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Original Article

The Diversity of Biologically Active Compounds in the Rhizomes of Recently Discovered Zingiberaceae Plants Native to North Eastern Thailand

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ABSTRACT

Objective: To identify and quantify the bioactive compounds, along with biological activities, of native Thai edible Zingiberaceae. **Methods:** The bioactive compounds evaluated were phenolic acids, flavonoid, vitamin C, curcumin, 6-gingerol, eugenol and essential amino acids; analyses involved HPLC and LCMS/MS. Antioxidant activities were assessed by DPPH and FRAP assays. **Results:** *Zingiber officinale* was the richest source of bioactive compounds, followed by *Z. officinale*, *Alpinia zerumbet* and *Alpinia conchigera*. Total phenolic content and total flavonoid contents ranged widely across these species (17 to 200 mg GAE/100 g DW and 17 to 66 mg RE/100 g DW). All the species studied possessed strong antiglycation properties, ranging from 82 to 98%, with strong positive correlations of total phenolic content and antioxidant activity. The contents of curcumin, 6-gingerol, eugenol and vitamin C were in the range of 1 to 26, 1 to 140 µg/ g DW, 5 to 1600 and 4 to 21 mg/100 g DW, respectively. Seven essential amino acids identified by using LCMS/MS were found in most samples studied ranging from 2 to 6752 µg/100 g DW. **Conclusion:** Z. *officinale* is an abundant source of bioactive compounds and antioxidant activity in all these samples. These plants are fresh sources for developing novel functional ingredients in either food or cosmetics.

Key words: Essential amino acids, Antiglycation, Antioxidants, Bioactive compounds, Ginger family.

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INTRODUCTION

Zingiberaceae is an important tropical plant found worldwide. Several members of Zingiberaceae or 'Ginger family' are generally found in the tropical regions of Southeast Asia. Thailand has one of the richest Ginger floras in the world. There are approximately 52 genera with over 1300 species discovered by scientists, out of which 26 genera and 250 species are found native to Thailand.[1] North eastern Thailand provides a unique environment for the nurture of specific genotypes, being unusually dry and elevated. The biologically active compounds of many of these genotypes have never been reported.

The Ginger plant sprouts from the bottom of the plant during early rainy reason. The dominant characteristics of all species in this family are that all part of plants are aromatic especially leaves and rhizomes. Leaves are crushed to be pseudostem. Leaf blades appear as elliptic to elliptic–oblong or subspheroidal or spheroidal shapes and are arranged in two ranks (or one rank only in *Kaempferia siamensis*) on the leafy shoot. In the Southeast Asian region, rhizomes of the Ginger species have been used as food ingredient as a spice or flavoring agent for culinary application. Moreover, the Zingiberaceae rhizomes have also been used as traditional medicine for treating various diseases.[2]

In Thailand, Rhizomes of some Zingiberaceae species are used in Thai native dishes and some species are also used in ancient recipes of folk medicine. Numerous studies on medicinal and nutritional properties have been conducted.[3,4] The Zingiberaceae species which are commonly consumed in Thailand include *Zingiber officinale*, *Alpinia conchigera*, *A. zerumbet*, *Z. mekongense*, *Curcuma singularis* and *C. angustifolia*. They are used as spices in many traditional Thai foods such as Tom yam, Tom Kha, green and red curry, etc.[5] In addition, some species are used as vegetables especially *C. angustifolia* (in Thai called Krajeo-daeng) and *C. singularis* (in Thai called Krajeo-khao). Currently, development of functional foods from indigenous plants has been gaining a great interest. For example, *Z. officinale* has been processed to powder as an instant beverage as it has been reported to possess medicinal properties for relief of diarrhea, stomach ache, nausea and antiobesity.[6,7] Furthermore, Bellik[8] brought to light their potential antimicrobial activity. A large number of bioactive compounds with novel structures have been identified from these plants; for example, 6-gingerol, curcumin, eugenol and vitamin C.[9,10] Wang *et al.*[11] have reported the identification of gingerol in Zingiberaceae grown in China. In addition, found that the major phytochemicals in *C. longa* were curcumin in Brazil.[12] Although there are many published reports related to the bioactive compounds of the Zingiberaceae family plant of many origins, there has been little information of amino acids, phytochemicals along with the biological activities of Zingiberaceae in Thailand. In addition, the composition and content of these compounds

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could be affected by growth conditions or growth locations.[13,14] For example, turmeric grown in the central region of Thailand had a higher content of curcuminoid than that grown in other parts of Thailand.[15]

Therefore, we aimed to generate information about essential amino acids, and bioactive compounds, in eight varieties of Zingiberaceae discovered in northeastern which is the biggest plain region in Thailand. The contents of curcumin, 6-gingerol, eugenol as well as total phenolic, total flavonoid contents, along with antiglycation and antioxidant activities were determined, and individual phenolic and flavonoid acids of the studied species were quantified. Correlations of antiglycation activity with total phenolic and flavonoid contents were also carried out. This present study expects to provide useful information for wider use of these plants. This study should provide a useful foundation for further studies on processing and or production of functional food products.

MATERIALS AND METHODS

Plant materials and sample preparation

Whole plants of eight edible species from Zingiberaceae were collected from the northeastern region of Thailand in 2017. Advice from local people was used as a basis for characterizing samples according to their uses, especially for being edible. They were identified by Dr. Surapon Saensouk, plant taxonomist, from Walai Rukhavej Botanical Research Institute, Mahasarakham University, where specimens were also deposited in the herbarium. The rhizomes of selected species were thoroughly washed in tap water, freeze-dried and crushed into fine powder using a dry grinder. The ground samples were stored at -20°C until further analysis.

