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Chemical profiles and antibacterial activities of acetone extracts of *Globba* macrocarpa

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Abstract

Globba macrocarpa Gagnep. is a rare species of Globba genus (Zingiberaceae family). The present study reported the chemical compositions and antibacterial effects of acetone extracts obtained from the *G. macrocarpa* rhizomes and aerial parts. By using Gas Chromatography Mass Spectrometry (GC/MS) assay, fifty and thirty-two chemical compounds were identified from rhizomes and aerial parts of the species, respectively. Of those, germacrene D (15.25 %), 1H-indole, 4-(3-methyl-2-butenyl)- (14.33 %), (*E*)- β -farnesene (11.28 %), and 2-biphenylamine, 3-methyl (10.27 %) were the major constituents in the rhizome extract while the aerial part extract was characterized by the predominance of linolenic acid (19.89 %), palmitic acid (13.05 %), phytol (7.52 %), and neophytadiene (4.76 %). In addition, the rhizome extract had antibacterial activities against five 5 out of 6 oral bacteria, including *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Salmonella typhimurium Bacillus cereus*, and *Staphylococcus aureus*. Meanwhile, the aerial part extract was active against 4 out of 6 test bacteria, except for *Escherichia coli* and *S. typhimurium*.

Introduction

Globba L. is a third largest genus belonging to Zingiberaceae with over 100 species. This genus is distributed widely throughout tropical and subtropical of Asia such as India, southern China, New Guinea and Southeast Asia. *Globba* plants are small perennial herbs, reaching to a height of 1 m, except for *G. racemosa* (about 3 m) (Williams *et al.* 2004). Members of *Globba* consist of a large number of useful plants. For instance, the *G. bulbifera* rhizomes have been used to cure

cough, asthma, and snakebite. G. multiflora was used to treat headache and rheumatic inflammation (Jain 1995). The anti-inflammatory, antioxidant, and antipyretic activities were recorded as the biological activities of malaccensis *G*. (Ngamrojanavanich et al. 2005). In addition, previous studies showed the phytochemical composition and bioactivities of several Globba species (Andila and Tirt 2019: Raj et al. 2020). Globba macrocarpa Gagnep. is a rare species of Globba genus. This species was described for the first time by Gagnepain (1901) and mainly

distributed in Cambodia, Thailand and Vietnam (Pham 2000; Nguyen 2017). In 2020, we conducted some field trips to the Binh Chau-Phuoc Buu Nature Reserve, Bung Rieng ward, Xuven Moc District, Ba Ria-Vung Tau Province, and encountered a flowering population of *G*. macrocarpa. To date, the chemical compositions and bioactivities of G. macrocarpa are still unknown. The present study, thus, firstly reported the chemical constituents and antibacterial effects of acetone extracts from the G. macrocarpa rhizomes and aerial parts.

Experimental

Plant materials

The samples of *G. macrocarpa* were collected from Binh Chau-Phuoc Buu Nature Reserve, Bung Rieng Ward, Xuyen Moc District, Ba Ria-Vung Tau Province, Vietnam, location of about 10°32'19.4"E 107°26'57.4"N, 18 m in elevation (Fig. 1).

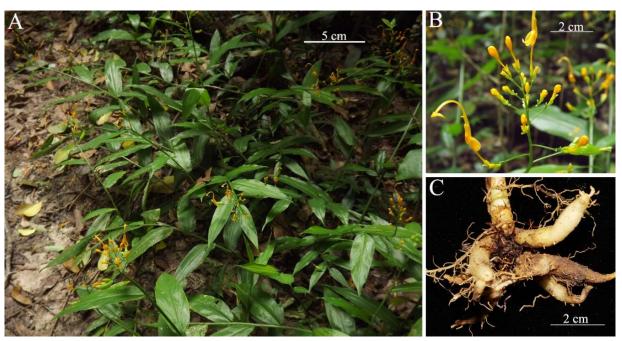


Fig.1. *Globba macrocarpa*. A – Species in habitat, B – Flowers, C – Rhizome.

