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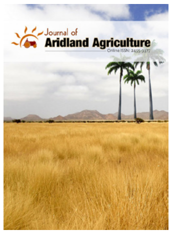

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Chemical diversity of essential oils of rhizomes of six species of Zingiberaceae family

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ABSTRACT

In this study, the essential oils from the rhizomes of six species belonging to the Zingiberaceae family, including *Zingiber zerumbet*, *Curcuma pierreana*, *Globba macrocarpa*, *Alpinia conchigera*, *Stahlianthus campanulatus* and *Amomum* sp., collected in Binh Chau-Phuoc Buu Nature Reserve were isolated using hydrodistillation, and their constituents were identified via Gas Chromatography-Mass Spectrometry. A total of 91 constituents have been identified from essential oils. These compounds were classified into 4 clusters by Agglomerative Hierarchical Clustering (AHC) and Principal Component Analysis (PCA) analysis. The principal constituents of the essential oils isolated from four species, *C. pierreana*, *S. campanulatus*, *A. conchigera*, and *Z. zerumbet* contained camphene (18.82%), α-copaene (11.75%), p-xylene (21.86%), and α-santalene (17.91%), which were significantly different from those in previous reports. Furthermore, this study revealed the chemical constituents of essential oils of *G. macrocarpa* and *Amomum* sp. for the first time. Accordingly, artemisia triene (22.21%), β-pinene (13.57%), 4,6,8-trimethylazulene (11.1%), 2-tert-butylquinoline (9.86%), β-patchoulene (7.06%), α-elemene (6.93%), and β-ocimene (6.0%) were the major compounds in essential oils of *G. macrocarpa* rhizomes whereas the oil of *Amomum* sp. was found to be rich in 2-carene (21.82%), fenchyl acetate (14.26%), 3-carene (8.28%), bornyl acetate (7.7%), and D-limonene (7.13%).

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INTRODUCTION

Gingers (Zingiberaceae) is a large family which has about 53 genera with over 1500 species occurring primarily in Asia, especially Southeast Asia (Kress *et al*., 2002; Leong-Škorničková & Newman, 2015; Van, 2021; Van *et al*., 2021). Members of Gingers are important natural resources, which provide many useful products for medicines, spices, cosmetics, and essential oil etc (Jantan *et al*., 2003; Jatoi *et al*., 2007; Koga *et al*., 2016; Zahara *et al*., 2018). Many medicinal compounds, including terpenes, alcohols, ketones, flavonoids and phytoestrogens were found in the essential oils isolated from rhizomes of some species belonging to the Zingiberaceae family (Zahara *et al*., 2018).

Vietnam is one of the large and diverse areas for Zingiberaceae which over 129 species have been recorded (Pham, 2000; Bach, 2005; Leong-Škorničková & Ly, 2010; Leong-Škorničková & Tran, 2013; Leong-Škorničková & Luu, 2013; Leong-Škorničková *et al*., 2015; Nguyen *et al*., 2017; Tran *et al*., 2019). Furthermore, the chemical constituents of some plants of this family have been identified. However, most of these studies have only analyzed components of individual species collected in the Northern or North Central regions of Vietnam (Dung *et al*., 1995; Dung *et al*., 1998; Huong *et al*., 2015; Dai *et al*., 2017; Hung *et al*., 2019).

Binh Chau-Phuoc Buu Nature Reserve is the only remaining sandy forest along the coastline of Vietnam for species of the Dipterocarpaceae family, which is located in Xuyen Moc District, Ba Ria-Vung Tau Province, Southern Vietnam. In 2019, we conducted some field trips to the Binh Chau-Phuoc Buu Nature Reserve and collected six species of the Zingiberaceae family. Here, we firstly investigated and compared the chemical composition of essential oils isolated from rhizomes of six Zingiberaceae family, including *Z. zerumbet*, *C. pierreana*, *G. macrocarpa*, *A. conchigera*, *S. campanulatus* và *Amomum* sp.