Sample extraction

Extraction was carried out using the method of Jelled *et al.*[16] with minor modifications. Samples (1.0 g) were extracted for 12 hours with 10 mL of 80% methanol at 37°C on an incubator shaker set at 150 rpm. After that the mixture was filtered with filter paper (Whatman No. 1) and the filtrate was collected. The pellet was re-extracted under the same conditions. The filtered samples were combined and used to analyze the total flavonoid and total phenolic contents, antiglycation activities and antioxidant activities.

Total phenolic contents (TPC)

TPC was determined by Thammapat *et al.*[17] using Folin–Ciocalteu's reagent as described 0.3 mL of extract was briefly mixed with 2.25 mL of Folin–Ciocalteu's reagent (1:10 diluted with purified water) and allowed to settle for 5 min at an room temperature. After 2.25 mL of a 6% Na_2CO_3 solution was added, the mixture was allowed to stand for 90 min at an ambient temperature and absorbance of mixture was measured at 725 nm using a UV–vis spectrophotometer (UV1700, Shimadzu, Japan). Results were reported as mg gallic acid equivalents per 100 grams of dried sample (mg GAE/100 g).

Total flavonoid contents (TFC)

TFC was determined using the colorimetric method described by Wanyo *et al.*[18] In brief, 500 µL of extract was mixed with 2.25 mL of purified water in 10 mL of graduated test tube followed by the addition of 150 µL of a 5% $NaNO_2$ solution. After standing for 6 min, 300 µL of a 10% $AlCl_3$ $6H_2O$ solution was added to the mixture and it was allowed to settle for another 5 min prior to the addition of 1.0 mL of 1 M NaOH. The mixture was mixed well by vertexing, and measured instantly at 510 nm. Results were expressed as mg rutin equivalents per 100 grams of dried sample (mg RE/100 g).

DPPH free radical scavenging assay

The DPPH free radial scavenging activity of the extracts was determined according to a previously published.[17] The extracted (100 µL) was added to 3 mL of 0.01 mM DPPH solution dissolved in methanol. The mixture was shaken vigorously and left to stand at an ambient temperature for 30 min in the dark. The absorbance of the mixture was measured at 517 nm and the activity was calculated using the equation: % scavenging effect = $(1–A_s/A_0) \times 100$, where A_s and A_0 denote the absorbance of sample and control, respectively. A standard of Trolox was run using five levels ranging from 1 to 500 µg/mL. A standard curve was then produced by plotting the percentage of activity of Trolox against its concentrations. The final results were expressed as mg Trolox equivalent antioxidant capacity in 1.0 g of dried sample (mg Trolox/g).

Ferric reducing/antioxidant power assay (FRAP)

The reducing power of the extracts was measured based on FRAP assay adapted from Siriamornpun *et al.*[19]. An aliquot of 1.8 mL of FRAP reagent was briefly mixed with 0.18 mL of deionized water and 0.06 mL of extract. FRAP reagent was freshly prepared by mixing 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3 6H_2O$ and purified water in a 10:1:1:12 ratio at 37°C. The mixture was shaken and incubated for 4 min at 37°C in a water bath and the absorbance was read at 593 nm. FRAP values were reported as mmol $FeSO_4$ in one gram of dried sample (mmol $FeSO_4$/g).

Antiglycation assay

The antiglycation activity of the extracts was estimated according to a previously published method with some modifications.[20] 500 µL of extract was briefly mixed with 500 µL of 20 mg/mL bovine serum albumin, 500 µL of 0.5 M glucose and 1.0 mL of 0.02% (w/v) sodium azide in phosphate buffer (100 mM, pH 7.4). After 5-days of incubation in the dark at 37°C, the mixture was analyzed for the amount of advanced glycation end-products (AGE) using a fluorescent spectrometer (LS 50B, Perkin Elmer, U.S.A.) with excitation (330 nm) and emission (410 nm). The percentage inhibition of AGE formation by each extract was calculated using the equation: % inhibition = $(1\text{-}F_s/F_0) \times 100$, where Fs and F_0 denote the fluorescence of the solution with and without sample, respectively.

Vitamin C by HPLC

Vitamin C was extracted and analyzed according to Siriamornpun,and Kaewseejan with slight modifications.[21] Samples (1.0 g) were extracted for 2 hours with 50 mL of 2% m-phosphoric acid at 37°C on a shaker incubator set at 150 rpm. After filtration using a filter paper (Whatman No.1), the pellet was re-extracted under identical conditions and the filtrate was combined and stored at -20°C until analysis. A Shimadzu LC-20AC series HPLC system with diode array detector (Shimadzu, Tokyo, Japan), was used for analyses. The separation was carried out at 40°C on a C-18 column (250 mm × 4.6 mm i.d., 5 µm, GL Sciences Inc., Tokyo, Japan) using isocratic elution with 100 mM KH_2PO_4: methanol (97:3, v/v) as the mobile phase. The flow rate was stable at 1.0 mL/min; the injection volume was 20 µL and the detection wavelength was 280 nm.

Curcumin, 6-gingerol and eugenol by HPLC

Extraction of curcumin was carried out by extracting samples (1.0 g) for 30 min with 10 mL of 80% methanol.[22] Meanwhile, extraction of 6-gingerol and eugenol was done by extracting samples (1.0 g) for 12 hours with 20 mL of 80% methanol, using the modified method of Usman *et al.*[23] Extraction was performed at 37°C on a shaker incubator set at 150 rpm. After centrifugation for 15 min at 19000×g to collect the supernatant, the pellet was re-extracted, and the supernatant was combined and evaporated under vacuum at 40°C to dryness. The residue was re-dissolved in 5 mL of methanol and filtered through a 0.45 µm nylon membrane filter before HPLC analysis.

