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# Chemical components and antioxidant properties of acetone extracts of four different species of Zingiberaceae

**Thi Hoi Huynh[1], Gia Hoa Tran[1], Ngoc Nam Trinh[2], Quoc Hung Nguyen[3], Thien Hoang Ho[1], Le Pham Tan Quoc[1], Nguyen Tuong An Huynh[1] & Hong Thien Van[1*]**

[1]Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, No. 12 Nguyen Van Bao Street, Go Vap District, Ho Chi Minh City, Vietnam
[2]Office of Science Management and International Affairs, Industrial University of Ho Chi Minh City, No. 12 Nguyen Van Bao Street, Go Vap District, Ho Chi Minh City, Vietnam
[3]Centre of Analytical Services and Experimentation HCMC, Vietnam, 2 Nguyen Van Thu, Dakao Ward, District 1, Ho Chi Minh City, Vietnam

*Email: vanhongthien@iuh.edu.vn

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## Abstract

In this report, the chemical components and antioxidant properties of the acetone extracts obtained from 4 Zingiberaceae species, including *Curcuma gracillima* Gagnep., *Curcuma pierreana* Gagnep., *Globba marantina* L. and *Amomum biflorum* Jack were investigated for the first time. A total of 142 chemical compounds were determined in 4 studied extracts using GC/MS techniques. Furthermore, the extract obtained from *C. gracillima* showed the strongest antioxidant effect with an $IC_{50}$ value of 1.76 mg/mL, followed by *G. marantina* ($IC_{50}$ = 2.45 mg/mL), *C. pierreana* ($IC_{50}$ = 2.97 mg/mL) and *A. biflorum* ($IC_{50}$ = 6.32 mg/mL).

## Keywords

Antioxidant; chemical compounds; GC/MS techniques; zingiberaceae

## Introduction

Zingiberaceae, the largest family in the order Zingiberales, includes around 1200 species belonging to 53 genera (1) being distributed mainly in subtropical and tropical zones, especially in Southeast Asia. Most members of this family are medicinal plants and are widely used in indigenous medicine and the pharmaceutical industry because of their good biological properties (2). In addition, the Zingiberaceae species are also used for dye and perfume (3), food, spices, ornamental plants and cosmetics, etc (4).

Vietnam is one of the countries with a high diversity of Zingiberaceae species. In 2000, Pham-Hoang recorded 24 genera and 113 species belonging to the Zingiberaceae family for the flora of Vietnam (5). In 2017, Nguyen in the book "Flora of Vietnam" reported 19 genera and 140 species of Zingiberaceae in Vietnam (6). Notably, a large number of plants belonging to this family have been discovered as new species for the flora of Vietnam by recent publications (7-11).

Binh Chau-Phuoc Buu is a large Nature Reserve with about 10451 ha located in the lowland dry forests in southern Vietnam (12). This Nature Reserve was established in 1996 and had about 732 vascular plants belonging to 113 genera and 63 families that were used as medicinal plants (12, 13). Recently, we have conducted some field trips to survey the biodiversity in Binh Chau-Phuoc Buu Nature Reserve, Ba Ria-Vung Tau province, and collected 4 species of Zingiberaceae family, including *Curcuma gracillima* Gagnep., *Curcuma pierreana* Gagnep., *Globba*

*marantina* L. and *Amomum biflorum* Jack. Among 4 studied species, except for *G. marantina*, an ethnomedicinal plant used in some Asian countries, the remaining three species are rare and they do not have any information about their use in traditional medicine. Accordingly, indigenous medicine in India has used *G. marantina* to treat some common diseases such as rheumatism, conjunctivitis, snake bite, cough, leucoderma, cold, asthma, etc (14, 15). Thus, in the current study, the chemical components and the antioxidant properties of the acetone extracts from these species were firstly reported.