MATERIALS AND METHODS

Plant Materials

Specimens of six species were collected from Binh Chau-Phuoc Buu Nature Reserve, Bung Rieng Ward, Xuyen Moc District, Ba Ria-Vung Tau Province (Figure 1). The vouchered specimens were stored at hebarium of Binh Chau-Phuoc Buu Nature Reserve, including *Zingiber zerumbet* (L.) Roscoe ex Sm. (VS Le 215), *Curcuma pierreana* Gagnep. (VS Le 210), *Globba macrocarpa* Gagnep. (VS Le 212), *Alpinia conchigera* Griff. (VS Le 218), *Stahlianthus campanulatus* Kuntze (VS Le 217) and *Amomum* sp. (VS Le 216).

Hydro Distillation of the Essential Oils and Yield Calculation

Hydro-distillation was performed in a Clevenger type apparatus at 100°C for 3 hours with normal pressure. Pulverized and introduced five hundred gram of the fresh rhizomes of each of the six studied species into a 1.5 L flask; subsequently, added distilled water until completely submerged in the samples. After 3 hours, collected the essential oils in the receiver arm of the apparatus and transferred them into clean and dark bottles. The essential oils were dried by Na_2SO_4 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) (Costa *et al*., 2014).

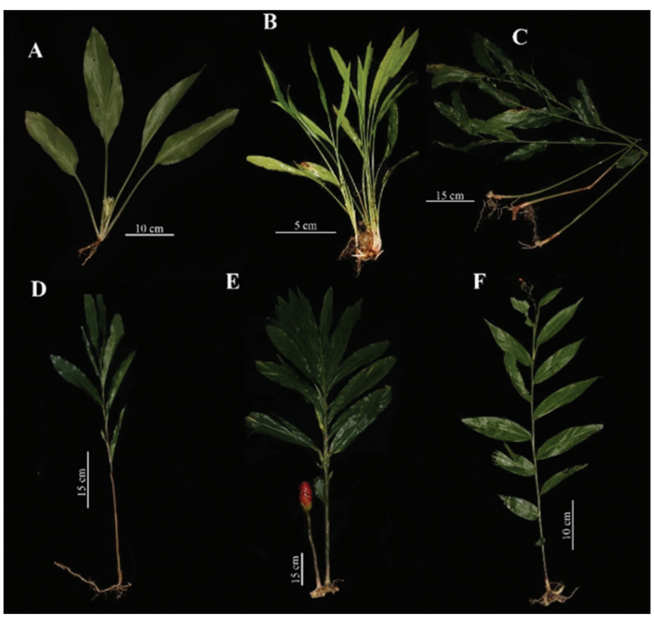

Figure 1: Six studied species in this study. A: *C. pierreana*, B: *S. campanulatus*, C: *A. conchigera*, D: *Amomum* sp., E: *Z. zerumbet*, F: *G. macrocarpa*.

Gas Chromatography-Mass Spectrometry (GC/MS) Analysis

The chemical composition of the essential oil was examined by gas chromatography/mass spectrometry (GC/MS) on an Agilent GC 7890B-MS 5975C using a HP-5MS capillary column (30 m x 250 µm x 0.25 µm). Briefly, 0.2 µL of the essential oil was injected into the GC-MS system. The separation process occurred in capillary column at the pressure of 13.209 psi with thermal program set as follows: start at 50°C and increase gradually to 320°C at a heating rate of 8 °C/min. The chemical components of the studied essential oils were identified based on the comparison between their mass spectra with NIST 2017 library.

Data Analysis

To classify the essential oils isolated from the rhizomes of six studied species into clusters, Agglomerative Hierarchical Clustering (AHC) was performed according to their similarities in the concentration of the 91 compounds identified by GC/MS. To reveal the differences among 91 compounds, Principal Component Analysis (PCA) was performed on the concentrations of them using a covariance matrix to identify the main constituents of each of the six essential oils. The analyses were conducted using XLSTAT Sensory (Addinsoft, Boston, USA).

RESULTS AND DISCUSSION

The studied essential oils from the rhizomes of six studied species were obtained in a yield of 0.01% (*C. pierreana*), 0.012% (*G. macrocarpa*), 0.03% (*Z. zerumbet*), 0.04% (*Amomum* sp.), 0.014% (*A. conchigera*), and 0.14% (*S. campanulatus*), calculated on a dry weight basis.

