Indian Journal of Natural Products and Resources
Vol. 11(1), March 2020, pp 30-37

Chemical composition and antibacterial activities of essential oils from *Homalomena pierreana* (Araceae)

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Received 27 April 2019; Revised 3 February 2020

Homalomena is a genus of the Araceae family which contains several remedies used extensively in traditional Vietnamese medicine. *H. pierreana* is a rare plant species of *Homalomena* genus and found only in Phu Quoc National Park, Phu Quoc Island, Kien Giang Province, Vietnam. Therefore, the number of studies about this species is limited and the bioactivity of this species is still unknown. In this study, the chemical composition of essential oils was investigated which was isolated from leaves and rhizomes of *H. pierreana* at the first time by GC-MS. Eight and twelve compounds were identified from the essential oils of rhizomes and leaves, respectively. The major component from both the rhizomes and the leaves was aromadendrene (44 and 48%, respectively). Furthermore, the antibacterial activity of essential oils collected from leaves and rhizomes of *H. pierreana* was investigated and it was observed that the essential oil of rhizomes could inhibit the growth of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, while the essential oil of leaves exhibited an inhibitory effect against *Staphylococcus aureus* and *Escherichia coli.*

Keywords: Antibacterial activities, Essential oils, GC-MS, *Homalomena pierreana*.

IPC code; Int. cl. (2015.01)- A61K 36/00, A61K 36/888, A61K 125/00, A61K 127/00, A61P 31/00

Introduction

In recent years, traditional medicine is an important component of primary health care system in many developing countries like Vietnam because of its safety, effectiveness and inexpensive properties. *Homalomena* is a genus which contains several medicinal plants of the Araceae family and has been used extensively in traditional Vietnamese medicine[1-2]. Many studies have shown the chemical composition, antimicrobial and antioxidant activities of the compounds which are extracted from leaves and rhizomes of several plants of *Homalomena* genus[3-7].

Homalomena genus consists of 250 species occurring primarily in temperate regions of Asia[8]. According to Van[9], five species of the genus have found in Vietnam, including *H. conchinchinensis, H. occulta, H. pendula, H. pierreana,* and *H. vietnamensis*. In 1912, *H. pierreana* was first described by Engler and Krause[10] with the specimens collected in Indochina. However, in Vietnam, Pham-Hoang[1] and Nguyen[11] suggested that *H. pierreana* was grown in the Southern Region of Vietnam but the exact location had not been known and no more specimen of this species were found or reported until 2015. Recently, this species was found again and collected in Phu Quoc National Park in 2015 by Van *et al*[12]. Due to lack of specimens of *H. pierreana*, the number of studies about this species is limited and the bioactivity of this species is still unknown. In this study, the authors identified the chemical composition of essential oils which were isolated from leaves and rhizomes of *H. pierreana* and investigated the antibacterial activity of essential oils of *H. pierreana.*

Materials and Methods

Plant samples

Samples of *H. pierreana* were collected from Phu Quoc National Park, Kien Giang Province, location of

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about 10°21'01"N; 103°06'52"E, 83 m in elevation. The voucher specimen (H.T. Van 109) was deposited at SGN (herbarium of the Southern Institute of Ecology, Vietnam Academy of Science and Technology).

Bacterial strains

Three bacterial strains were used to determine the antibacterial activity. One Gram-positive bacteria, including *Staphylococcus aureus* (ATCC 25923) and two Gram-negative bacteria, including *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were obtained from the microbiology collection, Department of Biotechnology, Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Vietnam. All bacterial strains were maintained at -20 ^{o}C in 20% glycerol solution and inoculated into Luria-Bertani broth at 37 ^{o}C for 24 h to be re-activated again before using in further experiments.

Taxonomic method

Specimens were sampled and processed using conventional methods guided by the Royal Botanic Gardens, Kew[13]. Detailed photos of taxonomically important characters of the species were taken from fresh materials in the field using digital camera. We examined aroid specimen collections which were housed at SGN, HN, HNU, and VNM. Digital images of related taxa at P and K were also consulted. Species identification was done by comparison of morphological vegetative and reproductive characteristics[1,9,11-12].

