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CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITIES OF *HOMALOMENA VIETNAMENSIS* BOGNER & V. D. NGUYEN (ARACEAE)

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ABSTRACT

Homalomena vietnamensis is a rare species of the *Homalomena* genus and only found in Middle region of Vietnam. In this study, we found 10 compounds in ethanol extracts of leaf and rhizome of *H. vietnamensis*, such as cadinane-4β,5α,10α-triol, *oplopanone*, 4-epi-oplopananol, 2α-hydroxy homalomenol A, 1β,4β,7β-Trihydroxyeudesmane, homalomentetraol, 4-acetoxyoplopananol, 5,7-diepi-2a-acetoxyoplopanone, eudesma 4β, 7α- diol-1β-fumarate), and homalomenol F, via liquid chromatography-mass spectrometry (LC/MS). Moreover, the antibacterial activity of ethanol extracts of leaf and rhizome from this species has been evaluated by disc diffusion method for the first time. The results showed that rhizome extract of could inhibit the growth of 5 tested micro-organisms, including of *Bacillus cereus* (28.3 ± 1.5 mm), *Salmonella enteritidis* (19.5 ± 1.5 mm), *Staphylococcus aureus* (16.3 ± 1.5 mm), *Escherichia coli* (14.7 ± 1.2 mm), and *Pseudomonas aeruginosa* (8.2 ± 0.8 mm), while the leaf extract showed antibacterial effect against *Bacillus cereus* (22.0 ± 2.0 mm), *S. enteritidis* (14.7 ± 0.6 mm), and *S. aureus* (12.5 ± 1.8 mm).

Keywords: ethanol extracts, antibacterial activities, LC/MS, *Homalomena vietnamensis*

INTRODUCTION

Homalomena Schott is a genus of the Araceae family and comprises of 250 species growing over the world **(Boyce *et al.*, 2012)**. Several members of the *Homalomena* genus are extensively used as traditional remedies in Vietnamese medicine **(Pham, 2000)**. Chemical composition, antimicrobial and antioxidant activities of the compounds extracted from many species of *Homalomena* genus have been well-documented in literature **(Singh *et al.*, 2000; Rana *et al.*, 2009; Liliwirianis *et al.*, 2011; Yang *et al.*, 2016)**. For example, the essential oil of *H. aromatica* contains 55 compounds, such as linalool, terpene-4-ol, δ-cadinene, T-muurolol, viridiflorol, α-cadinol, α-selinene, M-cymene, γ-Muurolene, and spatulenol… and exhibits a strong antifungal effect against dermatophytes and yeasts, such as *Trichophyton rubrum, Trichophyton mentagrophytes, Microsporum fulvum, Microsporum gypseum, Trichosporon beigelii* and *Candida albicans* (**Policegoudra *et al.*, 2012**). Furthermore, *H. aromatica* oil also has antibacterial activity against five common and significant pathogens such as *S. aureus*, *E. coli*, *P. aeruginosa*, *Klebsiella pneumoniae*, and *Proteus vulgaris* (**Laishram *et al.*, 2006**). These data suggest the essential oil of *H. aromatica* as a potential antimicrobial agent or the bioactive component of pharmaceutical preparations. According to **Hu *et al.* (2008)**, some sesquiterpenoids from *H. occulta*, such as oplodiol, oplopanone, homalomenol C, bullatantriol, could stimulate osteoblast proliferation and differentiation, whereas chloroform extract and oplodiol enhance osteoblast mineralization. Recently, **Eldeen *et al.* (2016)** also shows that diacylglycerolglycolipid isolated from *H. sagittifolia* possesses the strong anti-inflammatory and anticholinergic effects, as well as hinders the growth of two Gram negative bacteria, including *Klebsiella pneumonia* and *Pseudomonas stutzeri*.

In Vietnam, five species of this genus are recorded, including *H. pierreana*, *H. vietnamensis*, *H. occulta*, *H. pendula* and *H. tonkinensis* **(Van *et al*, 2017)**. Among them, *H. vietnamensis* is an extremely rare species and is described for the first time by Bogner and Nguyen in Bach Ma National Park, Thua Thien-Hue province, Vietnam (**Bogner and Nguyen, 2008)**. Nowadays, the presence of this species also has only been recorded in some provinces in Middle region of Vietnam, such as Thua Thien-Hue, Khanh Hoa and Quang Nam Provinces, Vietnam (**Bogner and Nguyen, 2008).** Due to the limit of the number of specimens, the bioactivity this species is still unknown. In this study, we identifies the chemical composition and proves the antibacterial activity of ethanol extracts of leaf and rhizome from this species for the first time, which will support the information for further application of this species in future.