## Materials and Methods

### *Plant materials and taxonomy*

The whole plants of 4 studied species, namely *C. gracillima*, *C. pierreana*, *G. marantina,* and *A. biflorum* were collected from Binh Chau-Phuoc Buu Nature Reserve from June to August 2023 by Mr. Van Son Le. The voucher specimens (Le VS 982-985) were deposited in the herbarium of this Nature Reserve. The process of collecting samples and processing specimens was carried out according to the instructions Kew Botanic Garden protocol (16). Comparative morphology was used to identify the scientific names of studied species based on the prior publications (5, 6).

### *Extraction procedures*

The whole plants of each species were dried at 50 °C and then ground into powder. 500 mL of acetone solution (Thermo Fisher Scientific, USA) was used to soak 100 g of sample powder for 72 h at room temperature. The soaking solution was filtered through the Whatman filter paper. The experiment was repeated two more times with the filter residue. The filtrate was evaporated on a vacuum evaporator at 45 °C and then the evaporation fluid was dried to completely remove acetone (17).

### *Gas chromatography-mass spectrometry (GC/MS) analysis*

The chemical components of the studied sample were determined using the gas chromatograph GC TRACE 1310 coupled with a single quadrupole mass spectrometer ISQ 7000 (Thermo Fisher Scientific Inc., Waltham, MA, USA) and the DB-5MS column (30 m x 0,25 mm x 0,25 µm) with a standard procedure (17, 18).

### *Determination of the antioxidant activity of the extracts*

The free radical scavenging property of the 4 studied extracts was performed by DPPH radical scavenging assay. The procedure was also performed (17, 19).

## Results

### *Chemical compounds of acetone extracts from four studied species.*

The chemical constituents of the acetone extracts isolated from 4 studied species are presented in Fig. 1 and Table 1. The major compounds of *G. marantina* extract were composed of n-hexadecanoic acid (13.42%), neophytadiene (7.35%), *(E)*-labda-8(17),12-diene-15,16-dial (6.65%), caryophyllene oxide (6.36%) and β-caryophyllene (5.07%). The *C. gracillima* extract was found to be rich in n-hexadecanoic acid (14.03%), β-caryophyllene (11.76%), *(E)*-labda-8(17),12-diene-15,16-dial (9.88%), caryophyllene oxide (8.81%), neophytadiene (8.78%) and linolenic acid (8.69%). The most abundant component of *C. pierreana* extract was n-hexadecanoic acid (12.07%), followed by *(E)*-labda-8(17),12-diene-15,16-dial (11.97%), cis-13-octadecenoic acid (6.86%), γ-bicyclohomofarnesal (6.11%), octadecanoic acid (5.34%), 9E,11E-octadecadienoic acid (4.97%) and caryophyllene oxide (3.78%) whereas (-)-alcanfor (14.12%), reynosin (8.74%), neophytadiene (8.31%) and n-hexadecanoic acid (6.75%), eucalyptol (4.24%) was the major compounds of