Analysis of curcumin was performed on the same HPLC system, adapted from the procedure of Naksuriya *et al.*[24] The separation was carried out at 38°C on Inertsil® ODS-3 column (250 mm × 4.6 mm i.d., 5 µm, GL Sciences Inc., Tokyo, Japan) using isocratic elution with acetonitrile: water (90:10, v/v) as the mobile phase. The flow rate was constant at 1.0 mL/min; the injection volume was 20 µL and the detection wavelength was 425 nm.

Analysis of 6-gingerol and eugenol was performed on the same HPLC system, adapted from the procedures of Wohlmuth *et al.* and Geng *et al.*[25,26]. The separation was carried out at 40°C on Inertsil® ODS-3 column (250 mm × 4.6 mm i.d., 5 µm, GL Sciences Inc., Tokyo, Japan). Solvent A (water) and solvent B (acetonitrile) as the mobile phase in the flow rate was constant at 1.0 mL/min. The gradients elution program were 0–40%, 0–10 min B; 40–60%, 10–40 min B; 60–100% ,40–45 min B; 100–40% ,45–50 min B. The injection volume was 20 µL; the detection wavelength was 254 nm for 6-gingerol and 280 nm for eugenol.

Amino acids by LCMS/MS

Samples were analyzed for amino acid method as previously reported by Nimbalkar *et al.*[27] with some modifications. The LCMS/MS was performed using a LCMS-8030 triple-quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) operated in the ESI mode and a Shimadzu LC-20AC series HPLC system (Shimadzu, Kyoto, Japan). The HPLC analysis was operated follow by these conditions; the flow rate was set 0.2 mL/min, the temperature of column oven was determined at 38°C and autosampler was determined at 4°C. The mobile phases were prepared by (A) the water was added with formic acid 0.1% (v/v) and (B) the methanol was diluted with the water (50:50) then the mixer was added with formic acid 0.1% (v/v). The autosampler needle was purged with methanol before and after aspiration of the sample.

For the operation in MS/MS mode, runs: Multiple Reaction Monitoring (MRM) mode; capillary voltage at 4.5 kV (positive mode; ESI (+)); cone voltage at 1.72k V; ion source temperature of 400 °C. The amino acid was identified by their m/z values and by comparison with the retention time of standards. All other settings were analyte-specific and were auto-optimized by flow injection of 2 µL of a 1 ppm. solution in methanol containing one analyte. The results of the auto-optimizations are summarized in Table 1.

Phenolic acids and flavonoids by HPLC

Phenolic acids and flavonoids were extracted according to Siriamornpun et al with some modifications.[28] Samples (1.0 g) were extracted for 12 hours at 37°C with 20 mL of HCl/methanol (1:100, v/v) on a shaker incubator set at 150 rpm in the dark. After filtration on a Whatman No. 4 filter paper, the pellet was re-extracted, and the filtrate was combined and evaporated under vacuum at 40°C to dryness. The residue was re-dissolved in 5 mL of water/methanol (50:80, v/v) and filtered through a 0.45-µm nylon membrane filter before HPLC analysis.

The analysis was performed on the same HPLC system, the separation was carried out at 38°C on an Inertsil® ODS-3 column (250 mm × 4.6 mm i.d., 5 µm, GL Sciences Inc., Tokyo, Japan) protected with an Inertsil® ODS-3 guard column (10 mm × 4 mm i.d., 5 µm, GL Sciences Inc., Tokyo, Japan), Gradient elution condition was performed following the method of Siriamornpun *et al.*[28] The flow rate was constant at 0.8 mL/min; the injection volume was 20 µL; the detection wavelength was 280 nm for hydroxybenzoic acids, 320 nm for hydroxycinnamic acids, and 370 nm for flavonoids. The spectra were recorded from 200 to 800 nm. Phenolic acids and flavonoids in the extracts were identified by comparing their relative retention times with external standards.

Statistical analysis

All data were analyzed by the statistical program. The results are reported as the mean ± one standard deviation (SD) of three replicates and data were analyzed using one-way analysis of variance (ANOVA) with the least significant difference (LSD) test to determine the significance relative to the control and Pearson's correlation test to assess the correlations among the means. In all cases, $p < 0.05$ was considered significant.

RESULTS

TPC and TFC are indicative of the levels of bioactive compounds which is beneficial to the human when found in high levels. The phenolic compounds have raised interest for scientific community in recent times. In this study, total phenolic contents were estimated in eight selected species from Zingiberaceae. The results showed in Table 2. It was found that the TPC of selected species varied considerably across species, ranging from 16.5 to 199.7 mg GAE/100 g DW. The species *A. zerumbet* exhibited the highest total phenolic content (199.7 mg/100 g DW), followed by *A. conchigera* (186.5 mg GAE/100 g DW) and *Z. officinale* (173.0 mg GAE/100 g DW). The species *Z. junceum* showed the lowest phenolic content of 16.4 mg GAE/100 g DW. Flavonoids are a diverse group of phenolic compounds distributed in higher plants and they have been documented to have great antioxidant potential. As presented in Table 2, there were significant variations in the TFC in selected species, which ranged from 17.0 to 65.9 mg RE/100 g DW. The highest TFC was found in *A. conchigera* (65.91 mg RE/100 g DW), followed by *Z. officinale* (64.40 mg RE/100 g DW) and *Z. mekongense* (55.89 mg RE/100 g DW). The lowest TFC was detected in *Z. junceum* (17.0 mg RE/100 g DW). The result section of this study suggested that flavonoids are not predominant in these selected species. Additionally, *A. conchigera* was the richest source of phenolic compound and flavonoids, followed by *Z. officinale*. Even though *A. zerumbet* was the most abundant source of phenolic compound, its flavonoid content was relatively low.

Curcumin was evaluated in all selected species with its concentrations observed to vary significantly across species. The curcumin contents of

Table 1: Multiple Reaction Monitoring (MRM) conditions for amino acid on LCMSMS.