Bacterial strains

In this study, the antibacterial properties of acetone extracts of the *G. macrocarpa* rhizome and aerial parts were investigated by using six bacterial strains such as *Salmonella typhimurium*-ATCC 13311, *Salmonella enteritidis*-ATCC 13976, *Pseudomonas aeruginosa*-ATCC 27853, *Escherichia coli*-ATCC 25922, *Staphylococcus aureus*-ATCC 25923, and *Bacillus cereus*-ATCC 11774.

Extraction procedures

The rhizome and aerial parts of *G. macrocarpa* were dried at 50 °C until constant weight. The dried samples were pulverized. 250 mL of acetone 99 %

solution was used to macerate 50 g of the dried powders at ambient temperature for three days. The studied samples were then filtered through the Whatman paper. The process was repeated twice and filtrates were concentrated under the reduced pressure at 60 °C to obtain the brown extracts. The final extracts were subjected to sublimation drying to completely remove the remaining acetone (Bobinaite *et al.* 2013).

Gas chromatography/mass spectrometry (GC/MS) assay

The TRACE 1310 Gas Chromatograph (Thermo Fisher Scientific, Waltham, USA) and ISQ 7000 single quadrupole mass spectrometer was used to identify the chemical compositions in the acetone extract. GC column DB-5MS 30 m, 0.25 mm, 0.25 µm (Agilent Technologies, Santa Clara, USA) was used and carrier gas was helium with the column flow rate of 1.2 mL.min⁻¹. The specimen was added into the system with the split ratio of 30 : 1, the splitless mode of 1 min, and the split flow of 36 mL.min⁻¹, the inlet temperature of 250 °C. The initial temperature was set for 5 min at 80 °C. The temperature was then raised to 280 °C at the rate of 20 °C.min⁻¹ and hold for 10 min. The electron ionization mode and the ion source temperature were 70 eV and 250 °C, respectively. The mass scan range was 29 - 650 m/z. The NIST 2017 library version 2.3 (NIST 2017) was used to identify the chemical constituents of studied samples.

Antibacterial assay

The antibacterial properties of the acetone extract of the G. macrocarpa were conducted by Disc diffusion assay following the CLSI guideline (CLSI 2010). Luria-Bertani Broth was used to activate the bacterial strains until their turbidity was equivalent to 0.5 McFarland. Mueller Hinton Agar plate was inoculated with 0.1 mL of the bacterial culture by spread – plate technique before sterile paper discs containing 10 µL of the extract solution were placed on its surface. Gentamycin antibiotic discs (10 µg.mL⁻¹) (Nam Khoa Biotek, Ho Chi Minh City, Vietnam) were used as the positive control. The plates were incubated at 37 °C for 18 - 24 h. The antibacterial effects of the studied samples were identified by recording the size of the zone of inhibition.

Three biological replicates were used for the experiment, and results were expressed as mean \pm standard deviation (SD). Differences between means groups were calculated by LSD procedure using the Statgraphics Centurion XV software (Statgraphics Technologies, Inc., The Plains, USA) with P < 0.05.

Results and Discussion

Constituents of G. macrocarpa extracts

The acetone extracts isolated from rhizomes and aerial parts of *G. macrocarpa* in this study