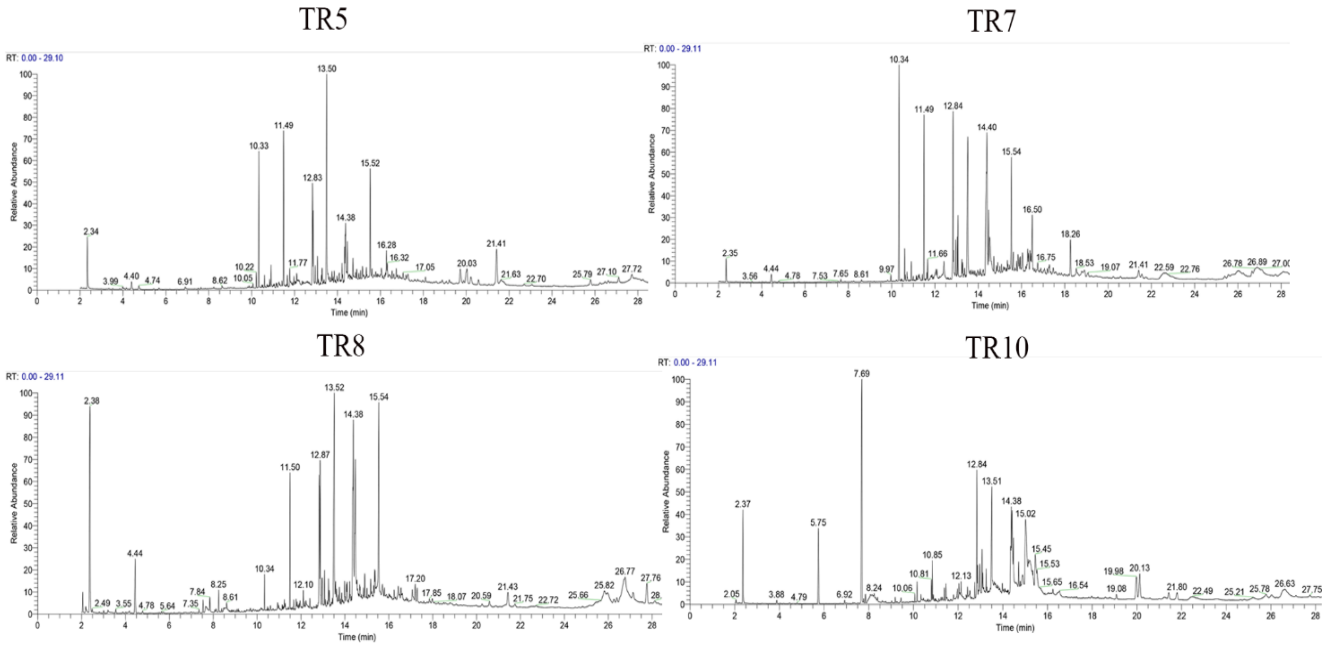


**Fig. 1.** The GC chromatogram of four studied extracts. TR5: *Globba marantina*, TR7: *Curcuma gracillima*, TR8: *Curcuma pierreana*, and TR10: *Amomum biflorum.*

