes of *A. glabrum* may be attributed to the monoterpenic hydrocarbons α-pinene and β-pinene which previously showed antimicrobial activity against broad-spectrum microorganisms [38]. The essential oil constituents such as sabinene, 1,8-cineole, terpinen-4-ol and β-caryophyllene were previously reported to inhibit significantly the growth and cell viability of potential infectious of broad-spectrum microorganisms [39, 40]. The antibacterial activity of β-caryophyllene against *S. aureus* was reported recently [41]. In recent years, greater attention has been paid to the screening of antimicrobial activity from essential oil as a source of developing new antimicrobial agents to combat microbial resistance. Therefore, effort is ongoing in our laboratory to isolate and characterize the specific antimicrobial bioactive principles in these essential oils as well as carry out *in-vivo* antimicrobial assays and object recognition test for the isolated compound(s).

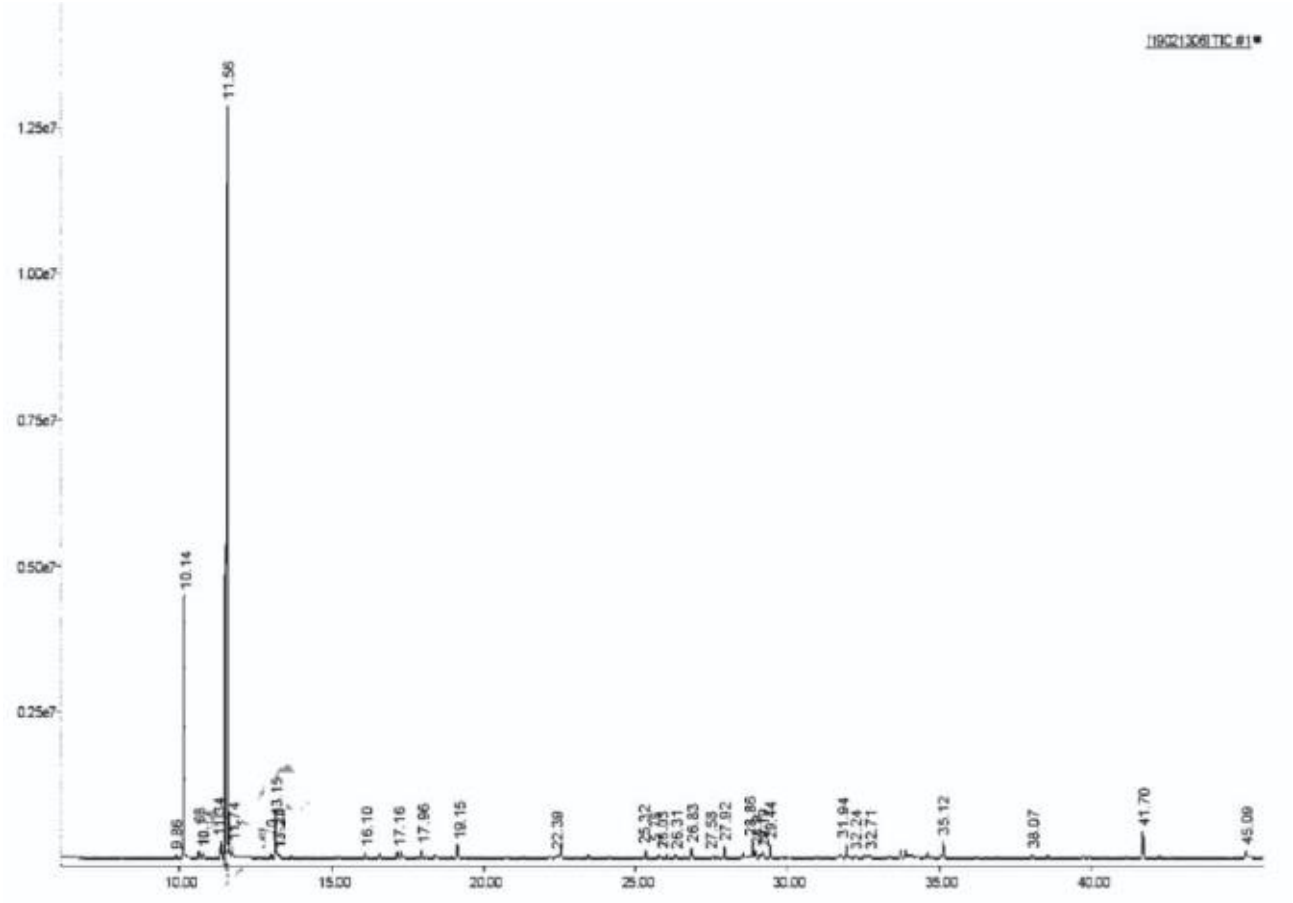

Figure 1. GC chromatogram of the leaf essential oil

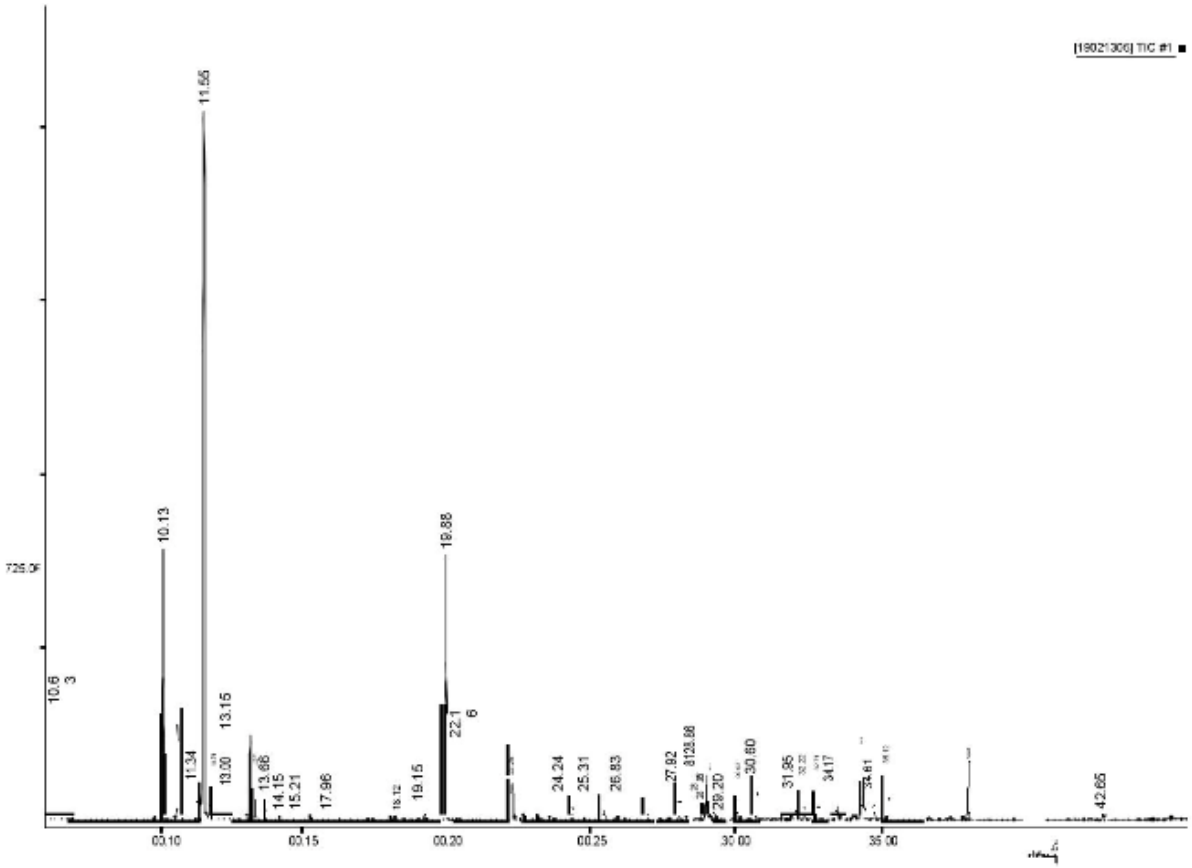

Figure 2. GC chromatogram of rhizome essential oil

Table 1. Chemical constituents of essential oils from the leaf and rhizome of *Amomum glabrum*

