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CHEMICAL DIVERSITY OF ESSENTIAL OILS FROM AERIAL PARTS OF EIGHT SPECIES OF ZINGIBERACEAE FAMILY FROM VIETNAM

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Abstract

The objective of this work was to analyze the content and the chemical diversity of the essential oils from the aerial parts of eight species belonging to Zingiberaceae family collected in Binh Chau-Phuoc Buu Nature Reserve, Vietnam. The essential oils were isolated by hydrodistillation and their constituents were investigated by gas chromatography-mass spectrometry. The essential oils from the aerial parts of eight studied species, including Zingiber zerumbet, Zingiber pellitum, Curcuma pierreana, Globba macrocarpa, Globba globulifera, Alpinia conchigera, Stahlianthus campanulatus and Amomum sp. were obtained in a yield of 0.01%, 0.008%, 0.01%, 0.012%, 0.012%, 0.02%, 0.015 and 0.3%, respectively. There were a total of 75 compounds identified from the essential oils obtained from eight studied species. Four main clusters and three subclusters were formed by the cluster analysis. Cluster I consisted of A. conchigera with the presence of β -elemene (51.76%) and caryophyllene (28.1%). Cluster II contained five species, including Z. zerumbet, Z. pellitum, C. pierreana, G. globulifera and S. campanulatus with high concentration of β-pinene (11.81%, 5.32%, 4.43%, 25.42% and 1.57%), caryophyllene (13.61%, 23.45%, 19.78%, 34.26% and 18.72%), caryophyllene oxide (4.66%, 4.00%, 13.65%, 8.15% and 24.69%). Cluster III consisted of *G. macrocarpa*, and presented 4-isobutylquinoline (24.43%), β-copaene (21.69%), α-farnesene (20.73%), 2-tert-butylquinoline (6.05%), 3-Dihydroxydiphenylamine (5.47%), β-panasinsene (5.13%) as major compounds. Cluster IV contained Amomum sp. and had eucalyptol (25.22%), camphor (24.51%), camphene (14.57%), β -pinene (13.45%), Isoborneol (9.17%), α-Selinene (6.21%) as main constituents.

Keywords: Zingiberaceae, GC-MS, essential oils, Binh Chau-Phuoc Buu Nature Reserve

INTRODUCTION

For thousands of years, plants have been used to flavor, food, medicines, in which medicinal plants are rich sources of antimicrobial agents, potential and powerful drugs. Many chemical constituents which isolated from medicinal plants are usually responsible for the biological properties including treatment of infectious diseases (Silva and Fernandes, 2010; Vashist and Jindal, 2012;

Awad and Abdelwahab, 2016). The potential of vascular plants as a source for new medicine is still largely unexplored. However, only a small percentage have been recorded phytochemically among the estimated about 500,000 species (Mahesh and Satish, 2008) in which the medicinal aspects, chemical constituents and bioactivities of Zingiberaceae plants were investigated by some previous studies (Jantan *et al.*, 2003; Jatoi *et al.*, 2007; Koga *et al.*, 2016).

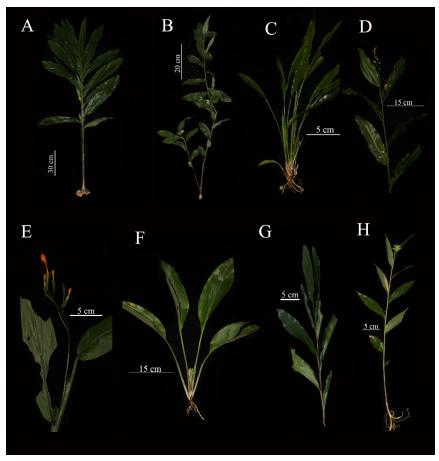
Zingiberaceae is a large family and has approximately 1400 species belonging to 53 genera which are distributed widely in Asia, especially Southeast Asia (Kress et al., 2002; Leong-Škornicková and Newman, 2012; Van et al., 2021; Van, 2021). In Vietnam, 129 species and 22 genera belonging to Zingiberaceae family have been recorded by previous reports (Pham, 2000; Nguyen, 2005; Leong-Škorničková and Ly, 2010; Leong-Škorničková and Tran, 2013; Leong-Škorničková and Luu, 2013; Leong-Škorničková et al., 2015; Nguyen et al., 2017; Nguyen, 2017). The family Zingiberaceae is wellknown for its medicinal values (Jantan et al., 2003). The aerial parts and rhizomes of Zingiberaceae species were used to treat cough, sore throat, improve digestion, reduce pain, heal bruises and scars (Pham, 2000; Jantan et al., 2003). Members of Gingers provided many useful products for medicines, spices, cosmetics, and essential oil as important natural resources (Jantan et al., 2003; Jatoi et al., 2007; Koga et al., 2016; Zahara et al., 2018). Furthermore, the essential oils isolated from the rhizomes and the aerial parts of Zingiberaceae species also contained many medicinal compounds, including terpenes, ketones, flavonoids phytoestrogens (Zahara et al., 2018).