**Table 1.** The relative percentage of chemical compounds of four studied extracts

| No. | Compounds | The relative percentage (%) | | | |
|---|---|---|---|---|---|
| | | TR5 | TR7 | TR8 | TR10 |
| 1 | 2-Pentanone, 4-hydroxy-4-methyl- | 2.50 | 1.27 | 9.70 | 4.30 |
| 2 | Ethanone, 1-(3-methylenecyclopentyl)- | - | 0.10 | 0.10 | 0.07 |
| 3 | 3-Methylcyclopentyl acetate | 0.09 | 0.10 | 0.13 | 0.07 |
| 4 | 2-Heptanol, acetate | - | - | 0.22 | - |
| 5 | Benzene, (1-methylethyl)- | 0.15 | 0.08 | 0.12 | - |
| 6 | α-Pinene | - | 0.14 | 0.23 | - |
| 7 | Benzene, propyl | 0.14 | 0.08 | - | - |
| 8 | Camphene | - | - | 0.09 | 0.25 |
| 9 | Toluene, m-ethyl- | 0.39 | 0.14 | 0.10 | - |
| 10 | Benzene, 1,2,3-trimethyl- | 0.17 | 0.19 | - | 0.12 |
| 11 | Mesitylene | - | 0.12 | 0.23 | - |
| 12 | β-Pinene | 0.77 | 0.91 | 2.79 | 0.13 |
| 13 | Benzene, 1,2,4-trimethyl- | 0.49 | 0.06 | - | - |
| 14 | o-Cymene | - | - | 0.08 | - |
| 15 | D-sylvestrene | - | - | 0.13 | - |
| 16 | Limonene | - | - | - | 0.04 |
| 17 | Eucalyptol | - | - | 0.11 | 4.24 |
| 18 | 3-Carene | - | - | 0.07 | - |
| 19 | Pyrazine, tetramethyl- | - | - | 0.09 | - |
| 20 | 2-Norbornanone | - | - | - | 0.05 |
| 21 | Linalool | 0.25 | - | - | 0.24 |
| 22 | rans-p-Mentha-2,8-dienol | - | - | - | 0.12 |
| 23 | α-Campholenal | - | - | 0.16 | - |
| 24 | 1,7,7-Trimethylbicyclo[2.2.1]hept-5-en-2-ol | - | - | - | 0.10 |
| 25 | (1R)-(+)-Nopinone | 0.13 | 0.09 | 0.47 | - |
| 26 | (-)-Alcanfor | 0.18 | 0.27 | 0.41 | 14.12 |
| 27 | Camphene hydrate | - | - | - | 0.24 |
| 28 | Sabinone | - | - | 0.52 | 0.45 |
| 29 | Terpinen-4-ol | - | - | - | 0.50 |
| 30 | Myrtenal | 0.20 | 0.14 | 0.76 | 0.69 |
| 31 | Levoverbenone | - | - | - | 0.30 |
| 32 | Ethylmethylmaleimide | 0.25 | 0.23 | 0.65 | 0.17 |
| 33 | (-)-Carvone | - | - | - | 0.15 |
| 34 | p-Mentha-1,8-dien-3-one | - | - | - | 0.16 |
| 35 | Bornyl acetate | - | - | 0.14 | 0.29 |
| 36 | (-)-Neoclovene-(I), dihydro | 0.20 | - | - | - |
| 37 | Bicyclo[2.2.1]heptane-2-carboxylic acid, 3,3-dimethyl | - | - | - | 0.30 |
| 38 | Spiro[2.7]dec-4-ene, 1,1,5,6,6,9,9-heptamethyl-10-methylene | - | - | - | 0.11 |
| 39 | Bicyclo[4.1.0]hept-3-en-2-one, 4,7,7-trimethyl- | - | - | - | 0.09 |
| 40 | α-Cubebene | - | 0.11 | - | 0.08 |
| 41 | 1,3,5,7-Tetramethyl-adamantane | 0.19 | - | - | - |
| 42 | Cypera-2,4-diene | 0.39 | - | - | - |
| 43 | Copaene | - | 0.37 | - | - |
| 44 | β-elemene | 0.35 | - | - | 0.35 |
| 45 | 9,9-Dimethyl-2-oxabicyclo[4.2.1]nonan-3-one | - | - | - | 0.84 |
| 46 | p-Cymene, 2,5-dimethoxy | - | - | 0.19 | - |
| 47 | α-Gurgujene | 0.80 | - | - | - |
| 48 | β-Caryophyllene | 5.07 | 11.76 | 1.08 | - |
| 49 | Isocaryophyllene | - | - | - | 0.43 |
| 50 | Geranylacetone | - | - | - | 0.25 |
| 51 | *(E)*-β-Famesene | 0.14 | - | - | - |
| 52 | Tetradecane, 2,6,10-trimethyl | 0.62 | - | - | - |
| 53 | Humulene | 0.61 | - | - | - |
| 54 | 1,1,4,8-tetramethyl-cis,cis,4,7,10-cycloundecatriene | - | 1.50 | 0.39 | - |
| 55 | Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl | - | 0.45 | - | - |
| 56 | Aristolochene | - | - | - | 0.81 |
| 57 | β-Selinene | 0.51 | - | - | 1.36 |
| 58 | α-Selinene | - | - | - | 0.32 |
| 59 | β-Bisabolene | 1.21 | - | - | - |
| 60 | β-Curcumene | - | 0.94 | - | - |
| 61 | Isogermacrene D | - | - | 0.28 | - |
| 62 | Cadina-1(10),4-diene | - | 0.38 | - | 0.28 |
| 63 | Guaia-10(14),11-diene | 0.33 | - | - | - |
| 64 | α-Guaiene | 0.29 | - | - | - |