contained a total of 50 and 32 components, respectively (Table 1 and 2). Accordingly, the rhizome extract was characterized by the predominance of germacrene D (15.25 %), 1H-indole, 4-(3-methyl-2-butenyl)- (14.33 %). (E)- β -farnesene (11.28 %) and 2-biphenylamine, 3-methyl- (10.27 %) (Fig. 2A). Meanwhile, the aerial part extract was found to be rich of linolenic acid (19.89 %), palmitic acid (13.05 %), phytol (7.52%) and neophytadiene (4.76%). Previous studies have been reported the biological properties of some compounds obtained from the acetone extracts of G. macrocarpa rhizomes and aerial parts in this study. For instance, germacrene D, a major component from both rhizome and extracts. possessed aerial part the strong insecticidal activities against Acyrthosiphon pisum, Sitobion avenae, and Myzus persicae (Bruce et al. 2005). Similarly, germacrene D had also potent larvicidal effects against three mosquitos such as Anopheles gambiae, Culex quinquefasciatus, and Aedes aegypti with LD50 values of 1.8, 2.1 and 2.8 mg.cm⁻³ (Ravi and Sita 2007). Recently, Schepetkin et al. (2020) demonstrated that germacrene D possessed the immunomodulatory activities. Accordingly, germacrene D could inhibit neutrophil Ca²⁺ mobilization, chemotaxis, and reactive oxygen species production with IC_{50} values of 0.51, 5.4 and 9.9 µg.mL⁻¹ (Schepetkin et al. 2020). In addition, (E)- β -farnesene, another component from both rhizome and aerial part acetone extracts of G. macrocarpa, showed the effects on natural enemies to cabbage aphid (Brevicoryne brassicae) control in Chinese cabbage fields (Cui et al. 2012). Also, this compound has been reported to possessed other biological activities, including repellent and aphicidal activities against Myzus persicae (Qin et al. 2016). Furthermore, α -pinene and β -pinene, two bioactive compounds found in the rhizome and aerial part extracts, has been suggested as the larvicidal and insecticidal agents against Aedes aegypti, Lasioderma serricorne, and Rhodnius nasutus (Aparna et al. 2012; Wu et al. 2015; Carta et al.

(Aparlia *et al.* 2012; will et al. 2013; Carta et al. 2017). Palmitic acid, a compound from both rhizome and aerial part acetone extracts of *G. macrocarpa*, possessed the anti-inflammatory activities (Aparna *et al.* 2012), physiological role, metabolism and nutritional implications in human (Carta et al. 2017).

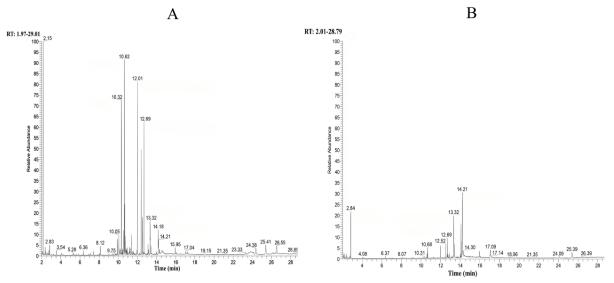


Fig. 2. GC/MS chromatograms of studied extracts from G. macrocarpa rhizomes (A) and aerial parts (B) with major components.

Table 1. Constituents of extract of G. macrocarpa rhizome.

No.	RT	Components	[%]	No.	RT	Components	[%]
1	2.15	Tyranton	0.23	26	10.83	γ-Amorphene	0.45
2	3.25	α-pinene	0.14	27	10.87	Guaia-10(14),11-diene	0.40
3	3.54	Camphene	0.69	28	10.91	α-Bulnesene	0.22
4	4.06	Sabinen	0.38	29	10.95	Epicubebol	0.48
5	5.28	Isosylvestrene	0.42	30	11.17	Germacrene B	0.53
6	5.38	Orthodene	0.28	31	11.21	Tricyclo[5.1.0.0(2,4)]oct-5-ene-5- propanoic acid, 3,3,8,8- tetramethyl-	0.34
7	5.63	β-Ocimene	0.21	32	11.27	Isoaromadendrene epoxide	0.59
8	6.36	3-(pyrrolidin-1-yl)cyclopent-2- en-1-one	0.56	33	11.40	1H-indole, 4-(3-methyl-2- butenyl)-	1.65
9	6.93	Triacetoneamin	0.14	34	11.51	1-epi-cubenol	0.24
10	7.10	4(1H)-quinolinone, octahydro-1- methyl-	0.39	35	11.56	Aromadendrene oxide-(1)	0.12
11	7.42	Alcanfor	0.51	36	11.60	Benzeneacetoneitrile, α- cyclopentyl-	0.21
12	7.97	Methyl salicylate	0.11	37	12.01	1H-indole, 4-(3-methyl-2- butenyl)-	14.33
13	8.12	Dihydroanethole	0.77	38	12.38	3-(2-methylbut-3-enyl)-1H-indole	8.01
14	9.89	β-elemen	1.33	39	12.53	Quinoline, 2-ter_butyl-	2.57
15	10.06	α-gurjunene	1.60	40	12.69	2-biphenylamine, 3-methyl-	10.27
16	10.17	Caryophyllene	0.49	41	13.11	3-Methyldiphenylamine	0.73
17	10.20	γ-elemene	0.43	42	13.32	Palmitic acid	2.52
18	10.32	(E)-β-farnesene	11.28	43	13.41	Benzenamine, 4-methyl-N- phenyl-	0.61
19	10.38	Aristolene	0.13	44	14.18	9(E),11(E)-conuated linoleic acid	2.0
20	10.44	Humulene	0.43	45	14.21	Linolenic acid	1.44
21	10.55	Isogermacrene D	0.40	46	15.95	Palmitin, 2-mono-	0.63
22	10.58	(Z)-α-farnesene	1.93	47	17.04	9,12-octadecadienoic acid (,),2,3- dihydroxypropyl ester	0.93
23	10.62	Germacrene D	15.25	48	17.23	Stearin, 2-mono-	0.44
24	10.72	Bicylogermacrene	1.35	49	24.13	Stigmasterol	3.08
25	10.75	β-bisabolene	0.36	50	25.42	γ-Sitosterol	0.94