The chemical compositions of essential oil from the rhizomes of six studied species are listed in Table 1 in which a total of 91 constituents have been identified. The results derived from clustering analysis and PCA is presented in Figure 2. The chemical compositions of essential oils could divide into four clusters. Accordingly, the cluster I contained 1 species (*G. macrocarpa*) with the high presence of artemisia triene (22.21%), β-pinene (13.57%), 4,6,8-trimethylazulene (11.10%), 2-tert-butylquinoline (9.86%), β-patchoulene (7.06%), and α-elemene (6.93%). The cluster II included 3 species (*A. conchigera*, *C. pierreana*, and *S. campanulatus*) with the presence of camphene (*A. conchigera*-8.88%, *C. pierreana*-18.82%, and *S. campanulatus*-9.56%), caryophyllene (*A. conchigera*-4.37%, *C. pierreana*-10.76%, and *S. campanulatus*-4.3%). Because it is characterized by a number of distinct components, cluster II was divided into two subclusters. For example, *A. conchigera* belonged to subcluster I with the appearance of p-xylene (21.86%), chavicol (7.87%) while subcluster II comprised *C. pierreana* and *S. campanulatus* with concentration of γ-terpinene (*C. pierreana*-2.88%, and *S. campanulatus*-6.7%), D-limonene (*C. pierreana*-8.85%, and *S. campanulatus*-2.93%). The cluster III contained 1 species (*Z. zerumbet*) with the high presence of α-santalene (17.91%); bicyclo[2.2.2]oct-2-ene, 1,2,3,6-tetramethyl- (11.65%) and

Table 1: Chemical compositions in the essential oils from rhizomes of six species of Zingiberaceae family in Binh Chau-Phuoc Buu Nature Reserve

Compounds	Code	*Z. zerumbet*	*C. pierreana*	*Amomum* sp.	*A. conchigera*	*G. macrocarpa*	*S. campanulatus*
1,3,5-Cycloheptatriene	C1	-	-	-	4.88	-	-
p-Xylene	C2	-	-	-	21.86	-	-
Camphene	C3	1.64	18.82	-	8.88	-	9.56
Tricyclene	C4	-	0.29	-	-	-	-
Artemisia triene	C5	-	-	-	-	22.21	-
α-Pinene	C6	9.99	5.83	2.77	-	0.97	-
2-Carene	C7	-	-	21.82	-	0.08	-
Cyclohexene, 5-methyl-3-(1-methylethenyl)-, trans-	C8	-	-	-	0.36	-	-
β-Pinene	C9	5.90	-	3.08	-	13.57	0.70
Eucalyptol	C10	-	-	2.83	7.33	-	-
γ-Terpinene	C11	4.84	2.88	-	-	-	6.70
β-Ocimene	C12	0.72	-	-	-	6.00	-
D-Limonene	C13	-	8.85	7.13	-	3.15	2.93
α-Thujene	C14	-	-	-	-	0.28	-
β-Thujene	C15	3.47	-	-	-	-	-
1,2-Diethylbenzene	C16	-	-	-	3.11	-	-
Cycloheptene, 5-ethylidene-1-methyl-	C17	-	-	-	3.86	-	-
3-Carene	C18	1.35	14.13	8,28	-	-	0.18
α-Fenchol	C19	-	0.48	-	-	-	-
trans-Alloocimene	C20	-	-	-	-	2.66	-
1,4-Hexadiene, 5-methyl-3-(1-methylethylidene)-	C21	1.84	-	-	0.60	-	-
(+)-2-Bornanone	C22	2.29	-	-		0.11	5.14
β-Terpinol	C23	-	-	-	4.50	-	-
Camphor	C24	1.04	0.94	3.66	-	-	0.85
Isobornyl acetate	C25	2.98	-	-	-	-	--
α-Terpinolen	C26	-	-	-	3.66	-	-
1-Terpineol	C27	-	-	-	2.66	-	-
1,4-Dimethyladamantane	C28	-	0.80	-	-	-	-
Fenchyl acetate	C29	-	10.6	14.26	-	-	-
Bornyl acetate	C30	5.84	1.67	7.70	-	-	0.54
Myrtenyl acetate	C31	-	-	-	-	-	0.25
α-Terpinene	C32	-	0.97	-	-	-	-
Benzene 1-(2-butenyl)-2 3-dimethyl-	C33	-	-	-	0.26	-	-
α-Copaene	C34	-	-	-	-	-	11.75
Chavicol	C35		-		7.87	-	-
4-Carene	C36	-	-	3.54	-	-	0.14
γ-Cadinene	C37	-	0.48	-	-	-	-
Geraniol	C38	0.80	-	-	-	-	-
α-Elemene	C39	-	-	-	-	6.93	-
β-Elemene	C40	-	4.84	1.97	5.00	-	-
β-Patchoulene	C41	-	-	-	-	7.06	-
Isocaryophyllene	C42	-	-	-	1.43	-	-
Humulene	C43	-	0.92	-	-	-	-
Caryophyllene	C44	-	10.76	3.14	4.37	-	4.30
β-Germacrene	C45	-	-	-	0.39	-	-
1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene	C46	8.68	-	2.15	-	-	-
Pentadecane	C47	-	-	-	1.28	-	-
γ-Muurolene	C48	-	1.34	-	-	-	-
β-Selinene	C49	-	3.15	-	0.23	-	-
α-Himachalene	C50	-	-	-	-	-	3.35
γ-Maaliene	C51	-	-	6.10	-	-	-
Aromandendrene	C52	-	-	-	4.33	-	2.26
α-Amorphene	C53	-	-	-	-	-	4.97
7-epi-α-Selinene	C54	-	-	-	0.47	-	-
Nerolidol	C55	-	-	0.32	-	-	-
α-Calacorene	C56	-	-	-	-	-	1.28
Citronellyl formate	C57	-	-	-	1.12	-	-
Naphthalene, 1,2-dihydro-1,1,6-trimethyl	C58	-	-	-	-	-	0.87
Bicyclo[2.2.2]oct-2-ene, 1,2,3,6-tetramethyl-	C59	11.65	-	-	-	-	-
Alloaromadendrene	C60	-	0.59	-	-	-	7.69