Amino acid	Retention time (min)	Precursor ion [M+H] + (m/z)	Product ion (m/z)	Q1 Pre Bias (V)	Collision energy (V)	Q3 Pre Bias (V)
Lysine	1.843	147.05	84.0	-17.0	-17.0	-18.0
Histidine	1.967	156.05	110.05	-12.0	-13.0	-23.0
Arginine	2.006	175.05	70.00	-26.0	-27.0	-29.0
Threonine	2.163	120.00	74.00	-14.0	-10.0	-18.0
Valine	3.177	118.05	72.05	-13.0	-13.0	-15.0
Methionine	3.748	150.05	104.00	-20.0	-18.0	-29.0
Isoleucine	5.658	132.10	68.00	-14.0	-14.0	-18.0
Leucine	6.107	132.10	86.00	-15.0	-14.0	-20.0
Phenylalanine	8.942	166.05	120.05	-12.0	-12.0	-14.0
Tryptophan	11.105	205.00	188.00	-18.0	-13.0	-14.0

the selected species ranged from 1.37 to 26.82 µg/ g DW. The highest curcumin content was detected in *Z. junceum* (26.82 µg/ g DW), followed by *C. singularis* (25.88 µg/ g DW). The species *A. zerumbet* exhibited the lowest curcumin content of 1.37 µg/ g DW, as shown in Table 2. A pungent compound unique to Zingiberaceae species called gingerol has been found to possess antioxidant and anti-inflammatory activities and also have several pharmaceutical effects, including cardiotonic and antipyretic effects, inhibition of spontaneous motor activity and prostaglandin biosynthesis. The results indicated that significant differences in 6-gingerol contents were found among the selected species such as *Z. rubens* (0.77 µg/g DW) and *Z. junceum* (137 µg/g DW). Other than *Z. junceum*, high 6-gingerol contents were also detected in *Z. officinale* and *A. conchigera* (Table 2).

Eugenol has been shown to be the most powerful antioxidant, antibacterial agent and monoamine oxidase inhibitor in addition to its neuroprotective effects. Eugenol contents in selected species were found to vary across species, ranging from 5.13 mg/100 g DW in *A. zerumbet* to 1570 mg/100 g DW in *A. conchigera*. The highest contents of eugenol were detected in *A. conchigera*, followed by that of *Z. officinale* and *Z. mekongense*, respectively (Table 2). Vitamin C is a good antioxidant and many Zingiberaceae species have been reported to contain vitamin C. The contents of vitamin C in selected species from Zingiberaceae are displayed in Table 2. The amount of vitamin C contents ranged from 4.24 mg/100 g DW in *A. conchigera* to 20.8 mg/100 g DW in *Z. officinale*. Similar results to eugenol, *Z. officinale* had the highest content of vitamin C, followed by *C. angustifolia* and *Z. junceum*, respectively.

DPPH is the stable free radical which has a purple color. It has been used to evaluate the free radical scavenging activity. The deep purple color of DPPH will become colorless or yellow color after adding antioxidant materials. Color changes occur due to transferring an electron or hydrogen atom to DPPH by antioxidant materials. The absorbance of the remaining DPPH was determined at 517 nm using a spectrophotometer. In this study, DPPH radical-scavenging activities measured as mgTrolox equivalent and the results were converted to percentage inhibition of all samples (Table 3). The DPPH radical scavenging activity exhibited in a wide range of 2.4 in *Z. junceum* to 9.5 mgTrolox/g in *A. zerumbet*. These DPPH radical scavenging activity differences appear to have no statistical significance in *A. zerumbet*, *A. conchigera* and *Z. officinale* displayed the highest scavenging effect with 9.5, 9.4 and 9.3 mg Trolox/g respectively, followed by *Z. rubens*, *C. angustifolia*, *C. singularis*, *Z. mekongense* and *Z. junceum* with the values of 4.3, 4.1, 3.6, 2.8 and 2.4 mg Trolox/g. ($p < 0.05$) (Table 3).

FRAP, Ferric Reducing Antioxidant Power is a direct method for determination antioxidant capacities. Antioxidant is a reductant for the redox-linked colorimetric reaction. This method is based on the reduction of Ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to Fe^{2+}-TPTZ (blue-colored) at low pH. The change in absorption of Fe^{2+}-TPTZ is monitored at 593 nm using spectrophotometer.[29] FRAP values of the Zingiberaceae samples are presented in Table 3. It was found that *Z. officinale* had an excellent reducing power (5.6 mmol $FeSO_4$/g), after that *A. zerumbet* fresh (5.4 mmol $FeSO_4$/g), *A. conchigera* (4.1 mmol $FeSO_4$/g), *C. angustifolia* (1.4 mmol $FeSO_4$/g), *Z. mekongense* (1.1 mmol $FeSO_4$/g), *Z. mekongense* (1.0 mmol $FeSO_4$/g), *Z. junceum* (0.9 mmol $FeSO_4$/g) and then *C. singularis* (0.6 mmol $FeSO_4$/g).

Glycation is known as non-enzymatic reaction between molecules of reducing sugar (e.g. glucose) and lage biomolecules of protein, lipid or nucleic acid. Advanced Glycation End-Products (AGE) are produced from this reaction. AGE plays an important role in aging damage and many chronic diseases, especially diabatic mellitus. Inhibition of AGE formation in glycation system may be a beneficial to reduce the risk factors of metabolic syndrome and aging process. In this study, the inhibition of protein glycation was evaluated and results were showed in Table 3. Among tested extracts, *Z. officinale*, *Z. rubens* and *A. conchigera* showed no significant ($p < 0.05$) differences in the highest anti-glycation activity were 98.2, 97.1 and 95.3%, respectively, whereas the lowest %inhibition of Zingiberaceae was found as 81.9% in *Z. junceum*.