Table 2. Constituents of extract of G. m	nacrocarpa aerial part.
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No.	RT	Components	[%]	No.	RT	Components	[%]
1	4.07	β-Pinene	0.70	17	12.69	Neophytadiene	4.76
2	4.24	Cyclohexane, 1,2,4-trimethyl	0.38	18	12.72	3,7,11,15- Tetramethylhexadec-2-ene	1.05
3	6.37	3-(pyrrolidin-1-yl)cyclopent-2-en-1- one	0.77	19	12.82	Phytol	0.57
4	6.93	Triacetonamin	0.21	20	12.69	Neophytadiene	1.13
5	7.01	Isophorone	1.62	21	13.32	Palmitic acid	13.05
6	7.10	(5R,8aR)-5- Propyloctahydroindolizine	0.22	22	13.41	2-biphenylamine, 3-methyl-	3.17
7	8.07	Pyrazine, 3-butyl-2,5-dimethyl	0.24	23	14.08	Phytol	7.52
8	9.02	5H-1-pyrindine	0.12	24	14.18	Linoleic acid	4.36
9	9.17	2-methoxy-4-vinylphenol	0.20	25	14.21	Linolenic acid	19.89
10	10.31	(E)-β-farnesene	0.41	26	14.30	Octadecanoic acid	1.48
11	10.57	trans-α-bergamotene	0.24	27	15.94	Palmitin, 2-mono-	1.94
12	10.61	Germacrene D	1.06	28	17.08	2-Monolinolenin	3.93
13	10.68	α-farnesene	2.17	29	23.58	Campesterol	0.55
14	11.26	(-)-Spathulenol	0.30	30	24.10	Stigmasterol	1.04
15	11.99	1H-indole, 4-(3-methyl-2-butenyl)-	2.84	31	25.39	β-Sitosterol	5.80
16	12.52	3-(2-methylbut-3-enyl)-1H-indole	2.91	32	26.37	Cholesteryl formate	0.70

Several chemical compounds obtained from G. macrocarpa aerial parts and rhizomes in this reported study have been to possess the antimicrobial properties by previous studies. Accordingly, Simionatto et al. demonstrated that (E)-\beta-farnesene had antibacterial effects against Bacillus subtilis, **Staphylococcus** aureus, Salmonella setubal, and Pseudomonas aeruginosa (Simionatto et al. 2007). In addition, linolenic acid bacterial showed strong activities against Staphylococcus aureus, and *Streptococcus* pyogenes (Zheng et al. 2005). Also, a-pinene presented the antimicrobial against a large amount and fungal strains, of bacterial including Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Streptococcus faecalis, Candida albicans, Sclerotinia sclerotiorum, Mycobacterium smegmatis, Cylindrocarpon mali, Aspergillus niger, and Stereum purpureum (Prudent et al. 1993). In another report, α -pinene has been also reported to possess the antibacterial activities against Staphylococcus aureus, S. epidermidis, Streptococcus pyogenes, and S. pneumonia. Moreover, β -pinene had the antibacterial activity against gram-positive bacteria causing potential infectious endocarditis such as Staphylococcus

aureus, S. epidermidis, Streptococcus pyogenes, and S. pneumonia (Medeiros et al. 2007).