Distillation of the essential oils

Essential oils were obtained by hydrodistillation which was carried out in an all glass Clevenger-type distillation unit. Briefly, fresh samples of leaves or rhizomes of *H. pierreana* (500 g) were pulverized and introduced into a 5 L flask; subsequently, distilled water was added until it covered the samples completely. Samples were subjected to hydrodistillation at 100 ^{o}C for 3 hours with normal pressure. The volatile oils distilled over water and were collected in the receiver arm of the apparatus into clean and dark bottles. The oils were kept under refrigeration until further experiments. The oil yields were determined using the equation

$$RO = M/B_m \times 100\%,$$

where M is the weight of the extracted oil (g) and B_m is the initial leaf biomass (g)[14].

Gas chromatography-mass spectrometry (GC-MS) analysis

Gas chromatography (GC) analysis was performed by an Agilent Technologies HP 6890 Plus Gas chromatograph which was equipped with a flame ionization detector (FID). The column was an HP-5MS (30 m x 0.25 mm) with film thickness 0.25 μm. Hydrogen was the carrier gas at a flow rate of 1 mL/min. The injector and detector temperatures were maintained at 250 and 260 ^{o}C, respectively. The column temperature was programmed from 40 ^{o}C with a 2 minutes hold to 220 ^{o}C with 10 minutes hold at 4 ^{o}C/min. Oil samples were injected into the GC by splitting method with the split ratio of 100:1, while 1.0 mL of each individual oils was injected into the GC. The inlet pressure was maintained at 6.1 kPa. Each analysis was performed in triplicate. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using correction factors.

An HP 6890N Plus Chromatograph (Agilent Technologies) which was fitted with a HP-5MS column (30 m x 0.25 mm, film thickness 0.25 μm) and interfaced with a mass spectrometer HP 5973 MSD was used for the GC-MS analysis. The analytical conditions were the same as used for GC analysis, except that He (1 mL/min) was used as the carrier gas. The ionization voltage was 70 eV with the emission current of 40 mA. The acquisitions scan mass range of MS was 35-350 amu with the sampling rate of 1.0 scan/s.

Antibacterial activities

The antibacterial assay of essential oils from rhizomes and leaves of *H. pierreana* was performed using Bauer *et al.* method[15]. The bacteria were cultured in Luria-Bertani broth until reached a turbidity of 0.5 McFarland standard; The bacterial suspensions (100 μL) were spread on sterile Mueller Hinton plate and a sterile 6 mm diameter discs were placed on the inoculated surface. A 20 μL of sample was pipetted onto each disc and the plates were maintained at 4 ^{o}C for 2 hours to allow oil diffusion into the medium. The plate was incubated at 37 ^{o}C for 24 hours and the antibacterial activity of sample was evaluated by measuring the diameter of the zone of inhibition. Sterilize distilled water was used as negative control and Gentamycin antibiotic discs (Nam Khoa BioTek, Vietnam) were used as positive control.

Results

Taxonomic treatment

Homalomena pierreana Engl. & K. Krause (Plate 1). Medium-sized, evergreen, ca. 10 cm tall.

Plate 1 – *Homalomena pierreana*. a) Habitat and leaf blade b) Rhizome c) Cross section of rhizome d) Inflorescences and spathe e) Spadix.