| | | | | | |
|---|---|---|---|---|---|
| 65 | Dihydroactinolide | 0.34 | 0.37 | 0.25 | 0.25 |
| 66 | 3,5,9-Trimethyl-deca-2,4,8-trien-1-ol | 0.32 | - | - | - |
| 67 | 2(1H)-Naphthalenone, octahydro-4a,7,7-trimethyl-, cis | - | - | 0.23 | - |
| 68 | Dodecanoic acid | - | - | 0.19 | - |
| 69 | Nerolidol | - | 0.31 | 0.45 | 0.37 |
| 70 | Caryophyllene oxide | 6.36 | 8.81 | 3.78 | 0.37 |
| 71 | 4,8,12-trimethyltrideca-1,3,7,11-tetraene | 0.21 | - | - | 0.39 |
| 72 | α-Costal | - | - | - | 1.82 |
| 73 | (-)-Spathulenol | - | - | - | 1.00 |
| 74 | Humulene-1,2-epoxide | 0.64 | 1.03 | 0.31 | - |
| 75 | Methyl (3-oxo-2-[(2Z)-2-pentenyl]cyclopentyl)acetate | 2.15 | - | - | - |
| 76 | Isospathuleno | - | - | - | 0.66 |
| 77 | Cyclolongifolene oxide, dehydro | - | 0.39 | - | - |
| 78 | Pogostole | - | - | 0.50 | - |
| 79 | Neointermedeo | - | - | - | 1.06 |
| 80 | Globulol | 0.58 | - | - | - |
| 81 | Mustakone | - | 0.63 | - | - |
| 82 | (+)-β-Costol | - | - | - | 1.02 |
| 83 | Longifolenaldehyde | 0.85 | - | 0.73 | - |
| 84 | Pentadecanal | - | - | 0.30 | - |
| 85 | 2-Phytene, isomer 1 | - | - | 0.43 | - |
| 86 | Isovalencenyl formate | 0.46 | - | - | - |
| 87 | 1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulene-4,7-diol | - | - | - | 0.63 |
| 88 | Tetradecanoic acid | 0.35 | - | 0.60 | - |
| 89 | Aromadendrene oxide-(1) | - | - | - | 0.89 |
| 90 | 3,7,11-Trimethyl-dodeca-2,4,6,10-tetraenal | - | - | - | 0.76 |
| 91 | trans-Methylisocosticate | - | - | - | 1.20 |
| 92 | Neophytadiene | 7.35 | 8.78 | 1.36 | 8.31 |
| 93 | γ-Bicyclohomofarnesal | - | - | 6.11 | - |
| 94 | 2-Pentadecanone, 6,10,14-trimethyl- | 3.33 | - | 3.49 | 1.33 |
| 95 | 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl | 1.45 | 2.48 | - | - |
| 96 | Valerenic acid | - | - | - | 1.29 |
| 97 | Farnesyl acetone | - | - | - | 1.41 |
| 98 | 2-Dodecen-1-yl(-)succinic anhydride | 1.07 | - | - | - |
| 99 | n-Hexadecanoic acid | 13.42 | 14.03 | 12.07 | 6.75 |
| 100 | *(E)*-15,16-Dinorlabda-8(17),11-dien-13-one | - | - | 0.45 | 0.97 |
| 101 | Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate | 0.64 | - | - | - |
| 102 | *trans*-Geranylgeraniol | 1.25 | - | - | - |
| 103 | Shyobunol | - | - | 0.68 | - |
| 104 | Heptadecanoic acid | 0.80 | 0.69 | 1.12 | 1.06 |