In 2019, we conducted some field trips to the Binh Chau-Phuoc Buu Nature Reserve, Bung Rieng ward, Xuyen Moc District, Ba Ria-Vung Tau Province, Southern Vietnam and collected eight species belonging to Zingiberaceae family, including Zingiber zerumbet, Zingiber pellitum, Curcuma pierreana, Globba macrocarpa, Globba globulifera, Alpinia conchigera, Stahlianthus campanulatus and Amomum sp. Here, we firstly investigated and compared the chemical constituents of the essential oils isolated from the aerial parts of eight Zingiberaceae species from Binh Chau-Phuoc Buu Nature Reserve.

MATERIALS AND METHODS

Plant Materials

Specimens of eight species, including Z. zerumbet, Z. pellitum, C. pierreana, G. macrocarpa, G. globulifera, A. conchigera, S. campanulatus and Amomum sp. were collected from Binh Chau-Phuoc Buu Nature Reserve, Bung Rieng Ward, Xuyen Moc District, Ba Ria-Vung Tau Province. The vouchered specimens of these species were stored at herbarium of Binh Chau-Phuoc Buu Nature Reserve (Fig. 1 and Tab. I).



1: Eight Zingiberaceae species in present study. A: Zingiber zerumbet. B: Zingiber pellitum. C: Stahlianthus campanulatus. D: Alpinia conchigera. E: Globba macrocarpa, F: Curcuma pierreana, G: Amomum sp. H: Globba globulifera.

Voucher number	Scientific names	Collected sites
VS Le 210	C. pierreana	N10°35'41,1"; E107°30'17,9"
VS Le 212	G. macrocarpa	N10°37'14,6"; E107°32'08,0"
VS Le 213	G. globulifera	N10°32'54,7"; E107°27'13,0"
VS Le 214	Z. pellitum	N10°32'22,9"; E107°28'57,5"
VS Le 215	Z. zerumbet	N10°32'49,8"; E107°27'18,0"
VS Le 216	Amomum sp.	N10°32'20,2"; E107°26'56,1"
VS Le 217	S. campanulatus	N10°32'51,5"; E107°27'08,9"
VS Le 218	A. conchigera	N10°31'45,4"; E107°27'33"

I: Detailed information of collected site of eight studied species in Binh Chau-Phuoc Buu Nature Reserve

Hydrodistillation of the Essential Oils and Yield Calculation

500 g fresh aerial parts of each species were hydrodisstilled in a Clevenger-type aggaratus for 4h. The essential oil was dried using anhydrous sodium sulphate and (Na₂SO₄) and stored at 4°C. The experiments were performed in triplicate. 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).

Gas Chromatography-mass Spectrometry (GC/MS) Analysis

The constituents of essential oil isolated from studied specimens were investigated by Gas Chromatography Mass Spectrometry GC-MS using an Agilent 7890A GC coupled to a 5975C VL MSD Triple-Axis selective Detector equipped with an ZB-5MS capillary nonpolar column (30.0 m length \times 0.25 mm i.d. \times 0.25 μ m film thickness). Helium was the carrier gas at constant pressure at 13.209 psi. The injection temperature was 250 °C; the injection volume was 0.1 µl; the split ratio was 10:1. The oven temperature was programmed to proceed from 60 °C to 240 °C at 3 °C/min. the chemical constituents were identified by comparing their mass spectra with internal library, NIST 2017 library and the Wiley 8th edition libraries.

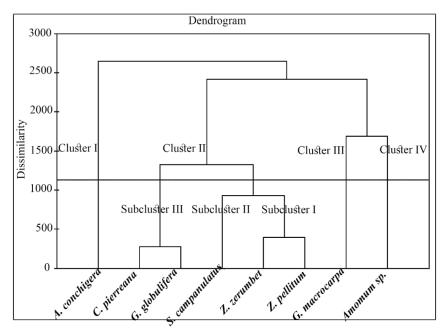
Data Analysis

The essential oils were classified into clusters based on their similarities in volatile compound constituents by Agglomerative Hierarchical Clustering (AHC). Besides, the volatile constituents were subjected to Principal Component Analysis (PCA) using covariance matrix to identify the typical constituents of each essential oil. All analyses were performed using XLSTAT Sensory (Addinsoft, Boston, USA).