The investigation of amino acids present in different kinds of plants has been a subject of interest for different studies.[30-33] This work identifies

Table 2: Contents of total phenolics, total flavonoids, curcumin, 6-gingerol, vitamin C, and eugenol in selected species from Zingiberaceae.

Species	Total phenolics (mg GAE/ 100 g)	Total flavonoids (mg RE/ 100 g)	Curcumin (µg/ g DW)	6-gingerol (µg/ g DW)	Eugenol (mg/ 100 g DW)	Vitamin C (mg/ 100 g DW)
Z. officinale	173.00 ± 8.29^{c}	64.40 ± 0.16^{b}	9.09 ± 0.11^{d}	115.37 ± 0.12^{b}	66.56 ± 2.94^{c}	20.83 ± 0.54^{a}
Z. mekongense	50.99 ± 1.12^{d}	55.89 ± 0.07^{c}	4.22 ± 0.14^{e}	1.14 ± 0.01^{f}	83.70 ± 0.63^{b}	10.43 ± 0.53^{d}
Z. rubens	22.25 ± 0.62^{f}	21.64 ± 0.32^{g}	1.84 ± 0.11^{f}	0.77 ± 0.01^{h}	16.56 ± 0.97^{e}	7.33 ± 0.23^{e}
Z. junceum	16.48 ± 0.36^{f}	17.00 ± 0.30^{h}	26.82 ± 0.27^{a}	137.03 ± 1.89^{a}	8.23 ± 0.36^{g}	12.34 ± 0.63^{c}
C. angustifolia	36.88 ± 2.37^{e}	38.69 ± 0.08^{d}	11.51 ± 0.62^{c}	1.16 ± 0.08^{g}	21.67 ± 0.56^{d}	17.33 ± 0.74^{b}
C. singularis	40.84 ± 1.50^{e}	32.59 ± 0.12^{e}	25.88 ± 1.13^{b}	3.14 ± 0.09^{e}	12.61 ± 0.67^{f}	5.86 ± 0.20^{f}
A. zerumbet	199.77 ± 3.46^{a}	29.24 ± 0.31^{f}	1.37 ± 0.04^{f}	18.62 ± 0.18^{d}	5.13 ± 0.38^{h}	4.55 ± 0.26^{g}
A. conchigera	186.57 ± 5.52^{b}	65.91 ± 0.19^{a}	3.88 ± 0.20^{e}	56.18 ± 0.89^{c}	1567.33 ± 1.74^{a}	4.24 ± 0.20^{g}

Values are expressed as mean ± SD of triplicate measurements (n = 3).
Means with different letters are significantly different at p < 0.05 within the same column.

Table 3: Antioxidant activities measured by means of DPPH radical scavenging and FRAP analyses, and antiglycation activities in selected species from Zingiberaceae.

Species	FRAP (mmol $FeSO_4$/ g)	DPPH (mg Trolox/ g)	Antiglycation (% inhibition)
Z. officinale	5.60 ± 0.10^{a}	9.35 ± 0.02^{a}	98.29 ± 0.68^{a}
Z. mekongense	1.08 ± 0.05^{e}	2.81 ± 0.03^{e}	90.49 ± 1.67^{b}
Z. rubens	1.10 ± 0.02^{e}	4.36 ± 0.02^{b}	97.12 ± 0.82^{a}
Z. junceum	0.91 ± 0.09^{f}	2.40 ± 0.07^{f}	81.97 ± 0.62^{d}
C. angustifolia	1.44 ± 0.08^{d}	4.13 ± 0.06^{c}	84.79 ± 1.93^{cd}
C. singularis	0.63 ± 0.08^{g}	3.62 ± 0.25^{d}	85.51 ± 2.33^{c}
A. zerumbet	5.41 ± 0.02^{b}	9.51 ± 0.06^{a}	91.68 ± 1.30^{b}
A. conchigera	4.41 ± 0.04^{c}	9.48 ± 0.06^{a}	95.29 ± 3.36^{a}

Values are expressed as mean ± SD of triplicate measurements (n = 3).
Means with different letters are significantly different at p < 0.05 within the same column.
FRAP: Ferric reducing antioxidant activities; DPPH radical scavenging activities.

both qualification and quantitation using LCMS/MS of 10 amino acids including 9 essential amino acids in a Zingiberaceae for the first time to provide useful information for further use of this plant. The results showed that isoleucine, lysine, phenylalanine, threonine and arginine were found in all samples studied. On the other hand, methionine and valine were absent in all samples (Table 4). The predominant essential amino acids were isoleucine phenylalanine and tryptophan (3255 µg/100 g DW) respectively. Whilst, arginine was found the highest content (14617 µg/100 g DW).

The Correlation analysis pearson test was used to perform the correlation coefficients (r) between the mean values of each parameter obtained from the study. A strong positive correlation was found between TPC, TFC and DPPH (r = 0.968 and r = 0.514 respectively) and reducing power (FRAP) (r = 0.968 and r = 0.509, respectively), as well as anti-AGE formation activity (r = 0.558 and r = 0.501, respectively) (Table 5).

The Zingiberaceae extract were identified and quantified using HPLC. The distribution of phenolic acids and flavonoid compound is presented in Figure 1A. Among all samples of Zingiberaceae, *p*-hydroxybenzoic acid was identified as the predominant phenolic acid, ranging from 165.3 µg/g with *A. zerumbet* to 419.5 µg/g. The minor phenolic acid, protocatechuic acid and sinapic acid, were detected in Zingiberaceae extract. The five flavonoids, quercetin, kaempferol, rutin, apigenin and myricetin were detected by HPLC the quantifications of the five flavonoids based on calibration curves of authentic standards are presented in Figure 1B.