As mentioned above, G. macrocarpa is a rare The phytochemicals and biological species. properties of this plant are limited. However, there are some publications for chemical compositions and antimicrobial effects of other member of the Globba species using GS/MS assay. For instance, the major components of ethanol extracts isolated from G. candida rhizomes grown in Indonesia included levoglucosan (19.07 %), allylhydrazone acetaldehyde (5.52 %), *trans*-2,3-epoxybutane (6.30 %), butan-3-enoic acid methyl ester (4.36 %) while the leaf extract has been reported to contain pinostrobin chalcone (75.63 %), 1-(1-(hydroxyphenyl-methyl)-cyclopropyl)-2-phenyl-ethano (2.4 %), benzenepentanal (2.08 %) as the major compounds (Andila and Tirta 2019). In addition, the chemical profiles of the essential oil extracted from the rhizomes of G. pendula from Vietnam were found to be rich in δ -selinen (36.45 %) and ishwarane (10.76 %) (Ngo et al. 2020). Furthermore, the chemical compositions of rhizome essential oils of three Globba species collected from India, including G. cernua, G. marantina, and G. ophioglossa have been reported (Menon and Dan 2009). As a result, caryophyllene was the most

components in the oils (19.3 - 24.2 %), followed by α -humulene, (Z)-nerolidol, and (Z,Z)-farnesol (Menon and Dan 2009).

Antibacterial activities of G. macrocarpa extracts

The extracts of *G. macrocarpa* rhizomes were found to be effective against five oral bacteria, except for *E. coli* (Fig. 3 and Table 3). As a result, the rhizome extract showed potent antibacterial effects against *P. aeruginosa* and *S. aureus* with the zone of inhibition of 19.0 ± 1.7 and 14.7 ± 2.5 mm, respectively which higher than that of positive control (Table 3). Also, the rhizome extract possessed moderate effects against *B. cereus* $(14.2 \pm 0.8 \text{ mm})$, S. enteritidis $(11.2 \pm 1.2 \text{ mm})$, and S. typhimurium $(10.8 \pm 1.1 \text{ mm})$.

The data in Table 3 and Fig. 3 presented the antimicrobial effects of aerial part extracts of G. macrocarpa. Among 6 tested bacteria, the extract was found to be effective against four studied bacteria, including Р. aeruginosa, B. cereus, S. enteritidis, and S. aureus. Generally, the aerial part extract possessed antibacterial effects weaker than that of rhizome extract. This extract only possessed the moderate effects against S. enteritidis with the zone of inhibition of 13.5 ± 1.3 mm while this sample presented the weak antibacterial activities against B. cereus $(9.3 \pm 0.3 \text{ mm})$, S. aureus $(9.3 \pm 0.3 \text{ mm})$, and *P. aeruginosa* $(7.2 \pm 0.3 \text{ mm})$.

Table 3. Zone of inhibition of acetone extracts from G. macrocarpa against six oral bacteria.

Microorganism	Size of zone of inhibition [mm]				
-	Rhizome	Aerial part	Gentamycin		
B. cereus	14.2 ± 0.8^{b}	$9.3\pm0.3^{\mathrm{a}}$	$22.3 \pm 0.6^{\circ}$		
E. coli	-	-	21.3 ± 1.5		
P. aeruginosa	$19.0\pm1.7^{\circ}$	$7.2\pm0.3^{\mathrm{a}}$	$13.5 \pm 1.8^{\circ}$		
S. enteritidis	$11.2 \pm 1.2^{\mathrm{a}}$	$13.5 \pm 1.3^{\mathrm{a}}$	$20.3\pm1.5^{\rm b}$		
S. typhimurium	$10.8 \pm 1.1^{\mathrm{a}}$	-	14.8 ± 1.1^{b}		
S. aureus	14.7 ± 2.5^{b}	$9.3\pm0.3^{\mathrm{a}}$	12.3 ± 2.1^{ab}		

a.b.c.The notable variation (P < 0.05) was represented by distinct superscript lower-case letters in the same row.