(Contd...)

Table 1: (*Continued*)

Compounds	Code	*Z. zerumbet*	*C. pierreana*	*Amomum* sp.	*A. conchigera*	*G. macrocarpa*	*S. campanulatus*
Propane, 1,3-bis(ethylthio)-	C61	-	-	-	-	4.67	-
Spathlenol	C62	-	-	2.57		-	-
Caryophyllene oxide	C63	-	3.11	-	0.93	-	5.07
α-Sinensal	C64	6.71	-	-	-	-	-
Cyclosativene	C65	-	-	-	-	-	3.37
tau-Muurolol	C66	-	-	-	-	-	1.36
Isolimonene	C67	-	-	-	0.17	-	-
4,6,8-Trimethylazulene	C68	-	-	-	-	11.10	-
9-(1-Methylethylidene)-bicyclo[6.1.0]nonane	C69	2.81	-	-	-	-	-
α-Selinene	C70	0.95	-	2.04	0.52	-	-
9-Methoxycalamenene	C71	-	-	-	-	-	2.71
α-Gurgujene	C72	-	-	-	2.77	-	-
Patchoulane	C73	-	-	0.92	-	-	-
3,4-Dimethylanisole	C74	-	-	-	-	-	2.43
2-tert-Butylquinoline	C75	-	-	-	-	9.86	-
α-Santalene	C76	17.91	1.42	-	-	-	-
Isoaromadendrene epoxide	C77		-	0.70		-	-
trans-Calammenene	C78	-	-	-	-	-	0.57
Diazoprogesterone	C79		-	0.12		-	-
3,4-Hexadienal, 2-butyl-2-2-ethyl-5-methyl-	C80	-	1.07	0.87	-	-	-
Isoangenomalin	C81	-	-	-	-	-	7.30
4'-(1-Pyrroyl)acetophenone	C82	-	-	-	-	2.49	-
7-Hydroxycadalenal	C83	-	-	-	-	-	0.39
o-Chloro-N,N-diethylaniline	C84	-	-	-	-	3.65	-
n-Hexadecanoic acid	C85	-	-	0.44		1.23	0.41
(6E,10E,14E,18E)-2,6,10,15,19,23-Hexamethyltetracosa-1,6,10,14,18,22-hexaen-3-ol	C86	3.31	-	-	-	-	-
8,14-Cedranoxide	C87	-	-	-	-	0.27	-
(Z)-p-mentha-1,8-dien-2-hydroperoxide	C88	1.56	-	-	-	-	-
7-Methoxytetraphene	C89	-	-	-	-	-	10.56
1-Methylcarbazole	C90	-	-	-	-	0.33	-
9-Amino-1-phenyl-3,6-diazahomoadamantane	C91	-	-	-	-	-	1.88
Monoterpene hydrocarbons:		29.75	50.8	38.34	10.01	48.92	20.21
Oxygenated monoterpenes:		14.51	14.66	28.45	18.15	0.11	6.78
Sesquiterpene hydrocarbons:		27.54	23.5	16.32	19.51	13.99	39.54
Oxygenated sesquiterpenes:		6.71	3.11	3.27	1.40	0.27	6.43
Non-terpenes:		2.81	0.80	0.12	3.86	0.00	12.28
Others:		14.96	1.07	1.31	40.38	33.33	14.27
Total:		96.28	93.94	87.81	93.31	96.62	99.51