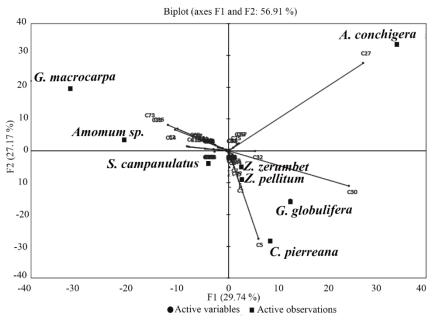
RESULTS AND DISCUSSION

The average of aerial parts oil yields of eight Z. zerumbet, studied specimens, including Z. pellitum, C. pierreana, G. macrocarpa, G. globulifera, A. conchigera, S. campanulatus and Amomum sp. presented highly significant differences, on average $0.01\%,\ 0.008\%,\ 0.01\%,\ 0.012\%,\ 0.012\%,\ 0.02\%,\ 0.015$ and 0.3%, respectively.

Tab. II presented the chemical compositions of essential oil from the aerial parts of eight studied species. A total of seventy-five chemical constituents have been identified in which the essential oils of eight studied specimens contained about monoterpene hydrocarbons (0.81% - 62.19%),oxygenated monoterpenes (0.12%–59.3%), sesquiterpene hvdrocarbons (10.65% - 90.3%)oxygenated sesquiterpenes (0.17%–34.2%), non-terpenes (0.24%– 30.58%) and others (0.31%–6.83%). Furthermore, Fig. 2 and 3 showed the results derived from clustering analysis and PCA. The chemical constituents of essential oils divided into 4 clusters. Accordingly, cluster I contained A. conchigera with the high presence of β-elemene (51.76%) and caryophyllene (28.1%). Cluster II, consisting of five plants, including Z. zerumbet, Z. pellitum, C. pierreana, G. globulifera and S. campanulatus had β-pinene (11.81%, 5.32%, 4.43%, 25.42% and 1.57%), caryophyllene (13.61%, 23.45%, 19.78%, 34.26% and 18.72%) and caryophyllene oxide (4.66%, 4.00%, 13.65%, 8.15% and 24.69%) as major compounds. Because it was characterized by a number of distinct constituents, cluster II was divided into three subclusters. Consequently, subcluster I consisted of Z. zerumbet and Z. pellitum with β -Pinene (11.818% and 5.32%) as a main constituent while S. campanulatus belonged to the second subcluster which was characterized by the following compounds: alloaromadendrene (9.74%), β-pinene (1.57%) and D-limonene (1.33%). Subcluster III contained two species (C. pierreana and G. globulifera) with concentration of α-pinene (11.72% and 1.9%), D-limonene (1.20% and 1.80%), humulene (1.87% and 9.56%). Furthermore, cluster III contained *G. macrocarpa* with the presence of 4-isobutylquinoline (24.43%), β-copaene (21.69%), α-farnesene (20.73%), 2-tert-butylquinoline (6.05%), 3-dihydroxydiphenylamine (5.47%) and β -panasinsene (5.13%) as major compounds. Finally, cluster IV, consisting of *Amomum* sp. had eucalyptol (25.22%), camphor (24.51%), camphene (14.57%), β-pinene (13.45%), isoborneol (9.17%), α-selinene (6.21%) as major constituents.



2: The AHC tree diagram presenting the four clusters of the essential oils of eight studied species



3: PCA biplot presenting the main constituents of the essential oils of eight studied species

The chemical constituents of the essential oils of eight studied specimens showed significant difference. Among eight studied species, two species belonging to *Zingiber* genus, including *Z. zerumbet* and *Z. pellitum* were classified into subcluster I whereas two *Globba* species (*G. macrocarpa* and *G. globulifera*) belonged to two different clusters (Fig. 2 and 3). The chemical diversity of essential oils of some species belonging to the same genus has been investigated by previous studies (Wong *et al.*, 2005; Kurup *et al.*, 2018). For instance, the chemical constituents of the essential oils of two *Alpinia*

species, such as A. conchigera and A. latilabris were high different. Accordingly, A. conchigera consisted of the following major compounds: β -bisabolene (28.9%), 1,8-cineole (15.3%), β -caryophyllene (10.0%), β -pinene (9.5%) whereas methyl (E)-cinnamate (89.5%) was the main constituent in essential oil of A. latilabris (Wong $et\ al.$, 2005). Similary, A. agastyamalayanum and A. newmanii, two species belonging to Amomum genus, had the diversity in the chemical constituents of the essential oils. For instance, A. agastyamalayanum oils contained allo-aromadendrene (16.23%),

