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Comparative study of phytochemical content and bioactivity of five Zingiberaceae species from Thailand with biodiversity implications

CHANAKRAN PAPAYRATA[1], SURAPON SAENSOUK[2], THEERAPHAN CHUMROENPHAT[3,♥]

[1]Laboratory Equipment Center, Division of Research Facilitation and Dissemination, Mahasarakham University. 41 Kham Riang, Kantharawichai District, Maha Sarakham 44150, Thailand

[2]Biodiversity Program, Diversity of Family Zingiberaceae and Vascular Plant for Its Applications Research Unit, Walai Rukhavej Botanical Research Institute, Mahasarakham University. 41 Kham Riang, Kantharawichai District, Maha Sarakham 44150, Thailand

[3]Cosmetic Science and Spa Program, Faculty of Thai Traditional and Alternative Medicine, Ubon Ratchathani Rajabhat University. 2 Ratcha Thani Road, Mueang Ubon Ratchathani District, Ubon Ratchathani 34000, Thailand. Tel./fax.: +66-45-352139, ♥email: theeraphan.c@ubru.com

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Abstract. *Papayrata C, Saensouk S, Chumroenphat T. 2025. Comparative study of phytochemical content and bioactivity of five Zingiberaceae species from Thailand with biodiversity implications. Biodiversitas 26: 4229-4242.* Zingiberaceae plants are widely used in traditional medicine and as food ingredients. Their phytochemicals and biological activities differ depending on species, genotype, and cultivation conditions. This study investigated five Thai Zingiberaceae species, including *Zingiber chrysostachys* (ZC), *Zingiber niveum* (ZN), *Zingiber ligulatum* (ZS), *Alpinia galanga* white cultivar (AGW), and yellow cultivar (AGY). ZC showed the highest total phenolic content (200.04 mg GAE/100 g DW) and total flavonoid content (157.16 mg RE/100 g DW), with myricetin at a concentration of 146.14 µg/g DW. Caffeic acid was the major phenolic acid found in all species, with the highest concentration in ZN (109.60 µg/100 g DW). ZC exhibited the highest antioxidant activity with a DPPH scavenging activity of 471.77 µg TE/g DW, IC_{50} of 251.04 µg/mL, and 104.71 mmol $FeSO_4$/g DW by FRAP assay, with AGE inhibition of 98.15%. AGY was prominent for its remarkably high curcumin content (263.42 µg/g DW), ZN contained the highest 6-gingerol (118.37 µg/g DW), AGW had the highest eugenol concentration (17.92 µg/g DW), and ZS showed the highest vitamin C content (16.29 mg/100 g DW). Multivariate analyses confirmed strong correlations between myricetin, caffeic acid, and rutin, as well as their corresponding biological activities. ZC demonstrated the best potential for development as a functional food or herbal ingredient.

Keywords: *Alpinia galanga*, bioactive profiling, DPPH antioxidant activity, phenolic acids, Zingiberaceae diversity

INTRODUCTION

Zingiberaceae plants, or the ginger family, are economically and culturally important in many countries, especially in Southeast Asia (Sharifi-Rad et al. 2017; Chumroenphat et al. 2019; Ragsasilp et al. 2022). This diverse plant family is valued in tropical and subtropical regions for its ecological, culinary, medicinal, and ornamental uses (Li et al. 2021; Sohrab et al. 2024). Their use in Asian cuisine is particularly prominent, as seen in dishes from India, Indonesia, Laos, and Thailand. Ground plant parts of ginger, galangal, and fingerroot are widely used as spices and food seasonings due to their unique aroma and taste. In Thailand, *Zingiber chrysostachys* Ridl., *Z. niveum* Mood & Theilade, *Z. ligulatum* Roxb., and *Alpinia galanga* (L.) Willd. (white galangal and yellow galangal) are widely used in local traditional dishes such as tom yum kung, kaeng kai (a chicken and mixed vegetable curry), and sweet pickled ginger. Ginger plants have also gained attention from the scientific and food industries due to their important source of phytochemicals.