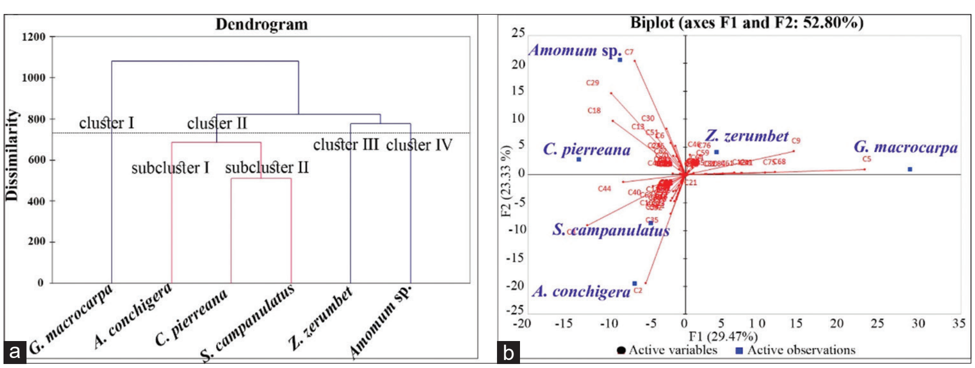

Figure 2: Comparison of chemical composition of essential oils of six studied species. (a) The AHC tree diagram presenting the four clusters of the essential oils. (b) PCA biplot presenting the main compound constituents of the essential oils.

α-sinensal (6.71%). Finally, *Amomum* sp. was characterized by 2-carene (21.82%), fenchyl acetate (14.26%), γ-maaliene (6.10%) and it therefore belonged to cluster IV.