Sr. No	RT (mins)	Compounds[a]	RI (Exp.)	RI (Lit.)	Leaves[b]	Rhizomes [b]
1	9.86	α-Thujene	930	921	0.2	-
2	10.14	α-Pinene [c]	939	932	13.1	10.0
3	10.63	Camphene	955	946	0.3	3.8
4	10.77	Thuja-2,4(10)-diene	960	960	0.3	-
5	11.34	Sabinene	978	972	1.0	0.8
6	11.56	β-Pinene [c]	985	988	62.2	43.7
7	11.74	Myrcene	992	990	0.6	0.6
8	13.00	o-Cymene	1030	1028	0.2	0.2
9	13.15	Limonene	1034	1030	1.9	3.3
10	13.20	β-Phellandrene	1035	1032	0.3	0.2
11	13.27	1,8-Cineole	1037	1034	0.2	-
12	13.28	(*Z*)-β-Ocimene	1038	1038	-	0.8
13	13.66	(*E*)-β-Ocimene	1049	1044	-	0.3
14	14.15	γ-Terpinene	1063	1065	-	0.1
15	15.21	Terpinolene	1094	1092	-	0.2
16	16.10	*allo*-Ocimene	1120	1128	0.3	-
17	17.16	*trans*-Sabinol	1150	1148	0.3	-
18	17.96	Pinocarvone	1173	1173	0.4	0.2
19	18.12	Borneol	1177	1177	-	0.2
20	19.15	Myrtenal	1207	1208	0.8	0.2
21	19.88	Fenchyl acetate [c]	1228	1228	-	11.3
22	22.16	Bornyl acetate	1294	1294	-	2.8
23	22.25	Isobornyl acetate	1296	1298	-	0.2
24	22.39	Dihydroedulan [c]	1301	1300	0.1	-
25	24.24	α-Terpinyl acetate	1357	1355	-	0.5
26	25.32	α-Copaene	1389	1389	0.5	0.5
27	25.78	*cis*-β-Elemene	1403	1404	0.2	-
28	26.03	Dodecanal	1411	1412	0.2	-
29	26.31	Aristola-1(10),8-diene [c]	1420	1422	0.1	-
30	26.83	β-Caryophyllene	1437	1437	0.6	0.5