Stem 8–14 cm long, 1.5–2.0 cm in diameter. Leaves 6–8; petiole 10–15 cm long, 0.4 cm in diameter, green-grey. Leaf blade 8–10 cm long, 3–5 cm wide, triangular or hastate, apex cuspidate, dark green adaxially, pale green abaxially, midrib impressed adaxially and prominent abaxially, lateral veins diverging from the midrib and then towards leaf margin. Inflorescences 2–5; peduncle much shorter than petiole, 4–5 cm long, 5 mm in diameter, grey to brown. Spathe longer than spadix, green at young, pale yellow at anthesis, elliptical, apex cuspidate. Spadix shorter than spathe, 2.5–3.0 cm long, conical, white-green; female part 8 mm long, cylindrical; ovaries bottle-shaped, 3-loped, pale green, ca. 2 mm tall, ca. 1 mm in diameter, ovules hemianatropous, many; staminode white, subcylindrical to slightly clavate; style 0.5 mm long; stigma globose, 0.5 mm in diameter, pale green. Male part 2.0–2.5 cm long, conical, apex cuspidate, density arranged, anthers dehiscent by long slits at apex.

Type: Pierre sine num (P, isotype), Cochinchina.

Studied specimens: Van Long Ha and Hong Thien Van H.T. Van 109 (SGN!), Phu Quoc National Park, Kien Giang Province, 11 August 2018, about 10°21'01"N; 103°06'52"E, 83 m in elevation; H.T. Van 108, Phu Quoc National Park, Kien Giang Province; Pierre sine num (P!, seen images), Cochinchina; Pierre sn. (K!, seen images), Cochinchina.

Essential oil composition

The studied essential oils from rhizomes and leaves of *H. pierreana* were obtained in a yield of 0.04 and

0.24%, respectively, calculated on a dry weight basis. By using the GC-MS analysis, we identified 8 and 12 compounds which existed in rhizomes and leaves of *H. pierreana* (Table 1 and Fig. 1). From the results presenting in Table 1, the main classes of compounds identified in rhizomes of this species were monoterpene hydrocarbons (4.1%), sesquiterpene hydrocarbons (72.7%). These main compounds were also found in leaves (monoterpene hydrocarbons (7.1%), sesquiterpene hydrocarbons (77.4%). The composition of leaves other than rhizomes is in the presence of oxygenated sesquiterpenes (0.32%). The constituents occurring in higher quantity in the leaves of *H. pierreana* were six compounds (δ^3-carene,

Table 1 — Phytochemical constituent of essential oil in rhizome and leaves of *H.pierreana*

S. No.	Retention time (min)	Compound name	Percentage (%) of rhizomes	Percentage (%) of leaves
1	8.74	δ^3-carene	1.6	1.5
2	10.25	2-β-pinene	1.3	1.0
3	12.12	Limonene	1.2	1.6
4	14.14	α-terpinolen	–	3.0
5	22.90	α -bergamotene	–	0.55
6	22.98	β-elemene	1.3	–
7	23.77	aromadendrene	44.0	48.0
8	24.20	bicycloelemen	1.4	1.7
9	24.70	cycloundecatriene	7.5	–
10	24.55	δ-selinene	18.5	13.5
11	24.66	Trans-α-Bisabolene	–	12.0
12	25.26	γ-curcumene	–	1.4
13	26.00	β-Bisabolene	–	0.2
14	29.70	3-Cyclohexen-1-ol, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	–	0.32
		Total	76.8	84.8
		Monoterpene hydrocarbons	4.1	7.1
		Oxygenated mooterpenes	–	–
		Sesquiterpene hydrocarbons	72.7	77.35
		Oxygenated sesquiterpenes	–	0.32