DISCUSSION

Phenolic compounds and flavonoids have been reported to be the main bioactives present in most medicinal plants.[34] The content of these compounds could be varied due to varieties and growth conditions. Curcumin is a yellow-orange pigment which naturally occurs in the spice plant. It has long been documented to have beneficial effects for human health. Curcumin is found to possess a wide range of biological activity, ranging from anticancer, anti-inflammatory to antioxidant. It is a compound in Zingiberaceae species particularly turmeric (*C. longa*) which showed high levels of curcumin.[12] In our present study, the concentration of curcumin was slightly higher than those reported in other species of Zingiberaceae family. Previous studies, the concentration of curcumin were found in *Z. officinale* (23 µg/g) from Taiwan.[22] In contrast, our result has lower the level of this compound than *A. officinarum* (5 mg/g) from Taiwan as well.[35]

The results of this study conform to the previous data which reported that *Z. officinale* was rich in 6-gingerol (245 µg/g DW).[25] Similarly, Puengphian and Sirichote[36] extracted 6-gingerol from *Z. officinale* using the supercritical CO_2 extraction method at 200 bar at 35°C and 230 bar at 40°C and reported 6-gingerol contents of 239 and 171 mg/g in the extract from the respective conditions. In our present study, 6-gingerol was found in all Zingiberaceae samples while a previous study reported 6-gingerol to occur primarily in *Z. officinale* species. Remarkedly, Eugenol detected in *A. conchigera* was about 8-fold greater than that found in Indian clove, which is reputed to possess high eugenol contents.[37] The findings obtained in this study conform to a previous study which presented a vitamin C content of 9.3 mg/100 g DW in *Z. officinale*. Additionally, vitamin C contents of 0.5-0.8 mg/g DW were also reported in Indian spices (ginger and turmeric).[9] It has been observed that there was a wide variation of antioxidant activities among Zingiberaceae samples used in this study. Although vitamin C is abundant in fruits.[38] Zingiberaceae species can also be alternative source of vitamin C, as indicated by this study.

Deviation in antioxidant activity values and bioactive compounds may be predictable due to variations in growth conditions. In the results obtained, it was revealed that the Zingiberaceae showed stronger anti-AGE formation capacities. This result reveals that all Zingiberaceae extracts, except *Z. officinale*, *Z. rubens* and *A. conchigera* had a strong ability to inhibit the formation of AGE in glycation system between glucose and protein. Inhibitory effects on AGE formation are beneficial for further studies to use Zingiberaceae extracts as anti-glycation agents for anti-aging products or the treatment of diabetic complications. The total levels of amino acids found in Zingiberaceae were considered to be lower than other plant previously reported in bean, potato, rice and mushroom, respectively. However, this is an important nutrient for human body.[39] The results from our present study have demonstrated that amino acid content and composition varied greatly among varieties however free amino acid variability may also be related to different

Table 4: Contents of amino acid in selected species from Zingiberaceae analyzed by LCMSMS.

Ziniberaceae species	Amino acid content (µg/100g DW)										TEA (µg/100g DW)	TAA (µg/100g DW)
	essential amino acid									Arginine		
	Histidine	Isoleucine	Leucine	Lysin	Methionine	Phenylalanine	Threonine	Tryptophan	Valine			
Z. officinale	nd	386.32±10.59^{d}	444.219±37.50^{c}	6.05±0.06^{e}	nd	123.56±0.76^{e}	64.21±0.98^{d}	14.83±0.20^{e}	nd	161.28±1.74^{e}	1039.17±5.57	1200.45±6.49
Z. mekongense	14.69±0.21^{c}	429.73±10.80^{d}	738.669±36.32^{b}	48.03±0.15^{c}	nd	342.57±6.09^{c}	219.56±1.89^{b}	76.62±1.25^{d}	nd	1044.73±10.37^{d}	1869.86±6.30	2914.59±8.42
Z. rubens	nd	11.93±0.27^{f}	3.029±0.12^{fg}	0.79±0.01^{f}	nd	14.17±0.59^{f}	28.80±0.14^{e}	nd	nd	7.30±0.11^{g}	58.71±0.14	66.01±0.17
Z. junceum	7.28±0.36^{d}	2056.28±44.91^{b}	233.369±10.17^{e}	40.38±0.66^{d}	nd	1649.38±3.37^{b}	87.11±0.61^{c}	154.06±2.21^{c}	nd	1285.41±6.49^{c}	4227.86±6.92	5513.27±8.60
C. angustifolia	24.87±0.12^{a}	3816.27±65.21^{a}	992.159±28.66^{a}	361.73±3.42^{b}	nd	4059.17±27.18^{a}	552.66±3.18^{a}	2788.97±16.18^{a}	nd	5323.25±28.33^{b}	12595.81±16.00	17919.06±21.54
C. singularis	19.88±0.49^{b}	1228.63±68.27^{c}	349.49±6.05^{d}	393.20±0.01^{a}	nd	1641.96±4.13^{b}	218.58±2.32^{b}	214.11±4.21^{b}	nd	6752.57±11.15^{a}	4065.81±9.50	10818.39±12.08
A. zerumbet	nd	309.05±2.99^{e}	39.420.67^{f}	0.17±0.02^{f}	nd	188.69±0.78^{d}	2.79±0.18^{f}	1.59±0.25^{f}	nd	2.44±0.14^{g}	541.71±0.58	544.15±0.67
A. conchigera	nd	13.510.20^{f}	nd	0.51±0.01^{f}	nd	22.33±1.22^{f}	3.22±0.25^{f}	5.07±0.14^{ef}	nd	39.58±0.73^{f}	44.64±0.27	76.79±0.40

TEA: Total essential amino acid; TAA: total amino acid; nd: Not detected; Values are expressed as mean ± SD of triplicate measurements (n = 3); Means with different letters are significantly different at p < 0.05 within the same column.

