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Chemical composition and antibacterial activities of ethanolic extract from rhizomes and aerial parts of *Typhonium lineare* Hett. & V.D. Nguyen (Araceae)

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Abstract. *Typhonium lineare* is a rare plant species of *Typhonium* genus and found only in Southern Vietnam. The species has not been any research on its phytochemical composition and antimicrobial activity. In present study, the chemical constituents and antibacterial activity of ethanolic extract of rhizomes and aerial parts of *T. lineare* was firstly investigated by Liquid chromatography–mass spectrometry (LC-MS) and disk diffusion methods, respectively. Consequently, six chemical constituents were reported from *T. lineare* aerial parts and rhizomes, including 5-(2'-methylpropyl)hydantoin, 5-(4'-hydroxybenzyl)hydantoin, uridine, cyclo(Leucyl-Tyrosyl), linolenic acid, gigantin and pheophorbide-a. Furthermore, the ethanolic extract of *T. lineare* rhizomes was not resistant to five studied bacteria whereas the extract from the aerial parts was proved to be able to resist against five bacterial strain, such as *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis* and *Salmonella typhimurium*.
Keyword: *Antibacterial activity, Chemical composition, Ethanolic extract Typhonium lineare.*

Introduction

Nowadays, medicinal plants are rich sources of antimicrobial agents as well as active substances. However, among the estimated about 500,000 species, only a small percentage of medicinal plants (around 20,000 species) have been recorded [KESKIN, 2018] in which the medicinal aspects and antimicrobial agents of the species belonging Araceae family ***were investigated by*** some previous studies [CHEN *et al.*, 2001; LAI *et al.*, 2010; MANKARAN *et al.*, 2013; MANNA *et al.*, 2016].

Typhonium Schott is the large genus in the tribe Areae (Araceae family) [SCHOTT, 1829], all of which are very small to medium-sized [BOYCE *et al.*, 2012]. This genus included about 100 species which are widely distributed from Himalaya, tropical Asia, New Guinea **and** Australia [BOYCE *et al.*, 2012]. Several *Typhonium* species have been known for their medicinal uses in Vietnam and many Asian countries, including *T. trilobatum, T. flagelliforme,* and *T. giganteum* [CHEN *et al.*, 2001; LAI *et al.*, 2010; MANKARAN *et al.*, 2013; MANNA *et al.*, 2016; PHAM, 2000]. Furthermore, many studies have identified proved phytochemical composition and proved some bioactivities of extracts from the aerial parts and the tuberous rhizomes of many species of *Typhonium* genus, such as antimicrobial and antioxidant activities [CHEN *et al.*, 2001; LAI *et al.*, 2010; MANKARAN *et al.*, 2013; MANNA *et al.*, 2016; LIU *et al.*, 2014].

Vietnam is a biodiversity hot spot for the Araceae family in which 17 *Typhonium* species have been recorded [PHAM, 2000; NGUYEN *et al.*, 2004; NGUYEN, 2005; NGUYEN, 2008; NGUYEN *et al.*, 2010; HETTERSCHEID *et al.*, 2001; LUU *et al.*, 2017; VAN *et al.*, 2017]. *Typhonium lineare* Hett. & V. D. Nguyen was described for the first time by Hetterscheid and Nguyen in *2001* [HETTERSCHEID *et al.*, 2001] which the type specimens were collected in Tuy Phong District, Binh Thuan Province, Vietnam. Recently, Van [VAN et al., 2017] who was the first author of this paper, also recorded the new distribution of this species in Binh Chau-Phuoc Buu Nature Reserve, Ba Ria-Vung Tau Province. To date, *T. lineare* is a rare species and has only been found from Bình Thuan and Ba Ria-Vung Tau Province, southern Vietnam. As the consequence, information on the chemical constituents and the bioactivity of this species is limited. In present study, we firstly identified chemical constituents and antibacterial activity of ethanolic extract of the rhizomes and the aerial parts of *T. lineare.*

Material and methods

Plant material

Ten kilograms of of *T. lineare* rhizome and aerial part were obtained by Binh Chau-Phuoc Buu Nature Reserve, Bung Rieng ward, Xuyen Moc District, Ba Ria-Vung Tau Province, Vietnam, location of about 10°31'11"N; 107°31'18"E, August 10, 2019, 29 m in elevation (Figure 1). The specimens were collected by Mr. Van Son Le which vouchered numbers were VS Le 322 and 323. All vouchered specimens were deposited at herbarium of Binh Chau-Phuoc Buu Nature Reserve.