MATERIALS AND METHODS

Plant material

Specimens (leaves and rhizomes) of *H. vietnamensis* were collected from Nam Tra My District, Quang Nam Province, location of about 15°07'22"N; 108°08'27"E, April 6, 2019, 426 m in elevation (Figure 1).

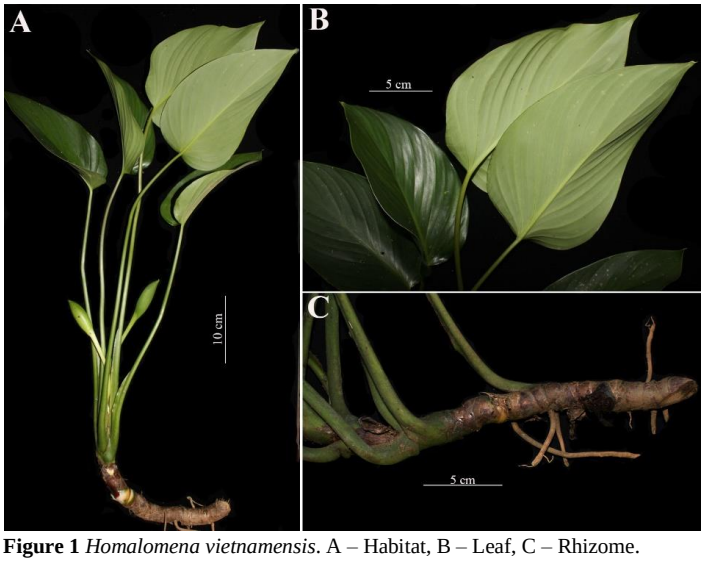

Figure 1 *Homalomena vietnamensis*. A – Habitat, B – Leaf, C – Rhizome.

Bacterial strains

Five bacterial strains, including two Gram-positive bacteria, *Bacillus cereus* (ATCC 11774) and *Staphylococcus aureus* (ATCC 25923), and three Gram-negative bacteria, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enteritidis* (ATCC 13976), were used to evaluate the antibacterial activity of ethanol extracts. Microorganisms were kindly provided from the microbiology collection, Department of Biotechnology, Institute of Food and Biotechnology, Industrial University of Ho Chi Minh city, Viet Nam. All bacterial strains were cultured in Luria-Bertani broth at 37ºC for 24 h to be re-activated again before using in further experiments.

Extraction procedure

Fresh rhizomes of *H. vietnamensis* were peeled and subsequently cut into slices. The fresh leaves and sliced rhizomes were moderate dried at 50-55ºC until masses of samples were unchanged. The samples were pulverized by an electric grinder into fine powder and kept at 4ºC. 50 g of the dried powder of leaves and rhizomes of *H. vietnamensis* were immersed with 450 mL of 98% ethanol for 5 weeks. The extracts were filtrated via Whatman filter paper, and subsequently concentrated in reduced pressure at 60ºC until the residue remained ½ volume of the initial filtrate **(Altemimi *et al.*, 2017)**, then sublimation drying was performed to remove ethanol in extracts. The obtained residue was stored at 4ºC until further use.

Liquid chromatography mass spectrometry (LC/MS)

Ethanol extracts were sent to the Central Laboratory for Analysis, University of Science, Vietnam National University of Ho Chi Minh City to conduct LC/MS analysis and elucidate the chemical composition of ethanol extract. In brief, aliquot of ethanol extract was injected to HPLC Agilent 1200 infinity liquid chromatography system (Agilent Technologies, CA, USA) coupled with MicroTOF-QII mass spectrometer (Bruker Daltonics, Germany). The chromatographic separation was carried out in an ACE3- C_{18} analytical column (4.6 ×150 mm, 3.5 µm). In mobile phase, deionized water with 0.1% formic acid was used as solvent A and acetonitril with 0.1% formic acid was used as solvent B. Gradient elution program for the chromatographic separation was presented in Table 1 with the flow rate at 0.3 mL/min. The mass spectrometer was implemented with electrospray ionization source (ESI) at positive mode and mass spectra data were recorded for a mass range 50-2000 m/z. Data analysis was performed using Data Analysis software (Bruker, Germany). To determine the compounds in extract, the mass spectra of compounds were compared with mass spectra of reference compounds which were identified in other species of *Homalomena* genus from previous studies (**Wang *et al.*, 2007; Hu *et al.*, 2008; Xie *et al.*, 2012; Wong *et al.*, 2012; Zhao *et al.*, 2016; Yang *et al.*, 2016).**