Table 5: Correlations among and between DPPH scavenging and FRAP activities, antiglycation activities, total phenolic content, total flavonoid content and total amino acid in selected species from Zingiberaceae.

	DPPH	FRAP	Antiglycation	TPC	TFC	TAA
DPPH	1	.969**	.641**	.968**	.514*	-.496*
FRAP	-	1	.589**	.968**	.509*	-.511*
Antiglycation	-	-	1	.558**	.501*	-.702**
TPC	-	-	-	1	.568**	-.520**
TFC	-	-	-	-	1	-.217
TAA	-	-	-	-	-	1

FRAP: Ferric reducing antioxidant activities; DPPH: Radical scavenging activities; TPC: Total phenolic content; TFC: Total flavonoid content; TAA: Total amino acid.

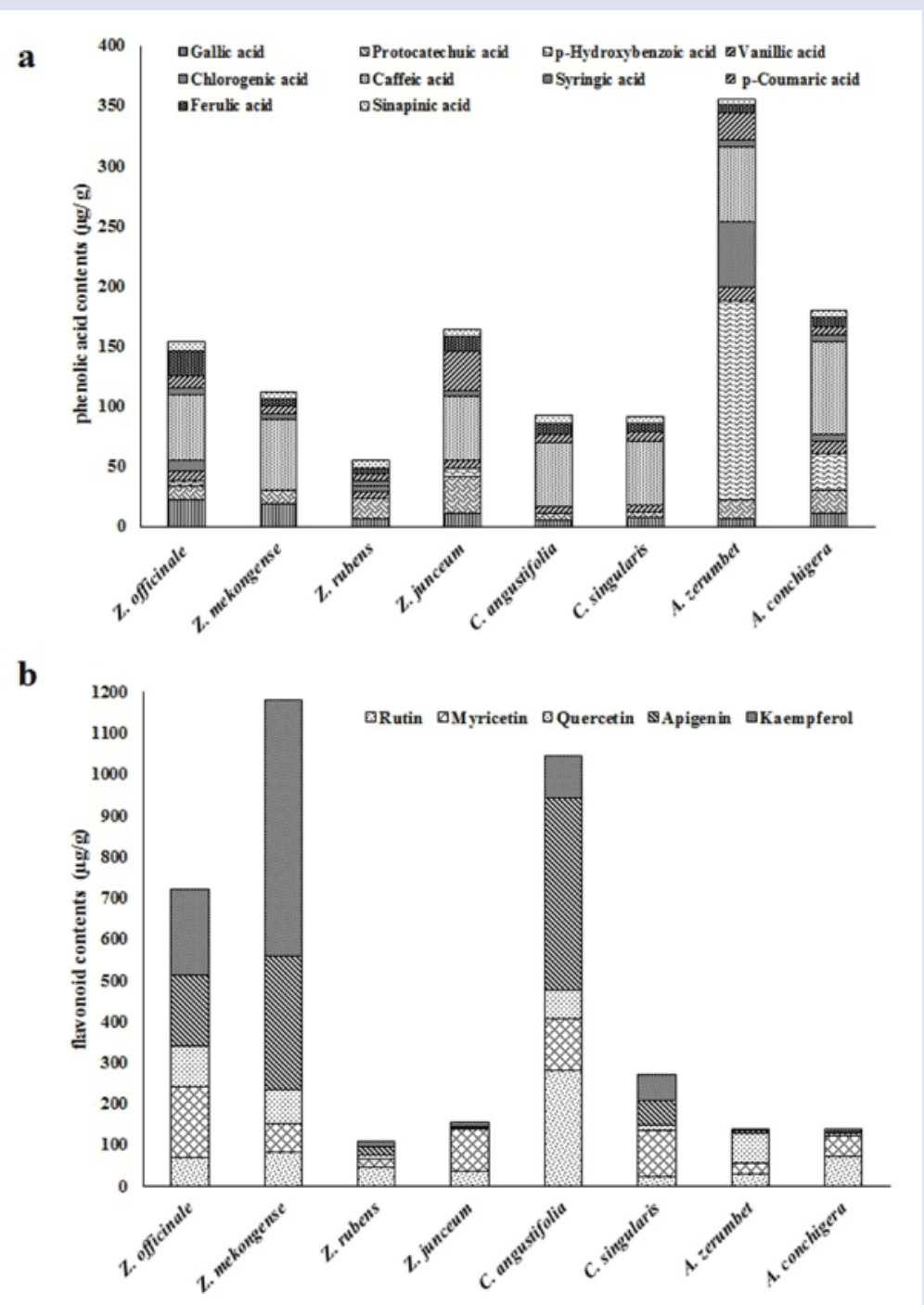

Figure 1: Contents of phenolic acids (a) and flavonoid (b) in selected species from Zingiberaceae.

environmental conditions using the discriminant and cluster analysis method.[40] Therefore, further studies on this aspect are needed.

This finding was agreed with previous studies which reported that phenolic content antioxidant and anti-AGE formation were well positively correlated.[41] Our findings indicated that the potency of anti-AGE formation depended on the capability of the antioxidants. Phenolic compounds have been reported to inhibit AGE formation through scavenging free radicals and antioxidant capacities.[42] The results have demonstrated that phenolic compounds (TPC and TFC) may be responsible for antioxidant and anti-glycation potentials in Zingiberaceae. Total amino acids content was significantly negatively associated with TPC DPPH FRAP and antiglycation activity (-.496*, -.511*, -.702**, -.520**). This discovery has never been reported previously however this may be explained that amino acid is not a substrate or precursor of those compounds and may not have direct impact to those biological activities. Chorismate, the final product of the Shikimate pathway is the precursor of the aromatic amino acid L-phenylalanine from which benzoic and cinnamic acid derivatives have their biosynthetic origin. These phenolic acids are naturally found in plants and classified by constitutive carbon structures. They are derived and biosynthetic origin from the aromatic amino acid L-phenylalanine which is synthesized by itself from chorismate, the final product in the shikimate pathway. Flavonoids were detected in all samples with significant differences among the sample ($p< 0.05$). Kaempferol was a major flavonoid ranging from 620 µg/g in *Z. mekongense* to 1179.8 µg/g.