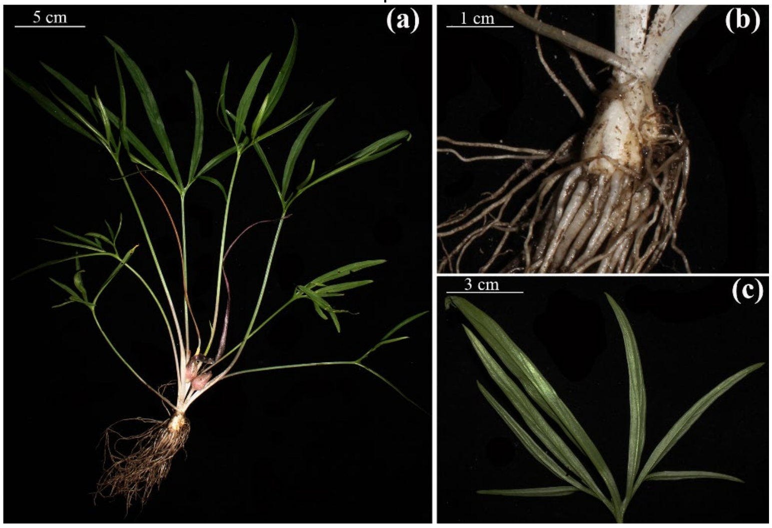

Figure 1. *T. lineare,* a) habitat, b) zhizome, c) leaf

Bacterial strains

To clarify the antibacterial activity of ethanolic extract of the rhizomes and the aerial parts of *T. lineare*, the present study used five bacterial strains, including *Bacillus cereus* (ATCC 11774), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enteritidis* (ATCC 13976), *Salmonella typhimurium* (ATCC 13311). Before using in antibacterial assays, bacterial strains were re-activated in Luria-Bertani broth at 37°C for 24h.

Methods

Extraction procedure

Peeled and subsequently cut into slices fresh rhizomes and aerial parts of *T. lineare*. Moderate dried the sliced specimens at 50-55°C until masses of samples were stability. Pulverized the samples by an electric grinder into fine powder then kept at 4°C. In the next 5 weeks, immersed in 4.5 L of 98% ethanol 500 g of the dried powder of leaves and rhizomes of *T. lineare*. Filtrated the extract via whatman filter paper, and subsequently concentrated in reduced pressure at 60°C to obtain the brown extract [ALTERMIMI et al., 2017]. To ensure the absolute absence of ethanol in the extract, sublimation dryer was utilized.

Liquid chromatography mass spectrometry (LC-MS)

An aliquot of ethanol extract of studied specimens was injected the aliquot of ethanol extract to HPLC Agilent 1200 infinity liquid chromatography system (Agilent Technologies, CA, USA) coupled with MicroTOF-QII mass spectrometer (Bruker Daltonics, Germany). The chemical components were separated on ACE3- C18 analytical column (4.6 ×150 mm, 3.5 µm). In mobile phase, used deionized water with formic acid (0.1%) as solvent A and acetonitril with formic acid (0.1%) as solvent B. In mass spectrometer, the extract was then ionized using electrospray ionization source (ESI) at positive mode and the mass spectra data were recorded on mode for a mass range 50-2000 m/z. Using Data Analysis software (Bruker, Germany) to analyze the data.

Antibacterial activity assay

Using the method detailly described by Bauer et al. (1996) for the antibacterial assay [BAUER *et al.*, 1996]. Inoculated the bacteria in LB Broth until a turbidity of 0.5 McFarland standards was reached. Subsequently, spread 100 µL bacterial suspensions on sterile Mueller Hinton plate and put a sterile 6mm diameter discs on the inoculated surface. Added 15 µL of the sample onto each disc and maintained the plates at 4°C for 2 hours to allow extract diffusion into the medium. Kept the plates at 37°C for 24h and the antibacterial activity of sample was determined via the inhibition zone diameter of tested bacteria.

Used sterilize distilled water as negative control and Gentamycin antibiotic discs (supplied by Nam Khoa BioTek, Viet Nam) as positive control. The antibacterial assay was investigaed in triplicate. The average and standard deviation of measurements were calculated using The Excel 2010. The data were presented as mean ± standard deviation (SD).

Results and discussion

Chemical compositions

The molecular weight of components in the chromatogram of Figure 2 was compared with other studies on *Typhonium* species.