Table 1 Gradient elution program for the chromatographic separation

Time (min)	Solvent A*	Solvent B*
0	90	10
15	0	100
30	0	100
31	90	10
40	90	10

(*): presented as the percentage of volume of mobile phase

Antibacterial activities

The antibacterial activity of ethanol extracts of leaf and rhizome of *H. vietnamensis* was analyzed according to Bauer protocol **(Bauer *et al.*, 1996)**. The bacteria were inoculated in LB Broth until reached a turbidity of 0.5 McFarland standard. 100 µl of bacterial suspensions were inoculated on Mueller Hinton plate, and a sterilized 6 mm diameter disc was placed on the plate. 10 µl of sample were put onto each disc and the plate was kept at 4ºC for 2 hours to fully diffuse extract into the medium. Diameters of zones of inhibition of extracts against tested bacteria were observed and measured after inoculation at 37ºC for 24 hours. Sterile distilled water was used as negative control and Gentamycin antibiotic disc (Nam Khoa BioTek, Viet Nam) was used as positive control.

Data analysis

The experiments were repeated in triplicate. The average and standard deviation of measurements were calculated using The Excel 2010 software. The data of experiments were expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSIONS

Ethanol extract composition

Based on comparison of mass of compounds (m/z) which were identified in other species of *Homalomena* genus in previous studies, we determined 10 compounds belonging to sesquiterpenoids in rhizome and leaf of *H. vietnamensis*, including of cadinane-4β,5α,10α-triol, *oplopanone*, 4-epi-oplopananol, 2α-hydroxy homalomenol A, 1β,4β,7β-Trihydroxyeudesmane, homalomentetraol, 4-acetoxyoplopananol, 5,7-diepi-2a-acetoxyoplopanone, eudesma 4β, 7α- diol-1β-fumarate, and homalomenol F (Figure 2 and Table 2). Among them, homalomenol F, 4-epi-oplopananol, 5,7-diepi-2a-acetoxyoplopanone, eudesma 4β, 7α- diol-1β-fumarate were found in both rhizome and leaf of *H. vietnamensis* whereas cadinane-4β,5α,10α-triol, *oplopanone*, 2α-hydroxy homalomenol A, 1β,4β,7β-trihydroxyeudesmane, and homalomentetraol were only found in ethanol extract of rhizome. On the other hand, leaf extract contained 4-acetoxyoplopananol which was not found in rhizome extract.

Moreover, most of 10 compounds determined in leaf and rhizome extract of *H. vietnamensis* were found extracts of rhizome and leaf of *H. oculta*, another member of *Homalomena* genus which is widely distributed and commonly used medicinal plant in several Asian countries **(Pham, 2000; Van, 2017; *Hu et al.*, 2008; Xie *et al.*, 2012; Yang *et al.*, 2016; Zhao *et al.*, 2016)**. For example, 1β,4β,7β-trihydroxyeudesmane and Homalomentetraol were 2 compounds identified from ethanol extracts of leaf and petioles of *H. occulta* **(Wang *et al.*, 2007)**. Cadinane-4β,5α,10α-triol, Homalomenol F, 4-epi-oplopananol, 2α-hydroxy homalomenol A, 5,7-diepi-2a-acetoxyoplopanone, and Eudesma 4β, 7α-diol-1β-fumarate were 6 compounds identified from rhizome extract of *H. occulta* **(Hu *et al.*, 2008; Xie *et al.*, 2012; Yang *et al.*, 2016; Zhao *et al.*, 2016)**. Furthermore, Wong et al. also found o*plopanone, a member of* sesquiterpenoids, in methanol extract of rhizome *H. sagittifolia (***Wong *et al.*, 2012)**.

All of 10 compounds identified in *H. vietnamensis* rhizome and leaf extracts are the members of sesquiterpenoids, a group of several bioactive compounds. According to **Chadwick *et al.* (2013)**, sesquiterpenoids lactones, a class of sesquiterpenoids containing lactone ring in its structure, have anti-inflammatory and anti-cancer effects and are used to treat several diseases such as diarrhea, influenza, neurodegradation, and cardiovascular diseases. Furthermore, antimicrobial, antitumor, and cytotoxic effects of sesquiterpenoids have been documented **(Chen *et al.*, 2011).**