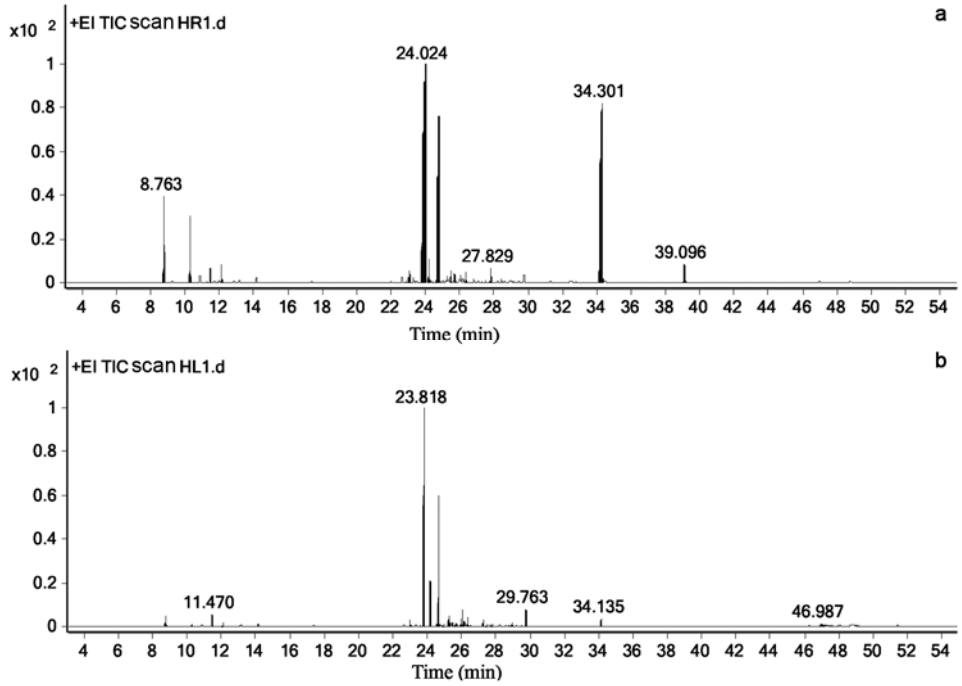

Fig. 1 — GC chromatogram of *H. pierreana* essential oils, a) Rhizome and b) leaf.

2-β-pinene, limonene, aromadendrene, bicycloelemen, δ-selinene) were found in both rhizomes and leaves of this species whereas two compounds (β-elemene and cycloundecatriene) were identified only in essential oil collected from rhizomes, and six compounds (α-terpinolen, α -bergamotene, Trans-α-Bisabolene, γ-curcumene, β-Bisabolene and 3-Cyclohexen-1-ol, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-) were only detected in essential oil collected from leaves. Among them, aromadendrene had highest percentage in both essential oils collected from rhizomes and leaves (44.0 and 48.0%, respectively). Although existing in both rhizomes and leaves, there was a remarkable difference in percentage of δ-selinene in essential oils from rhizomes and leaves (18.5 and 13.5%, respectively). Furthermore, cycloundecatriene and β-elemene were two compounds which were only found in essential oils from rhizomes but not from rhizomes and accounted for 7.5 and 1.3%, respectively. On the other hand, Trans-α-bisabolene (12.0%) was found only in essential oil isolated from leaves but not from rhizomes.

Antibacterial activity

Essential oils from rhizomes of *H. pierreana* showed the inhibition of the growth of three tested bacteria (Table 2 and Plate 2). We observed that essential oils of *H. pierreana* rhizomes exhibited the highest antibacterial activity against *S. aureus* with inhibition zone about 15.0±2.5 mm, while the inhibition zone of *E. coli* and *P. aeruginosa* were 13.0±3.5 mm, and 7.6±0.1 mm, respectively. Additionally, the essential oil from leaves of *H. pierreana* showed the inhibitory effect against two bacterial strains, such as *S. aureus* (12.0±3.5 mm) and *E. coli* (11.0±3.0 mm) (Table 3 and Plate 2). Based on analysis of oil composition, the antibacterial activity of the oil could be offered the presence of bioactive compounds such as aromadendrene, limonene, 2-β-pinene. In chemical composition of essential oil from rhizomes and leaves, aromadendrene accounted for highest percentage (44 and 48%, respectively).