II: Chemical compositions in the essential oils from aerial parts of eight species of Zingiberaceae family in Binh Chau-Phuoc Buu Nature Reserve

			The amount percentage in samples (%)							
Compounds	RT	Code	Z. zerumbet	Z. pellitum	C. pierreana	G. globulifera	G. macrocarpa	Amomum sp.	A. conchigera	S. campanulatus
α-Pinene	9.55	C1	29.30	15.95	11.72	1.9	-	-	0.13	0.16
Eucalyptol	9.60	C2	-	-	-	-	0.23	25.22	-	-
4-Carene	9.70	СЗ	-	-	0.63	-	0.43	-	-	-
Camphene	9. 72	C4	-	0.18	-	0.39	-	14.57	-	-
β-Pinene	10.10	C5	11.81	5.32	41.04	25.42	-	13.45	-	1.57
Cyclofenchene	10.10	C6	-	11.31	-	-	-	-	-	-
3-Carene	10.50	C7	2.30	6.21	6.99	-	-	-	-	-
D-Limonene	10.61	C8	10.18	-	1.2	1.8	-	-	-	1.33
Sylvestrene	10.68	C9	-	5.35	-	-	-	-	-	-
β-Ocimene	10.78	C10	2.67	-	-	-	-	-	-	-
y-Terpinene	10.87	C11	0.25	-	-	-	-	-	-	-
1,6-Octadien-3-ol, 3,7-dimethyl-	11.15	C12	-	-	-	0.6	-	-	-	-
Cyclopropane, 1,1-dimethyl-2- (3-methyl-1, 3-butadienyl)-	11.20	C13	-	4.43	-	-	-	-	-	-
Camphor	11.48	C14	-	-	-	-	-	24.51	-	-
2-Octanol, acetate	12.01	C15	-	-	-	0.51	-	-	-	-
Isoborneol	12.28	C16	-	-	-	-	-	9.17	-	-
4-Ethyl-2-hexynal	12.46	C17	-	-	-	-	-	1.09	-	-
Bornyl acetate	12.48	C18	-	-	-	-	-	-	-	0.14
Isobornyl acetate	12.55	C19	-	-	-	-	-	0.4	-	-
Dihydroedulan II	12.61	C20	0.14	-	-	-	-	-	-	-
Tetracyclo[3.3.1.1(1,8).0(2,4)]Decane	12.75	C21	0.12	-	-	-	-	-	-	-
y-Elemene	12.89	C22	0.20	-	-	-	-	-	-	-
α-Cubebene	12.95	C23	-	-	-	-	-	-	-	0.22
Aciphyllene	13.04	C24	-	-	-	-	-	-	-	0.23
γ-Pyronene	13.08	C25	-	-	-	-	-	0.25	-	-
α-Copaene	13.19	C26	-	-	-	-	-	-	-	7.28
β-Elemene	13.22	C27	7.59	-	-	4.44	-	3.15	51.76	-
Germacrene D	13.26	C28	-	-	-	-	0.53	-	-	-
β-Bourbonene	13.33	C29	-	-	-	-	0.46	-	-	-
Caryophyllene	13.58	C30	13.61	23.45	29.11	34.26	-	1.29	28.1	18.72
β-Cadinene	13.77	C31	-	-	-	-	2.37	-	-	-
Humulene	13.84	C32	2.92	-	1.87	9.56	-	-	6.24	-
2-Norpinene	13.86	C33	-	-	-	-	-	-	0.49	-
α-Curcumene	13.89		-	-	-	-	-	0.24	-	-
β-copaene	13.98		6.38	-	-	-	21.69		-	-
α-Farnesene	14.00	C36	-	-	-	-	20.73	-	-	-