| 105 | *(E)*-15,16-Bisnorlabda-8(17),12-diene-14-al | 1.56 | - | 1.06 | - |
| 106 | Naphthalene, decahydro-1,1,4a-trimethyl-5-(3-methylbutyl)-6-methylene-, (4aS,5S,8aS) | - | 0.64 | 0.85 | - |
| 107 | 3,7,11,15-Tetramethyl-1-hexadecyn-3-ol | 3.80 | - | - | - |
| 108 | 2,6-Dodecadien-1-ol, 3,7,11-trimethyl-, (Z,E)- | - | - | 0.94 | - |
| 109 | 9E,11E-octadecadienoic acid | 2.03 | - | 4.97 | - |
| 110 | 10E,12Z-octadecadienoic acid | - | - | - | 1.97 |
| 111 | Linolenic acid | 1.81 | 8.69 | - | - |
| 112 | *cis*-13-Octadecenoic acid | - | - | 6.86 | - |
| 113 | *cis*-Vaccenic acid | - | - | - | 3.80 |
| 114 | 7-Hydroxy-6,9a-dimethyl-3-methylene-decahydro-azuleno[4,5-b]furan-2,9-dione | - | - | - | 3.16 |
| 115 | Octadecanoic acid | 2.42 | 3.68 | 5.34 | 2.35 |
| 116 | 2,6-Di-tert-butylbenzoquinone | - | - | - | 1.92 |
| 117 | Androst-4-en-3-one, 17-methyl | - | 0.93 | - | - |
| 118 | 7β-Hydroxydehydroepiandrosterone | - | - | 1.35 | - |
| 119 | Ambria | - | - | 0.80 | - |
| 120 | Reynosin | - | - | - | 8.74 |
| 121 | Eicosane | - | 1.07 | - | - |
| 122 | Ambrosin | - | - | - | 2.52 |
| 123 | Androst-5-en-3-one, 4,4-dimethyl | - | - | 1.48 | - |
| 124 | 4,8,12,16-Tetramethylheptadecan-4-olide | 0.97 | - | 1.06 | - |
| 125 | *(E)*-labda-8(17),12-diene-15,16-dial | 6.65 | 9.88 | 11.97 | 1.55 |
| 126 | Androst-4-en-3-one, 17-(1-oxo-3-phenylpropoxy)-, (17β)- | 1.12 | - | - | - |
| 127 | Eicosanal | - | - | 1.26 | - |
| 128 | Pentacosane | 1.43 | 2.10 | - | - |
| 129 | Pentacosanal | - | - | 0.97 | 1.68 |
| 130 | 2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro | 1.88 | - | - | - |
| 131 | Pregn-20-yn-17-ol, (5β,17α)- | - | 2.11 | - | - |
| 132 | Villosin | 1.40 | 1.14 | 3.81 | - |
| 133 | 1-Heptatriacotanol | 1.81 | 6.71 | - | - |
| 134 | Tetracosanoic acid | - | - | 1.52 | - |
| 135 | 13-Docosenamide, (Z)- | - | - | 1.12 | 0.77 |
| 136 | Squalene | - | 4.23 | - | - |
| 137 | 2-(3-Hydroxy-4-methoxyphenyl)-3,7-dimethoxy-4H-chromen-4-one | - | - | - | 3.17 |
| 138 | 4',7-Dimethoxyflavonol | - | - | - | 3.14 |
| 139 | Cholesta-4,6-dien-3-ol, (3β)- | 5.55 | - | - | - |
| 140 | Tetratriacontane | - | - | - | 1.45 |
| 141 | Stigmasta-3,5-dien-7-one | 3.11 | - | - | - |
| 142 | γ-Sitostenone | 3.95 | - | - | - |
| | Total | 97.48 | 98.13 | 97.70 | 99.78 |