Among six studied species, the chemical compositions of the essential oils of four species, including *C. pierreana*, *S. campanulatus*, *Z. zerumbet*, and *A. conchigera* have been reported in previous studies (Dung *et al*., 1995; Ibrahim *et al*., 2009; Dai *et al*., 2017; Padalia *et al*., 2018) in which the first two species were sampled in Northern or Central regions of Vietnam (Dung *et al*., 1995; Dai *et al*., 2017) while the remaining were sampled in other countries (Ibrahim *et al*., 2009; Padalia *et al*., 2018). The chemical constituents of essential oils of these species were a significant difference in their concentrations as compared to those in previous studies. Note that, the concentrations of chemical compositions of plant essential oils are found to vary depending on the geographical regions where they are collected (Hassiotis *et al*., 2010; Devkota *et al*., 2013). Accordingly, in this study, the major compounds of *C. pierreana* rhizome oil were found to be camphene (18.82%), caryophyllene (10.76%), 3-carene (14.13%), fenchyl acetate (10.60%) while the predominant constituents of this species in Dung *et al*. (1995) were isoborneol (22.9%), isobornyl acetate (18.8%), and camphor (7.2%). According to Dai *et al*. (2017), the main constituents of the oil of *S. campanulatus* rhizomes were stahlianthusone (27.6%), α-copaene (16.7%) and camphor (14.7%) but the rhizome oil of this species in present study was clearly dominated by α-copaene (11.75%), 7-methoxytetraphene (10.56%), camphene (9.56%), alloaromadendrene (7.69%), isoangenomalin (7.3%), γ-terpinene (6.7%). Ibrahim *et al*. (2009) suggested that 1,8-cineole, β-bisabolene were the main constituents of the essential oil of *A. conchigera* rhizomes with a concentration of 17.90% and 13.9%, respectively while in this study, p-xylene (21.86%), camphene (8.88%), chavicol (7.87%), and 1,8-cineole (7.33%) were the major components. In another study, Padalia *et al.* (2018) demonstrated that zerumbone (72.86%), α-humulene (7.09%), camphene (5.04%) were the major constituents of the essential oil of *Z. zerumbet* rhizome whereas the main compounds of this species in present study were α-santalene (17.91%), bicyclo[2.2.2]oct-2-ene, 1,2,3,6-tetramethyl- (11.65%), α-pinene (9.99%),5,9,9-tetramethyl-1,4,7-cycloundecatriene (8.68%), and α-sinensal (6.71%).

G. macrocarpa Gagnep., another species in this study, was firstly described by Gagnepain (1901). Nowadays, this species is considered as it mainly distributed in Cambodia, Thailand and Vietnam (Pham, 2000; Nguyen, 2017; Nguyen *et al*., 2017). In Vietnam, *G. macrocarpa* is found in Son La, Dak Lak, Lam Dong, and Tay Ninh Province (Pham, 2000; Nguyen, 2017; Nguyen *et al*., 2017). 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 and encountered a flowering population of *G. macrocarpa*. To date, little is known about the chemical constituents of this species. Thus, in this study, the constituents of essential oils extracted from the rhizomes of *G. macrocarpa* were firstly investigated. Accordingly, the principal constituents of the essential oil of *G. macrocarpa* rhizomes were artemisia triene (22.21%), β-pinene (13.57%), 4,6,8-trimethylazulene (11.1%), 2-tert-butylquinoline (9.86%), β-patchoulene (7.06%), α-elemene (6.93%), and β-ocimene (6.0%). Furthermore, the essential oil of an unknown species belonging to *Amomum* genus, *Amomum* sp., were also recorded in present study which the oil was found to be rich in 2-carene (21.82%), fenchyl acetate (14.26%), 3-carene (8.28%), bornyl acetate (7.7%), and D-limonene (7.13%).

CONCLUSION

The average leaf oil yields from the rhizomes of six studied species belonging Zingiberaceae family collected in Binh-Chau_Phuoc-Buu Nature Reserve, including *C. pierreana*, *G. macrocarpa*, *Z. zerumbet*, *Amomum* sp., *A. conchigera*, *S. campanulatus* were 0.01%, 0.012%, 0.03%, 0.04%, 0.014%, and 0.14%, respectively. A total of 91 constituents have been identified from essential oils which were classified into 4 clusters by Agglomerative Hierarchical Clustering (AHC) and Principal Component Analysis (PCA) analysis. The principal constituents of the essential oils isolated from four species, *C. pierreana*, *S. campanulatus*, *A. conchigera*, and *Z. zerumbet* contained camphene (18.82%), α-Copaene (11.75%), p-Xylene (21.86%), and α-Santalene (17.91%). Furthermore, the chemical constituents of essential oils of *G. macrocarpa* and Amomum sp. were recorded for the first time. Accordingly, artemisia triene (22.21%), β-Pinene (13.57%), 4,6,8-Trimethylazulene (11.1%), 2-tert-Butylquinoline (9.86%), β-Patchoulene (7.06%), α-Elemene (6.93%), and β-Ocimene (6.0%) were the major compounds in essential oils of *G. macrocarpa* rhizomes whereas the oil of *Amomum* sp. was found to be rich in 2-Carene (21.82%), fenchyl acetate (14.26%), 3-Carene (8.28%), bornyl acetate (7.7%), and D-Limonene (7.13%).

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