	-		The amount percentage in samples (%)							
Compounds	RT	Code	Z. zerumbet	Z. pellitum	C. pierreana	G. globulifera	G. macrocarpa	Amomum sp.	A. conchigera	S. campanulatus
β-Panasinsene	14.08	C37	-	-	-	-	5.13	-	-	-
α-Selinene	14.09	C38	-	-	-	-	-	6.21	0.15	-
Alloaromadendrene	14.11	C39	-	0.56	-	-	-	-	4.05	9.74
γ-muurolene	14.14	C40	0.84	3.02	-	-	4.30	-	-	-
δ-Cadinene	14.24	C41	-	-	-	-	2.56	-	-	2.71
1,3-Bis-(2-cyclopropyl, 2-methylcyclopropyl)-but-2-en-1-one	14.25	C42	-	-	-	-	-	0.22	0.31	-
α-Elemene	14.27	C43	-	-	-	-	-	-	-	2.1
Nerolidol	14.30	C44	0.62	-	0.42	5.55	-	0.17	-	-
1,1,5-Trimethyl-1, 2-dihydronaphthalene	14.35	C45	-	-	-	-	-	-	-	2.6
Arachidonic acid	14.37	C46	-	-	0.14	-	-	-	-	-
2,2-Dichloro-1-phenyl-1,3-propanediol	14.38	C47	-	-	-	-	0.78	-	-	-
Aromadendrene	14.40	C48	0.18	-	-	-	-	-	-	-
Caryophyllene oxide	14.66	C49	4.66	4.00	4.38	8.15	-	-	-	24.69
cis-Thujopsene	14.67	C50	-	3.68	-	-	-	-	-	-
3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl-	14.77	C51	0.41	-	-	-	-	-	-	5.23
Carvone Epoxide	14.83	C52	-	-	-	-	0.24	-	-	-
Isooctadecane	14.87	C53	-	-	-	-	-	-	0.27	-
Adamantane	14.89	C54	-	-	0.61	-	-	-	0.19	-
Bicyclo[3.2.2]non-6-en-3-one	14.96	C55	-	-	-	-	-	-	-	5.45
4-hydroxymethylbenzaldehyde	14.98	C56	-	-	-	-	0.35	-	-	-
Epiglobulol	15.01	C57	-	-	-	-	-	-	4.04	-
Caryophylla-4(12),8(13)-dien-5.betaol	15.03	C58	-	-	-	4.36	-	-	-	-
Longifolene	15.04	C59	0.43	-	-	-	-	_	-	-
9-Tetradecenol	15.06	C60	-	-	0.47	-	-	-	-	-
β-Selinene	15.09		_	-	-	-	1.16	_	-	_
Isoaromadendrene epoxide	15.11	C62	0.54	-	-	-	-	-	-	9.51
Alloisolongifolene	15.29	C63	_	1.79	-	-	-	_	-	_
γ-Cadinene	15.35		-	1.72	-	-	1.03	-	-	-
2-tert-Butylquinoline	15.36	C65	-	-	-	-	6.05	-	-	-
α-Cadinol	15.43	C66	-	3.02	-	-	-	-	-	-
α-Gurjunene	15.48	C67	-	3.00	-	-	-	-	-	-
Hexahydrofarnesyl acetone	15.75		0.17	-	-	-	-	-	-	-
Tryptophol	15.75	C69	_	-	-	-	0.33	_	-	_
7-Methyl-3,4-octadiene	15.77		-	-	0.27	-	-	-	-	-
zerumbone	15.87		-	4.99	-	-	-	-	-	-
Di(1'-decahydronaphthyl)methane	15.88		-	-	-	0.44	-	-	-	-
4-Isobutylquinoline	15.91		-	-	-	-	24.43	-	-	-
3-Dihydroxydiphenylamine	15.97		-	-	-	-	5.47	-	-	-

	The amount percentage in								n samples (%)			
Compounds	RT	Code	Z. zerumbet	Z. pellitum	C. pierreana	G. globulifera	G. macrocarpa	Amomum sp.	A. conchigera	S. campanulatus		
Farnesol, acetate	15.99	C75	-	-	0.12	_	-	-	1.85	-		
Monoterpene hydrocarbons:	15.99	50.47	62.19	29.51	1.46	28.27	0.81	3.06				
Oxygenated monoterpenes:	16.08	-	-	0.6	0.23	59.3	-	0.14				
Sesquiterpene hydrocarbons:	16.15	35.5	30.98	48.26	58.93	10.65	90.3	41				
Oxygenated sesquiterpenes:	5.82	12.01	5.19	18.06	-	0.17	5.89	34.2				
Non-terpenes:	0.41	-	-	0.44	30.58	0.24	-	13.28				
Others:	0.31	_	0.61	0.51	6.83	1.31	0.58	-				
Total:	95.32	97.98	98.97	97.38	98.03	99.94	97.58	91.68				

β-pinene (8.47%), (E)-caryophyllene (8.45%) as major compounds while the main constituents of A. newmanii oils were santolina triene (42.16%), α -pinene (17.09%), β -pinene (3.20%) and α -copaene (2.39%) (Kurup et al., 2018).