Zingiberaceae plants, including *Zingiber* and *Alpinia*, are known for their rich secondary metabolite profiles, particularly in their rhizomes (Van et al. 2021). These phytochemicals are responsible for a range of biological activities, which include antioxidant and antiglycation effects. The biosynthesis and accumulation of these bioactive compounds in Zingiberaceae species are highly influenced by environmental variables, including climate (Huang et al. 2019), soil conditions (Setyawati et al. 2021), and cultivation conditions (Peng et al. 2022). These factors directly influence the expression of key biosynthetic enzymes, resulting in variations in phytochemical composition and biological efficacy among different species and growing environments (Jangpangi et al. 2025). Consequently, the quantities of active compounds such as phenolic acids, flavonoids, curcumin, 6-gingerol, eugenol, and vitamin C may differ significantly across species or cultivation sites. Plants synthesize a wide array of chemical compounds through complex metabolic processes, broadly categorized into primary and secondary metabolites. Primary metabolites, including carbohydrates, amino acids, and lipids, are essential for fundamental physiological functions such as growth, respiration, and photosynthesis (Sharifi-Rad et al. 2017; Edo et al. 2025). In contrast, secondary metabolites such as phenolics, flavonoids, terpenoids, and alkaloids are derived from specialized biosynthetic pathways, including the phenylpropanoid, mevalonate (MVA), and methylerythritol phosphate (MEP) pathways, and are modified through reactions such as methylation, glycosylation, and hydroxylation (Divekar et al. 2022).

Phenolic acids, flavonoids, and active compounds in ginger plants such as curcumin, 6-gingerol, and eugenol have antioxidant, anti-inflammatory, and AGE-inhibiting properties (Sharifi-Rad et al. 2017; Prasad et al. 2019; Ivanović et al. 2021; Khan et al. 2024; Sumi et al. 2024; Youn et al. 2024; Yuliawati et al. 2025). These compounds can donate electrons or efficiently bind free radicals (Chouni and Paul 2017; Tanweer et al. 2020). Phenolic compounds such as caffeic acid, ferulic acid, and p-coumaric acid are commonly found in herbal plants and inhibit the formation of Advanced Glycation End-products (AGEs), a key process involved in chronic ailments such as diabetes and heart disease (Prasad et al. 2019; Rashedinia et al. 2023; Chociej et al. 2024). Flavonoids, particularly flavonols such as myricetin, quercetin, and kaempferol, have shown anti-inflammatory, antibacterial, antioxidant, and antiglycation properties in several plants (Chagas et al. 2022; Zheng et al. 2025). Guerrini et al. (2023) reported that *Z. officinale* Roscoe demonstrated a more potent antioxidant potential, with lower IC_{50} values of 5.478 mg/mL (DPPH) and 0.56 mg/mL (ABTS). Furthermore, the extract of *A. galanga* rhizome exhibited antioxidant activity with IC_{50} values of 79.34 and 88.94 µg/mL against DPPH and ABTS, respectively (Aziz et al. 2024). Additionally, phenolic acids and flavonoid compounds have been identified in Zingiberaceae species and are linked to their biological activities. A previous study reported that curcumin, 6-gingerol, eugenol, and vitamin C in Zingiberaceae play important roles in biological activity. *Zingiber* spp. and *Curcuma* spp. have high curcumin content, with 6-gingerol as the major constituent in ginger (*Z. officinale*) and *Z. chrysostachys*, while eugenol and vitamin C are found in the genus *Alpinia* (Chouni and Paul 2017; Alolga et al. 2022).

The phytochemical richness of Zingiberaceae underscores their importance in natural product research and drug discovery (Alam et al. 2022). Although many Zingiberaceae species have been individually studied, comparative phytochemical and bioactivity analyses across multiple underexplored species remain limited. This study is the first to simultaneously investigate and compare phenolic acids, flavonoids, and key bioactive compounds (curcumin, 6-gingerol, eugenol, and vitamin C) of *Z. chrysostachys*, *Z. niveum*, *Z. ligulatum*, and two cultivars of *A. galanga* using multivariate statistical tools. The study also explores the antioxidant (DPPH and FRAP assays) and antiglycation activities of these species. Additionally, correlations between compound groups and biological activities were assessed to select cultivars of medicinal plants that showed potential for further development into healthy foods and dietary supplements.

MATERIALS AND METHODS

Plant materials and sample preparation

Five species of Zingiberaceae (ZC: *Z. chrysostachys*, ZN: *Z. niveum*, ZS: *Z. ligulatum*, AGW: *A. galanga* (white), AGY: *A. galanga* (yellow)) were collected from the northeastern region of Thailand in 2022. All samples were identified by Dr. Surapol Saensuk, a plant taxonomist at the Walailak Rukhavej Botanical Research Institute, Mahasarakham University, and stored as reference specimens in the herbarium with voucher specimen numbers ZN082201-ZN082205. The rhizomes of the selected plants were washed with tap water, freeze-dried, and ground into a fine powder before storing at -20°C for chemical and biological analysis. Cultivar selection was conducted based on information gathered from local people regarding their traditional use.