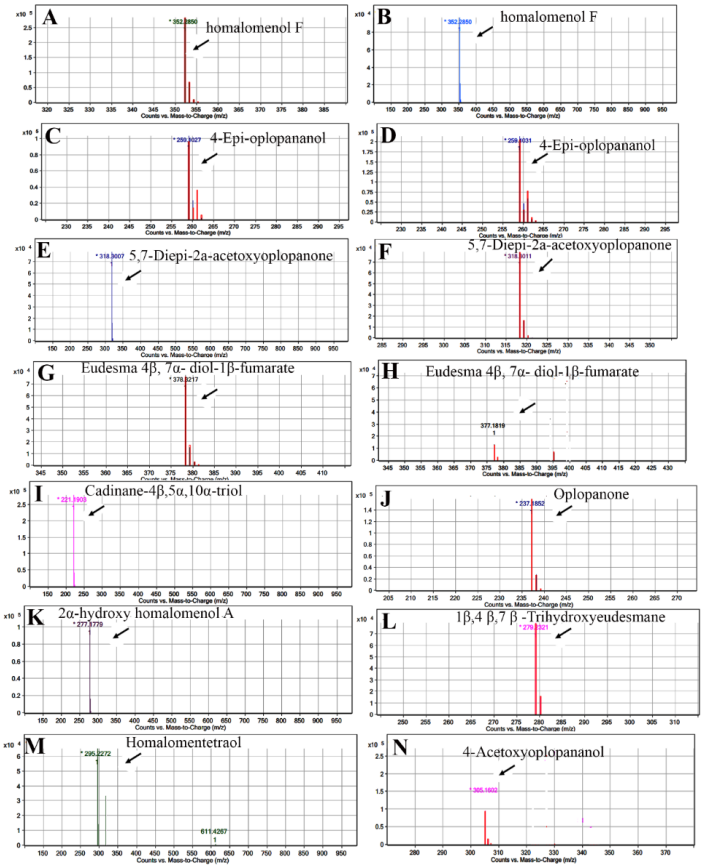

Figure 2 Mass spectrometry diagrams of 10 compounds of ethanol extracts of leaf and rhizome of *H. vietnamensis*. A, C, E, G, I, J, K, L, M are compounds of rhizome, B, D, F, H, N are compounds of leaf.

Antibacterial activity

Antibacterial activity of ethanol extracts from rhizome and leaf of *H. vietnamensis* was evaluated by the diameter of inhibition zone against tested bacteria (Table 3 and Figure 3). Ethanol extract of rhizome of this species showed the antibacterial effect against 5 tested microorganisms while those from leaf inhibited the growth of 3 bacterial strains, including of *B. cereus*, *S. enteritidis*, and *S. aureus*. We observed that diameters of inhibition zones of the

rhizome extract against *B. cereus*, *S. enteritidis*, *S. aureus*, *E. coli*, and *P. aeruginosa* were 28.3 ± 1.5, 19.5 ± 1.5, 16.3 ± 1.5, 14.7 ± 1.2, and 8.2 ± 0.8 mm, respectively. On the other hand, the leaf extract showed the strongest antibacterial effect against *B. cereus* (22.0 ± 2.0 mm), following by *S. enteritidis* (14.7 ± 0.6 mm) and *S. aureus* (12.5 ± 1.8 mm). The results suggested that antibacterial effect of rhizome extract was stronger that of leaf extract, both the number of bacterial strains and the diameter of inhibition zone of each strain.

Table 2 Phytochemical composition of ethanol extracts of rhizome and leaf of *H. vietnamensis*

Compounds		m/z	References
Rhizome	Leaf		
Cadinane-4β,5α,10α-triol	-	221	Xie et al. (2012)
Oplopanone	-	237	Wong et al. 2012
Homalomenol F	Homalomenol F	352	Hu et al. (2008)
4-epi-oplopananol	4-epi-oplopananol	259	Yang et al. (2016)
2α-hydroxy homalomenol A	-	277	Zhao et al. (2016)
1β,4β,7β-trihydroxyeudesmane	-	279	Wang et al. (2007)
Homalomentetraol	-	295	Wang et al. (2007)
-	4-acetoxyoplopananol	305	Yang et al. (2016)
5,7-diepi-2a-acetoxyoplopanone	5,7-diepi-2a-acetoxyoplopanone	318	Yang et al. (2016)
Eudesma 4β, 7α-diol-1β-fumarate	Eudesma 4β, 7α-diol-1β-fumarate	378	Zhao et al. (2016)