In present study, the aerial specimens were used to analyze the chemical composition of essential oils instead of rhizomes, as commonly used in other Zingiberaceae species. Among eight studies species, only one species belonging to Curcuma genus, *C. pierreana*, was investigated on chemical constituents of essential oils from the aerial parts. Accordingly, Nguyen et al. (1998) showed that the essential oils from the stem and leaf of C. pierreana contained isobornyl acetate (14.4% and 6.4%), isoborneo (12.4% and 12.8%), (2)-P-farnesene (10.8% and 8.5%), caryophyllene (10.4% and 9.1%), caryophyllene oxide (7.1% and 7.0%) whereas those of essential oils in this study were β-pinene caryophyllene (29.11%), (11.72%), 3-carene (6.99%) as major constituents.

Furthermore, four of the seven remaining species, including Z. zerumbet, Z. pellitum, A. conchigera and S. campanulatus have been reported on chemical constituents of essential oils by previous studies but those of essential oils were isolated from rhizomes instead of the aerial specimens, as in this study (Sirat and Nordin, 1995; Ibrahim et al., 2009; Phan et al., 2011; Di et al., 2017; Trinh et al., 2017). The present study, therefore, was firstly investigated the essential oils isolated from the aerial parts of these species in which Z. zerumbet was found to be rich in α -pinene (29.3%), β -pinene (11.8%) and D-limonene (10.18%); Z. pellitum contained high present of caryophyllene (23.45%), α-pinene (15.95%) and cyclofenchene (11.31%); A. conchigera consisted of the following β-elemene (57.75%) and caryophyllene (28.1%) as major compounds; S. campanulatus was characterized by the following main compounds: caryophyllene oxide (24.69%), caryophyllene (18.72%) and alloaromadendrene (9.74%).

Two Globba species, G. macrocarpa and G. globulifera, were rare species and mainly distributed in Thailand and Cambodia. In Vietnam, these species were discovered in four locations of Vietnam, such as Son La, Dak Lak, Tay Ninh and Ba Ria-Vung Tau Provinces (Pham, 2000; Jantan et al., 2003). To date, little is known about the chemical constituents of these species. Thus, in this study, the constituents of essential oils extracted from G. macrocarpa and G. globulifera were firstly investigated. Accordingly, 4-isobutylquinoline (24.43%), β -copaene (21.69) and α -farnesene (20.73) were the major compounds of *G. macrocarpa* while *G. globulifera* was found to be rich in caryophyllene (34.86%), β-pinene (25.42%), humulene (9.56%)

Finally, the essential oils of Amomum sp., an unknown species belonging to Amomum genus, were also recorded in present study. The chemical compounds of this species consisted of following compounds: eucalyptol (25.22%),camphor (25.51%), camphene (14.57%), β-pinene (13.45%) and isoborneol (9.17%).

CONCLUSION

The present study identified a total of 75 compounds of the essential oils from the aerial parts of eight species of the Zingiberaceae family collected in Binh-Chau Phuoc-Buu Nature Reserve, Southern Vietnam. Four main clusters and three subclusters were formed by the cluster analysis. Cluster I consisted of A. conchigera with the presence of β-elemene (51.76%) and caryophyllene (28.1%). Cluster II contained five species, including Z. zerumbet, Z. pellitum, C. pierreana, G. globulifera and *S. campanulatus* with high concentration of β-pinene (11.81%, 5.32%, 4.43%, 25.42% and 1.57%), caryophyllene (13.61%, 23.45%, 19.78%, 34.26% and 18.72%), caryophyllene oxide (4.66%, 4.00%, 13.65%, 8.15% and 24.69%). Cluster III consisted of *G. macrocarpa*, and presented 4-isobutylquinoline (24.43%), β-copaene (21.69%), α-farnesene (20.73%), 2-tert-butylquinoline (6.05%), 3-dihydroxydiphenylamine (5.47%), β-Panasinsene (5.13%) as major compounds. Cluster IV contained *Amomum* sp. and had eucalyptol (25.22%), camphor (24.51%), camphene (14.57%), β-pinene (13.45%), isoborneol (9.17%), α-selinene (6.21%) as main constituents.

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