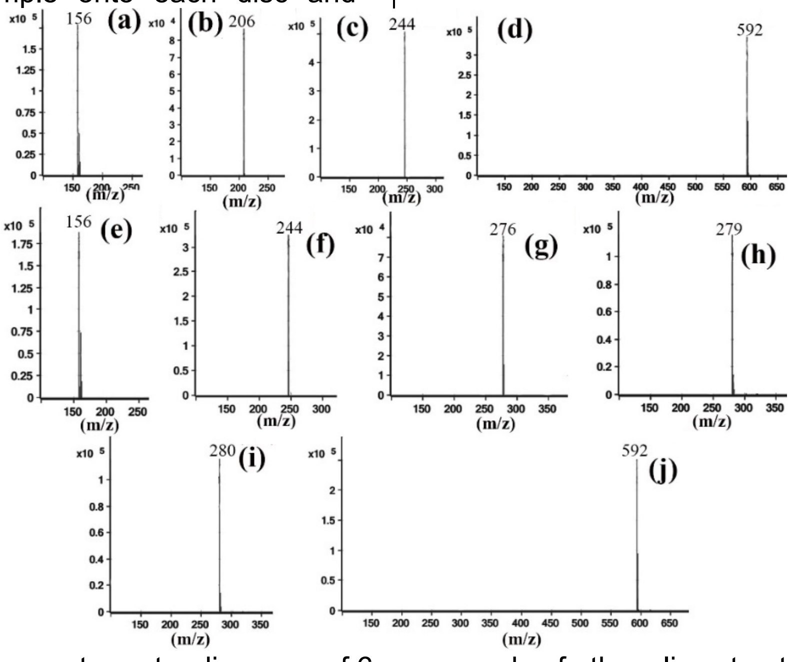

Figure 2. Mass spectrometry diagrams of 6 compounds of ethanolic extracts from rhizmes and aerial parts of T. lineare. a-d) rhizome. a) 5-(2-methylpropyl) hydantoin, b) 5-(4-hydroxybenzyl) hydantoin, c) uridine. d) pheophorbide-a; e-i) aerial part. e) 5-(2-methylpropyl) hydantoin, f) uridine, g) cyclo(leucyl-tyrosyl), h) linolenic acid, i) gigantin, j) pheophorbide-a.

As a result, there were 6 compounds present in the ethanolic extract of rhizomes and aerial parts of *T. lineare* with similar molecular weights of the compounds found in previous studies (Table 1). Accordingly, four constituents, including 5-(2-methylpropyl) hydantoin, 5-(4-hydroxybenzyl) hydantoin, cyclo(leucyl-tyrosyl) and gigantin were found in rhizomes of *T. giganteum* [LIU *et al.*, 2014].

Furthermore, pheophorbide-a and linolenic acid were the compounds found in *T. flagelliforme* [LAI *et al.*, 2010] while another species of *Typhonium* genus, *T. giganteum*, contained uridine [AI *et al.*, 2010].

Among 6 compounds present in the ethanolic extract of rhizomes and aerial parts of *T. lineare*, 5-(2-Methylpropyl) hydantoin, uridine and pheophorbide-a were found in both rhizomes and aerial parts of *T. lineare* whereas 5-(4-hydroxybenzyl) hydantoin only found in ethanolic extract of rhizomes. On the other hand, the aerial part extracts contained cyclo(leucyl-tyrosyl), linolenic acid and gigantin (Figure 2 and Table 1).

The bioactivities of some constituents isolated from rhizomes and aerial parts of *T. lineare* in this study have been documented in previous studies. For instance, pheophorbide-a was isolated from *T. flagelliforme* rhizome which could inhibit cancer cells, including NCI-H23 and S578T which cause lung and breast cancer [LAI *et al.*, 2010].

Table 1.

Phytochemical composition of ethanolic extracts from rhizomes and aerial parts of *T. Lineare*

Compounds Rhizome	Leaf	m/z	References
-	Linolenic acid	279	[LAI *et al.*, 2010]
Pheophorbide-a	Pheophorbide-a	592	[LAI *et al.*, 2010]
5-(2'-methylpropyl)hydantoin	5-(2'-methylpropyl)hydantoin	156	[LIU *et al.*, 2014]
5-(4'-hydroxybenzyl)hydantoin	-	206	[LIU *et al.*, 2014]
-	Cyclo(Leucyl-Tyrosyl)	276	[LIU *et al.*, 2014]
-	Gigantin	280	[LIU *et al.*, 2014]
Uridine	Uridine	244	[AI *et al.*, 2010]

Furthermore, linolenic acid which was found in some seed oils, such as walnuts, soybeans, navy beans, flaxseed, perilla, and chia seed was demonstrated that it could enhance absorption of insulin in mice [MORISHITA *et al.*, 1998]. Many previous studies showed the uridine function in nervous system. This constituent was a main type of pyrimidine nucleosides which was absorbed by brain. Uridine was changed to nucleotides by phosphorylation. These nucleotides then were used for the synthesis of DNA, RNA and membrane ingredients [DOBOLYI et al., 2011; PIERO et al., 2015]. Moreover, another study demonstrated that cyclo(leucyl-tyrosyl) was proved to be able to resist against *Staphylococcus epidermidis*. It was thus able to resist against this bacterial strain [SCOPEL et al., 2013]. In a contrary manner, the bioactivities of three constituents, including 5-(2'-methylpropyl) hydantoin, 5-(4'-hydroxybenzyl) hydantoin and gigantin have not been reported before in which gigantin, a new compound, was isolated from T. giganteum rhizomes [LIU et al., 2014].