**Note:** TR5: *Globba marantina*, TR7: *Curcuma gracillima*, TR8: *Curcuma pierreana*, and TR10: *Amomum biflorum*

the *A. biflorum* extract.

### *Antioxidant activity of acetone extracts from four studied species*

The DPPH radical scavenging agents of 4 studied extracts are presented in Table 2. Accordingly, the extract obtained from *C. gracillima* possessed the highest antioxidant property ($IC_{50}$ value of 1.76 mg/mL) compared to the other 3 extracts, including *G. marantina* ($IC_{50}$ = 2.45 mg/mL), *C. pierreana* ($IC_{50}$ = 2.97 mg/mL) and *A. biflorum* ($IC_{50}$ = 6.32 mg/mL).

## Discussion

The chemical compounds of the acetone extracts isolated from *G. marantina*, *C. gracillima*, *C. pierreana,* and *A. biflorum* were first presented in this study. However, their chemical constituents in the essential oils and other solvent extractions obtained from these species using GC/MS have been shown by prior studies. For instance, the chemical components of the essential oils (EOs) from *G. marantina* contained β-caryophyllene (19.30%), α-humulene (14.20%), (Z)-nerolidol (7.50%) and isoborneol (7.30%) as the main compounds (20) whereas the ethanolic extract of this species was found to be rich in pinocarvone (54.27%), β-caryophyllene (13.65%), terpineol (6.31%) and heptadecane (6.19%) (21). Furthermore, the EO isolated from *C. pierreana* was reported to contain camphene (18.82%), 3-carene (14.13%), β-caryophyllene (10.76%) and fenchyl acetate (10.60%) (22). The chemical compositions of the aerial part of the *C. gracillima* EOs were characterized by the predominance of caryophyllene (19.8%), caryophyllene oxide (13.7), and 3-carene (9.3%) while the rhizome oil contained α-curcumin (18.9%), 3,4-dimethyl anisole (17.6%) and 2-bornanone (8.6%) as the main components (23). The chemical compounds of the EO obtained from *Amomum biflorum* were reported by the dominance of camphor (17.60%), α-bisabolol (16.00%), camphene (8.20%), and α-humulene (5.10%) (24).

Many bioactive compounds contained from 4 studied extracts in this study have been also found in Zingiberaceae species. Accordingly, *(E)*-labda-8(17),12-diene-15,16-dial was reported as a compound obtained from the extracts of various Zingiberaceae plants (25-28). This component has been described to have cytotoxic properties against some cancer cell lines in humans (29). It has also been shown that this compound was used as an agent to treat fungal infections, a-glucosidase inhibition, or as an antibiotic (30, 31). Also, *(E)*-labda-8(17),12-diene-15,16-dial isolated from *Curcuma amada* extract showed potent antibacterial activity against *Escherichia coli* (26). β-caryophyllene has also been shown as a bioactive component to have anticancer, antioxidant, antimicrobial, re-epithelialization, and anti-inflammatory activities (32-35). Prior studies demonstrated that *n*-hexadecanoic acid also possessed cytotoxic (36), antibacterial and antioxidant (37), and anti-inflammatory (38) properties. Moreover, caryophyllene oxide has been announced to possess anti-inflammatory, analgesic, (39) antifungal (40), and cytotoxic (41) activities. The prior study showed that neophytadiene exhibited an anti-inflammatory effect in Sprague dawley rats and RAW 264.7 macrophages (42). This compound was also shown as an agent in neuropharmacological activity, a type of anticonvulsant, antidepressant, sedative, and anxiolytic (43).

## Conclusion

The present study firstly showed the chemical compositions and antioxidant activity of the acetone extracts isolated from 4 Zingiberaceae species in which the *C. gracillima* extract was found to be rich in n-hexadecanoic acid (14.03%), β-caryophyllene (11.76%), *(E)* -labda-8(17),12-diene-15,16-dial (9.88%) while the most abundant component of *C. pierreana* extract was n-hexadecanoic acid (12.07%), followed by *(E)*-labda-8 (17),12-diene-15,16-dial (11.97%), cis-13-octadecenoic acid (6.86%). The major compounds of *G. marantina* extract were composed of n-hexadecanoic acid (13.42%), neophytadiene (7.35%), *(E)*-labda-8(17),12-diene-15,16-dial (6.65%) while *Amomum biflorum* contains (-)-alcanfor (14.12%), reynosin (8.74%), neophytadiene (8.31%) as the main compounds. The acetone extracts from *C. gracillima, G. marantina, C. pierreana,* and *A. biflorum* showed antioxidant activity with $IC_{50}$ values of 1.76, 2.45, 2.97, and 6.32 mg/mL respectively.

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## Authors' contributions

This research was designed and conducted by THH. This author was also the person who wrote the manuscript. All authors searched literature and revised the manuscript as well as contributed many valuable options. HTV was a

**Table 2.** DPPH radical scavenging activity of four studied extracts

| | *G. marantina* | *C. gracillima* | *C. pierreana* | *A. biflorum* | Vitamin C |
|---|---|---|---|---|---|
| $IC_{50}$ (mg/mL) | 2.45 | 1.76 | 2.97 | 6.32 | 0.0075 |

person who revised the final version of the manuscript as well as was responsible for working with the Journal.

## Compliance with ethical standards

**Declaration :** Authors do not have any conflict of interest to declare.

**Ethical issues:** None.

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