Discussion

The bioactivities of some main compounds identified in the essential oils isolated from the species in this study have been documented in previous studies. Several studies have reported functions and bioactivities of aromadendrene. In plant, terpene such as aromadendrene contributes to its particular smell and flavor. Additionally, antioxidant and antibacterial activities of aromadendrene have been well documented in several reports. In previous study, Bombarda *et al*[16] suggested that aromadendrene in essential oil of *Melaleuca quinquenervia* had a strong antioxidant activity[16]. These results are in line with results from Mulyaningsih *et al*[17-18] studies, in which aromadendrene isolated from essential oil of some species of *Eucalyptus* genus showed antibacterial effect against many pathogenic bacteria such as *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa,* and *Acinetobacter*. Similarly, many recent studies demonstrated that the essential oil of *Pogostemon cablin contained* aromadendrene as a major component was able to resist against pathogenic bacteria (*K. pneumonia*, *H. pylori*, *E. coli*, *B. subtilis*, *S. aureus*, *P. aeruginosa*, *E. faecalis)*[19-24], fungi (*Aspergillus species,* C. albicans)[25-26] and *Influenza A (H2N2) virus*[27-29].

Furthermore, Leite *et al*[30] also proved that β-pinene could inhibit the growth of Gram positive bacteria which belonged to *Streptococcus* and *Staphylococcus* genus including *S. aureus,S. epidermidis*, *S. pyrogenes*, and *S. pneumoniae*. On the other hand, limonene, a favor ingredient widely used perfume, cream, soaps, food products, also exhibits strong antibacterial effect against *E. coli*. According to Espina *et al*[31] limonene exhibits not only the bacteriostatic activity but also the bactericidal activity through alteration of phospholipid and lipopolysaccharide cell fraction (in neutral condition) and protein fraction (in acidic condition). Moreover, limonene can combine with other food preservation technologies to generate a synergistic inhibition effect on the growth of bacteria. The presence of limonene in chemical composition of oil may explain the inhibitory effect of essential oil on *E. coli* growth and suggests the potential use of essential oils as food preservatives[31].

In addition, the antibacterial effect of essential oil against *S. aureus and P. aeruginosa* implies that the essential oil could apply into dermatology as a remedy to treats the skin diseases, and prevention of bacterial infection as well as their cross-infection in hospital[31]. Recently, *Silva et al.*[32] showed that essential oil of *Psidium guajava contained δ-selinene* as a main constituent was resistant to 5 *Streptococcus*

Table 2 — The inhibition zone of essential oils from rhizomes of *H. pierreana* against four test bacteria

Test bacteria	Growth inhibition zone (mm)
E. coli	13.0±3.5
S. aureus	15.0±2.5
P. aeruginosa	7.6±0.1