Bacterial activities

In this study, the antibacterial activities of ethanolic extracts from the aerial parts and the rhizomes of *T. lineare* were conducted. However, only the aerial part sample showed the antibacterial activity while the later sample could not inhibit the growth of the tested bacteria.

Table 2.

Inhibition zone ethanolic extracts from aerial parts of *T. lineare* against five bacterial strains

Tested bacteria	Growth inhibition zone (mm)
B. cereus	11.2 ± 1.0
E. coli	11.7 ± 1.5
P. aeruginosa	9.8 ± 0.3
S. enteritidis	10.7 ± 1.2
S. typhimurium	12.8 ± 0.8

Accordingly, data stated in Table 2 and Figure 3 showed that ethanolic extract of aerial parts of *T. lineare* was able to resist against five bacteria studied.

Among them, the ethanolic extract exhibited strong antibacterial activity against *S. typhimurium* (12.8 ± 0.8 mm), following *E. coli* (11.7 ± 1.5 mm), *B. cereus* (11.2 ± 1.0 mm), *S.* enteritidis (10.7 ± 1.2 mm) and *P. aeruginosa* (9.8 ± 0.3 mm). *T. lineare* is a rare and endemic to Vietnam which was only dicovered in Ninh Thuan and Ba Ria-Vung Tau Province [HETTERSCHEID *et al.*, 2001; VAN *et al.*, 2017].

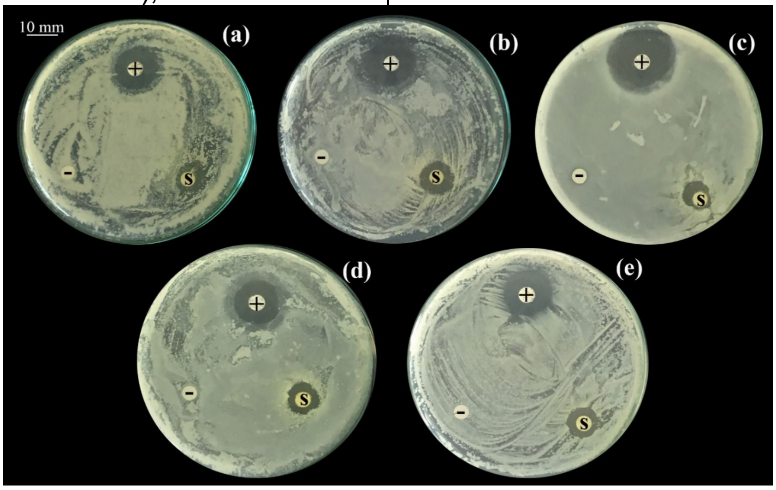

Figure 3. Antibacterial activity of ethanolic extracts from aerial parts of *T. lineare* against 5 bacterial strains. a*) B. cereus*, b) *E. coli*, c) *P. aeruginosa*, d) *S. enteritidis*, e) *S. typhimurium*. (-) Negative control with sterilized distilled water, (+) Positive control with discs containing gentamicin.

The present study, therefore, was firstly research showing phytochemical composition and antibacterial activity of ethanolic extract of *T. lineare.* However, the antimicrobial activities of the extracts from different solvents of other species belonging to genus *Typhonium* have been reported in previous studies. For instance, the hexane extract from tuber of *T. flagelliforme* could resistant to *Pseudomonas aeruginosa, Salmonella choleraesuis, Staphylococcus aureus* and *Bacillus subtilis* [MOHAN *et al.*, 2018] while the extract of *T. flagelliforme* leaf capable to show antibacterial activity against *P. aeruginosa* [MOHAN *et al.*, 2008].

Furthermore, the ethnaolic extract of *T. trilobatum* could be resistant to *Salmonella typhimurium*, *Proteus mirabilis, Staphylococcus aureus* and *Proteus mirabilis* [ROY *et al.*, 2012] whereas other solvents from the aerial parts of this species, including methanol, chloroform and ethyl acetate extract was found to could inhibit the growth of four pathogenic bacteria, including *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli* and *Pseudomonas aeruginosa* [ROY *et al.*, 2013].

Conclusions

Based on this study, six compounds were firstly identified in ethanolic extract of rhizomes and aerial parts of *T. lineare*. The ethanolic extract of *T. lineare* rhizomes was not able to resist against the studied bacteria whereas those from the aerial parts was proved to be able to inhibit an antibacterial activity against five bacterial strain, including *B. cereus*, *E. coli*, *P. aeruginosa*, *S. enteritidis* and *S. typhimurium*.

Conflict of Interest: The authors declare that they have no conflict of interest.

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