Some previous studies have proven the antibacterial activity of methanol and ethanol extracts of rhizome of some plant species of the *Homalomena* genus. For example, Liliwirianis *et al*. suggested that methanol extract of rhizome of *H. propinque* collected at Pahang Natural Reserve, Malaysia exhibited the antibacterial effect against 3 tested bacteria, such as *E. coli*, *B. subtilis*, and *S. aureus* **(Liliwirianis *et al.*, 2011)**. Those results are in line with the results from Wong *et al*. study, in which ethanol extract of rhizome of *H. sagittifolia* collected in China inhibited the growth of 2 Gram positive bacteria, including of *B. subtilis* and *S. aureus*, as well as 3 Gram negative bacteria, such as *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas stutzeri* **(Wong *et al.*, 2012)**. Furthermore, methanol extracts of rhizome and leaf of *H. aromatica*, another member of the *Homalomena* genus, also showed the antibacterial activity against 3 bacteria, such as *B. subtilis*, *E. coli*, and *S. aureus*, and the anti-fungal effect against 3 fungal strains, including *Aspergillus niger*, *Fusarium moniliforme*, and *Candida albicans* ***(Talukdar and Baruah, 2015).*** *Of note, some of identified compounds in rhizome and leaf of H. vietnamensis* also showed antibacterial effect in previous studies. In previous study, **Wang *et al.* (2007)** indicated that 1β,4β,7β-trihydroxyeudesmane could inhibit the growth of pathogenic bacteria, *Streptococcus pneumoniae and Mycobacterium tuberculosis, while* Homalomentetraol inhibited the growth of *Mycobacterium tuberculosis.*

Table 3 The inhibition zone of ethanol extract from rhizome and leaf of *H. vietnamensis* against five tested bacteria

Tested bacteria	Zone of inhibition (mm)	
	Rhizome	Leaf
Bacillus cereus	28.3 ± 1.5	22.0 ± 2.0
Escherichia coli	14.7 ± 1.2	-
Pseudomonas aeruginosa	8.2 ± 0.8	-
Salmonella enteritidis	19.5 ± 1.5	14.7 ± 0.6
Staphylococcus aureus	16.3 ± 1.5	12.5 ± 1.8

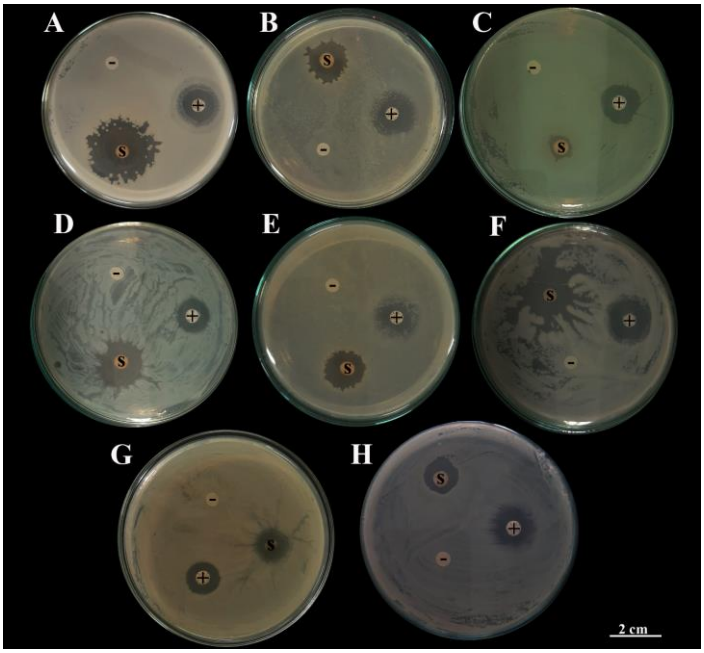

Figure 3 Antibacterial activity of ethanol extracts from rhizome and leaf of *H. vietnamensis* against tested bacteria.
Rhizome. A – *B. cereus*, B – *E. coli*, C – *P. aeruginosa*, D – *S. enteritidis*, E – *S. aureus*. Leaf. F – *B. cereus*, G – *E. coli*, H – *S. aureus*. (-) Negative control with sterilized distilled water, (+) Positive control with discs containing gentamycin, (S) sample of ethanol extract.

CONCLUSION

In this study, 10 compounds of sesquiterpenoids are determined in rhizome and leaf of *H. vietnamensis*, including of cadinane-4β,5α,10α-triol, *oplopanone*, 4-epi-oplopananol, 2α-hydroxy homalomenol A, 1β,4β,7β-Trihydroxyeudesmane, homalomentetraol, 4-acetoxyoplopananol, 5,7-diepi-2a-acetoxyoplopanone, eudesma 4β, 7α- diol-1β-fumarate, and homalomenol F. Moreover, ethanol extract of rhizome of this species showed the antibacterial effect against 5 tested microorganisms (*B. cereus*, *E. coli*, *P. aeruginosa*, *S. enteritidis*, and *S. aureus*) whereas leaf extract just inhibited the growth of 3 bacteria strains, such as *B. cereus*, *S. enteritidis*, and *S. aureus.*

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