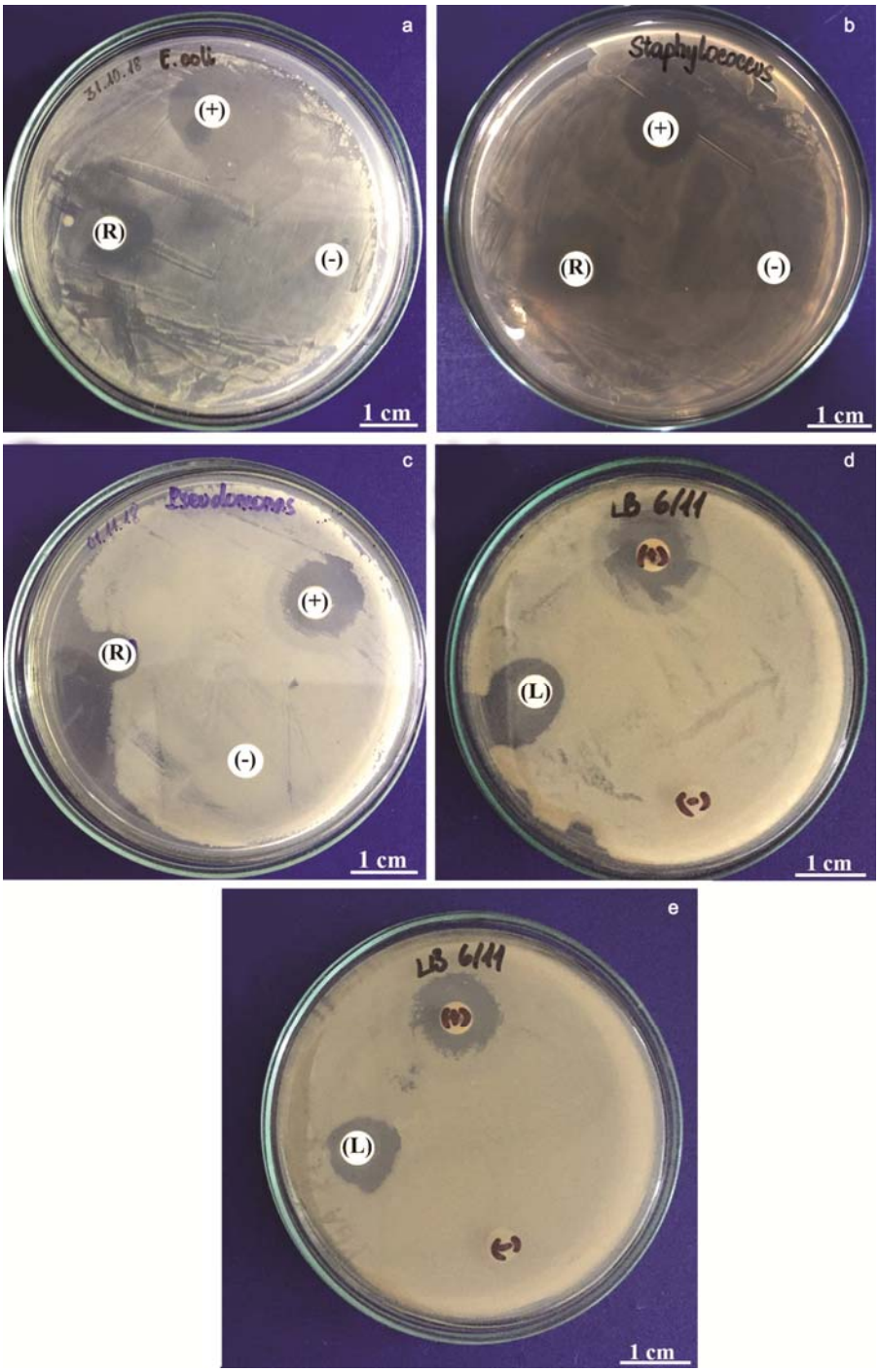

Plate 2 – Antibacterial activity of essential oils from rhizomes and leaves of *H. pierreana* against test bacteria, a) *E. coli* (rhizome), b) *S. aureus* (rhizome), c) *P. aeruginosa* ((rhizome), d) *S. aureus* (leaf), e) *E. coli* (leaf), (-) Negative control with sterilized distilled water, (+) Positive control with discs containing gentamicin, (R) sample of essential oil of rhizome, (L) sample of essential oil of leaf.

Table 3 — The inhibition zone of essential oils from leaves of *H.pierreana* against four test bacteria

Test bacteria	Growth inhibition zone (mm)
E. coli	11.0±3.0
S. aureus	12.0±3.5

strains, including *S. mutans, S. mitis,S. sanguinis, S. sobrinus, and S. salivarius.*

Conclusion

In this study, we identified 8 and 12 compounds in essential oils isolated from rhizomes and leaves of *H.*

pierreana, and it was observed that aromadendrene had the highest abundance in both the rhizomes and the leaves (44 and 48%, respectively). Furthermore, we investigated the antibacterial activity of essential oil collected from rhizomes of *H. pierreana* and proved that the essential oils could inhibit the growth of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa.* This finding will provide the information about bioactivity of *H. pierreana* and applications of this species in future.

Acknowledgement

The authors are thankful to the staff of Phu Quoc National Park, especially Mr. Van-Long Ha for his help in the field. The authors also thank Dr. Dieu-Hanh Thi Nguyen and Ms. Hoang-Yen Nguyen Ho for their helpful cooperation.

Conflict of interest

The authors declare that there are no conflicts of interest.

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