Chorismate, the final product of the Shikimate pathway is the precursor of the aromatic amino acid L-phenylalanine from which benzoic and cinnamic acid derivatives have their biosynthetic origin. These phenolic acids are naturally found in plants and classified by constitutive carbon structures. They are derived and biosynthetic origin from the aromatic amino acid L-phenylalanine which is synthesized by itself from chorismate, the final product in the shikimate pathway.

This research has identified and compared essential amino acids, key bioactive compounds and biological activities of eight selected species from Zingiberaceae discovered in the Northeastern region of Thailand. The composition and content of three essential amino acids (isoleucine, phenylalanine and tryptophan) have been revealed for the first time for Zingiberaceae. These genotypes were rich in phenolic compounds, flavonoids, curcumin, 6-gingerol, eugenol and vitamin C with their concentrations varying widely. *A. conchigera* was the richest source of phenolic compounds and flavonoids, followed by *Z. officinale*. We also found that *Z. junceum* was most abundant in curcumin and 6-gingerol, while *Z. officinale* and *A. conchigera* were richest in vitamin C and eugenol, respectively.

Our findings suggest that *Z. officinale* was the most promising source of bioactive compounds. Regarding the biological activities, *Z. officinale* appears to display the highest antioxidant activity, followed by *A. conchigera* and *A. zerumbet*. Gallic acid, protocatechuic acid, *p*-coumaric acid, ferulic acid sinapinic acid and all flavonoids were detected in all samples. The findings obtained from our study have provided useful information for potential use of Zingiberaceae as functional foods or cosmetics products. Further studies should include the influence of environmental and growth conditions and processing methods on these bioactive compounds and biological activities.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUMMARY

- Native Zingiberaceae spices grown in northeastern Thailand were studied.
- Main essential amino acids were isoleucine, phenylalanine and tryptophan.
- Curcumin has been reported for the first time in *Zingiber jencuem*.
- Eugenol is a major phenolic acid in all Zingiberaceae studied.
- *Alpinia conchigera* was the richest source of phenolic compounds.

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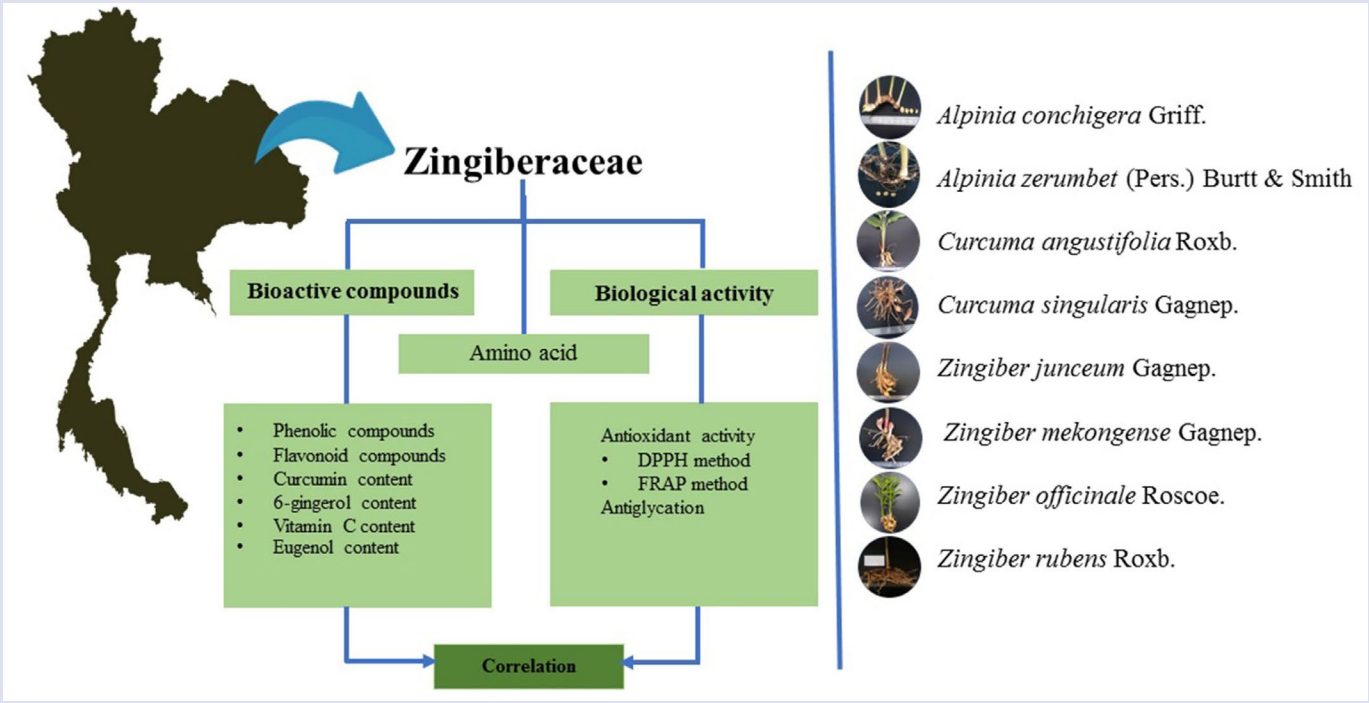
GRAPHICAL ABSTRACT



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