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

Hong Thien Van1,*, Quang Phuc Nguyen1, Gia Bua Tran1, Nguyen Tuong An Huynh2

Address(es): Hong Thien Van, PhD.
1Institute of Biotechnology and Food-technology, Industrial University of Ho Chi Minh City, 12 Nguyen Van Bao Street, Go Vap District, Ho Chi Minh City, Vietnam.
2Office of Postgraduate Management, Industrial University of Ho Chi Minh City, 12 Nguyen Van Bao Street, Go Vap District, Ho Chi Minh City, Vietnam.

*Corresponding author: vanthien@iuh.edu.vn

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, homalomenetraol, 4-acetoxypolopanol, 5,7-diepi-2α-acetoxypolopanol, 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; Liliwiranis et al., 2011; Yang et al., 2016). For example, the essential oil of H. aromatica contains 55 compounds, such as linalool, terpene-4-ol, β-cadinen, α-muurolol, α-cadinen, α-selinene, M-cymene, γ-Muurolene, and spathulenol… and exhibits a strong antifungal effect against dermatophytes and yeasts, such as Trichophyton rubrum, Trichophyton mentagrophytes, Microsporum fulvum, Microsporum gypseum, Trichophoron beigeli 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, bullantanniol, could stimulate osteoblast proliferation and differentiation, whereas chloroform extract and oplodiol enhance osteoblast mineralization. Recently, Eldeen et al. (2016) also shows that dicyclergolglycolglycolipid 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°30’27”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 29213), 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 37°C 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 moderately dried at 50-55°C until masses of samples were unchanged. The samples were pulverized by an electric grinder into fine powder and kept at 4°C. 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 filtered via Whatman filter paper, and subsequently concentrated in reduced pressure at 60°C until the residue remained ¼ 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 4°C 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 ACE5- C18 analytical column (4.6 x 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 the 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          |
| 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 4°C 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 37°C 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, *oplopamine*, 4-epi-oplopolan, 2α-hydroxy homalomenol A, 1β,4β,7β-trihydroxyeudesmane, homalomentetraol, 4-acetoxyoplopan, 5,7-diepi-2α-acetoxyoplopan, eudesma 4β, 7α- diol-1β-fumarate, and homalomenol 5 (Figure 2 and Table 2). Among them, homalomenol F, 4-epi-oplopolan, 5,7-diepi-2α-acetoxyoplopan, eudesma 4β, 7α-diol-1β-fumarate were found in both rhizome and leaf of *H. vietnamensis* whereas cadinane-4β,5α,10α-triol, *oplopamine*, 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-acetoxyoplopanol 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. oculata*, 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. oculata* (*Wang et al.*, 2007). Cadinane-4β,5α,10α-triol, Homalomenol F, 4-epi-oplopolan, 2α-hydroxy homalomenol A, 5,7-diepi-2α-acetoxyoplopan, and Eudesma 4β, 7α-diol-1β-fumarate were 6 compounds identified from rhizome extract of *H. oculata* (*Hu et al.*, 2008; *Xie et al.*, 2012; *Yang et al.*, 2016; *Zhao et al.*, 2016). Furthermore, Wong et al. also found *oplopamine*, 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](image-url) **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                      | Leaf | m/z | References |
|--------------------------------|------|-----|------------|
| Cadinane-4β,5α,10α-troto        | -    | 221 | Xie et al. (2012) |
| Oplopanone                      | -    | 237 | Wu et al. 2012 |
| homalomenol F                   | -    | 252 | Hu et al. 2009 |
| 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) |
| 5,7-diepi-2α-acetoxyoplopanone   | 4-   | 305 | Yang et al. (2016) |
| Eudesma 4β,7α,1β,4β,7β-homalo   | 5,7- | 318 | Yang et al. (2016) |
| Eudesma 4β,7α,1β,4β,7β-homalo   | -    | 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, Liliwiranis 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* (Liliwiranis et al., 2011). These 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 |

In this study, 10 compounds of sesquiterpenoids are determined in rhizome and leaf of *H. vietnamensis*, including of cadinane-4β,5α,10α-troto, oplopanone, 4-epi-oplopananol, 2α-hydroxy homalomenol A, 1β,4β,7β-trihydroxyeudesmane, homalomentetraol, 4-acetoxyoplopanol, 5,7-diepi-2α-acetoxyoplopanone, eudesma 4β,7α,1β,4β,7β-homalomenol. 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*.

### CONCLUSION

In this study, 10 compounds of sesquiterpenoids are determined in rhizome and leaf of *H. vietnamensis*, including of cadinane-4β,5α,10α-troto, oplopanone, 4-epi-oplopananol, 2α-hydroxy homalomenol A, 1β,4β,7β-trihydroxyeudesmane, homalomentetraol, 4-acetoxyoplopanol, 5,7-diepi-2α-acetoxyoplopanone, eudesma 4β,7α,1β,4β,7β-homalomenol. 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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