31	27.58	(*Z*)-β-Farnesene	1460	1460	0.2	-
32	27.95	α-Humulene	1471	1471	0.7	0.8
33	28.81	β-Chamigrene	1490	1488	-	0.6
34	28.86	Aristolochene	1501	1500	0.9	2.0
35	28.96	β-Selinene	1505	1505	0.5	0.2
36	29.29	(*E*,*E*)-α-Farnesene	1512	1511	0.5	-
37	29.11	γ-Amorphene	1515	1515	-	0.3
38	29.44	β-Curcumene	1521	1522	0.7	-
39	29.92	7-*epi*-α-Selinene	1537	1537	-	0.5
40	31.95	Caryophyllene oxide	1605	1608	0.8	0.7
41	32.24	Tetradecanal	1615	1616	0.2	-
42	32.22	Guaiol	1616	1618	-	0.6
43	32.71	Humulene epoxide II	1632	1632	0.2	0.6
44	35.12	Pentadecanal	1718	1718	1.0	1.1
45	34.17	*neo*-Intermedeol	1683	1683	-	1.0
46	34.26	Bulnesol	1686	1686	2.1	2.4
47	38.07	γ-Bicyclohomofarnesal [c]	1828	1830	0.3	2.1
48	42.65	(*E*)-15,16-Bisnorlabda-8(17),11-dien-13-one [c]	2013	2008	-	0.3
49	45.09	Phytol [c]	2128	2119	0.4	-
Total					**92.3**	**93.6**
Monoterpene hydrocarbons					**80.4**	**60.4**
Oxygenated monoterpenes					**2.3**	**15.4**
Sesquiterpene hydrocarbons					**3.4**	**5.4**
Oxygenated sesquiterpenes					**4.4**	**7.4**
Diterpenes					**0.4**	**0.3**
Non-terpenes					**1.4**	**1.1**

[a] Elution order on HP-5MS column; [b] Standard deviation were insignificant and excluded from the Table to avoid congestion; [c] Further identification by co-injection with known compounds; RI (Exp.) Retention indices

Table 2. Antimicrobial activity of the essential oils from the leaves and rhizomes of *Amomum glabrum*

Sample	Gram (+)			Gram (-)			Yeast
	Enterococcus faecalis ATCC299212	*Staphylococcus aureus* ATCC25923	*Bacillus cereus* ATCC14579	*Escherichia coli* ATCC25922	*Pseudomonas aeruginosa* ATCC27853	*Salmonella enterica* ATCC13076	*Candida albicans* ATCC10231
			IC_{50} (µg/mL)				
Leaf	8.0 ± 0.10	16.0 ± 0.00	128.0 ± 0.50	-	-	-	8.0 ± 0.50
Rhizome	64.0 ± 0.50	16.0 ± 0.11	32.0 ± 0.26	-	-	-	32.0 ± 0.56
			MIC (µg/mL)				
Leaf	4.23	5.67 ± 0.16	67.98 ± 0.50	-	-	-	1.56 ± 0.00
Rhizome	18.67 ± 0.68	5.67 ± 0.12	9.78 ± 0.50	-	-	-	10.23 ± 0.50

- Not active

Conclusion

This study concludes that α-pinene and β-pinene were the main constituents common to the essential oils hydrodistilled from the leaf and rhizome of *A. glabrum.* In addition, these compounds may be thought as the contributing factor to the observed antimicrobial activity of the essential oils against *E. faecalis, S. aureus, B. cereus* and *C. albicans*. The essential oils may be sources of promising antimicrobial agents.

Abbreviations

GC: Gas chromatography
GC/MS: Gas chromatography/mass spectrometry
SD: Standard deviations
MIC: Minimum inhibitory concentrations
GPS: Global Positioning System
FID: Flame ionization detector
PTV: programmable temperature vaporization
RI: Retention indices
ATCC: American Type Culture Collection
IC50: Median inhibitory concentration
OD: Optical density

Authors' Contribution

LTH and DND designed the study and wrote the protocol. Authors IAO performed the statistical analysis while both NTH, LTH and TMH managed the analyses of the study. Authors IAO' wrote the first and final drafts of the manuscript. 'IAO' and 'DND' managed the literature searches. All authors read and approved the final manuscript.

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Conflict of interest

Authors have declared that no competing interests exist.

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