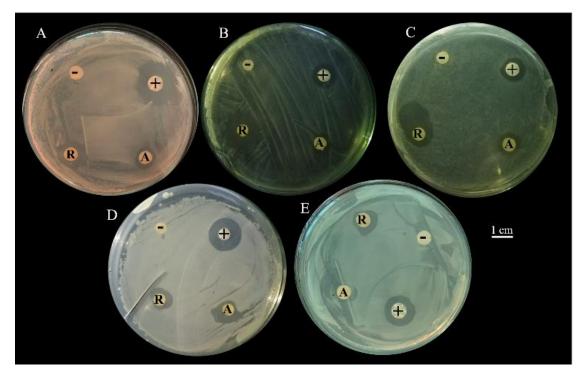


Fig. 3. Antibacterial effects of the rhizome (R) and aerial part (A) extracts of *G. macrocarpa*. $\mathbf{A} - B$. *cereus*; $\mathbf{B} - P$. *aeruginosa*; $\mathbf{C} - S$. *aureus*; $\mathbf{D} - S$. *enteritidis*; $\mathbf{E} - S$. *typhimurium*. (-) – Negative control, (+) – Positive control.

The chemical components of hexane and dichloromethane extracts obtained from different parts of G. schomburgkii collected from Thailand have been reported. Accordingly, hexane extract of rhizomes was characterized by the predominance of %), 3-acetoxy-5-pregnene β -patchoulene (9.8) (7.8 %), and γ -bicyclohomofarnesal (6.4 %) while phytol was the most abundant components in the hexane extract of stalks (13.8 %) and leaves (34.6 %). The major constituents in the hexane extract of flowers were β -caryophyllene (16.5 %) and γ -bicyclohomofarnesal (14.6 %). Meanwhile, the most major constituent the dichloromethane extracts of rhizomes, stalks, leaves, and flowers were α -gurjunene (16.3 %), β -caryophyllene (11 %), phytol (19 %), and caryophyllene oxide (15.8 %), respective (Doungchawee et al. 2019). In addition, the antimicrobial activities of the hexane, dichloromethane, and methanol extracts from four plant parts of G. schomburgkii have been investigated. All twelve extracts had an inhibitory effect on Streptococcus sobrinus. The hexane and dichloromethane extracts displayed activity against Streptococcus mutans, Salmonella typhimurium, and Staphylococcus aureus whereas Aspergillus flavus was inhibited by the dichloromethane extracts (leaves, stalks, and flowers) and the methanol extracts of leaves (Doungchawee et al. 2019). Similarly, the dichloromethane extract and its fractions and sub-fractions obtained from G. schomburgkii rhizomes were mainly composed of γ -bicyclohomofarnesal (4.1 – 20.8 %), (E)-15,16dinorlabda-8(17),12-dien-14-one (7.6 - 58.2 %). Moreover, crude dichloromethane extract and its fractions were found to be effective against four pathogenic bacteria, including S. aureus, M. luteus, E. coli, and P. aeruginosa (Suekaew et al. 2020).

Conclusion

This study identified 50 and 32 chemical compositions in the acetone extracts of *G. macrocarpa* rhizomes and aerial parts, in which some components have been reported to possess many biological properties. The rhizome extract was demonstrated to be active against 5 bacterial strains with the diameter of inhibition zones of *P. aeruginosa* (19.0 \pm 1.7 mm), *S. aureus* (14.7 \pm 2.5 mm), *B. cereus* (14.2 \pm 0.8 mm), *S. enteritidis*

 $(11.2 \pm 1.2 \text{ mm})$ and *S. typhimurium* $(10.8 \pm 1.1 \text{ mm})$ while the aerial part extract showed antibacterial effects *S. enteritidis* $(13.5 \pm 1.3 \text{ mm})$, *S. aureus* $(9.3 \pm 0.3 \text{ mm})$, *B. cereus* $(9.3 \pm 0.3 \text{ mm})$ and *P. aeruginosa* $(7.2 \pm 0.3 \text{ mm})$. The results from the present study can be used as reference for pharmaceutical products and relative fields from *G. macrocarpa* which could increase the economic valuation of this species.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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