Determination of phenolic acids and flavonoid compounds

The extraction and analysis of phenolic acids and flavonoid compounds were performed using the methods described by Chumroenphat et al. (2021b). Briefly, samples (1.0 g) were extracted with 20 mL of HCl/methanol (1:100, v/v) and filtered through a 0.45 µm membrane prior to HPLC analysis. Ten phenolic acids (gallic acid, protocatechuic acid, genistic acid, chologenic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, sinapinic acid, and cinnamic acid) and five flavonoid compounds (rutin, myricetin, quercetin, apigenin, and kaempferol) were analyzed using HPLC (model 20 Series, Shimadzu, Kyoto, Japan) following the conditions described in a previous study (Chumroenphat et al. 2021a). Each phenolic acid and flavonoid compound were prepared as part of a mixed standard solution. Individual stock solutions (1 mg/mL) were combined and serially diluted to obtain concentrations ranging from 2 to 250 µg/mL. Each concentration was injected in triplicate into the HPLC system. Calibration curves for each compound were generated by plotting peak areas against concentration. The results expressed as micrograms per 100 grams of dry weight (µg/100 g DW) and micrograms per gram of dry weight (µg/g DW) for phenolic acids and flavonoid compounds, respectively.

Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The extraction and analysis of TPC and TFC were performed according to the methods by Papayrata et al. (2024). One gram of sample powder was extracted with 10 mL of 80% methanol and shaken at 37°C at 150 rpm for 12 h. The mixture was filtered through Whatman No. 1 filter paper, and the residue was re-extracted under the same conditions. The combined filtrates were adjusted to a final volume of 20 mL with 80% methanol and used for TPC and TFC analyses. The Folin-Ciocalteu method was used for TPC determination by a microplate reader (Varioskan Lux, Thermo Fisher Scientific, USA) at 725 nm. Results were displayed as mg gallic acid equivalent per 100 g dry weight sample (mg GAE/100 g DW). Gallic acid was used as the standard, and a calibration curve was constructed using five concentrations ranging from 1 to 250 µg/mL. The TFC was determined using a previously established method (Papayrata et al. 2024), as measured by a microplate reader (Varioskan Lux, Thermo Fisher Scientific, USA) with absorbance at 510 nm immediately after mixing. Rutin was used as the standard, and a calibration curve was constructed using five concentrations ranging from 1 to 250 µg/mL. Results were expressed as

mg rutin equivalent per 100 g of sample dry weight (mg RE/100 g DW).

Determination of curcumin, 6-gingerol, and eugenol

The extraction and analysis of curcumin, 6-gingerol, and eugenol were performed following a previously published method with slight modifications (Chumroenphat et al. 2021a). 1.0 g of the sample was extracted with 80% methanol, and the extract was filtered through a 0.45 μm nylon membrane before HPLC analysis. The curcumin content was determined using an HPLC Shimadzu LC-20AC series (Tokyo, Japan) with an Inertsil ODS-3 column (250×4.6 mm, five μm particle size, GL Sciences Inc., Tokyo, Japan). The column oven was operated at 38°C using an isocratic mobile phase consisting of acetonitrile and water in a 90:10 (v/v) ratio, with a constant flow rate of 1.0 mL/min and a system injection volume of 20 μL. Measurements were conducted at a wavelength of 425 nm. 6-gingerol and eugenol were analyzed using the same HPLC system at a column oven temperature of 40 °C. The gradient elution's of the mobile phase using water (solvent A) and acetonitrile (solvent B) were 0-10 min: 0-40% B, 10-40 min: 40-60% B, 40-45 min: 60-100% B, and 45-50 min: 100-40% B at a flow rate of 1.0 mL/min. The injected volume was 20 μL, and measurements were performed using a diode array detector at 254 nm for 6-gingerol and 280 nm for eugenol. Standard solutions of curcumin, 6-gingerol, and eugenol were prepared at concentrations ranging from 2 to 250 μg/mL. Each concentration was injected into the HPLC system in triplicate. Calibration curves were constructed by plotting peak area against concentration for each compound. The concentrations of curcumin, 6-gingerol, and eugenol in the samples were calculated using their respective calibration curves, and the results were expressed as micrograms per gram of dry weight (μg/g DW).

Determination of Vitamin C

Vitamin C was extracted and analyzed using a previously established method (Chumroenphat et al. 2019). The dried samples (1.0 g) were extracted with 50 mL of 2% meta-phosphoric acid solution, and the extract was filtered through a 0.45 μm nylon membrane before HPLC analysis. Vitamin C was analyzed using an HPLC (Shimadzu LC-20AC series, Tokyo, Japan) with a C18 column (250×4.6 mm i.d. 5 μm, GL Sciences Inc., Tokyo, Japan) at a column temperature of 40°C. The isocratic elution of 100 mM KH_2PO_4 in methanol (97:3, v/v) was used as the mobile phase at a constant flow rate of 1.0 mL/min. Vitamin C was quantified using an external standard method. Standard solutions were prepared at concentrations ranging from 2 to 250 μg/mL and injected in triplicate into the HPLC system under the same conditions as the sample analysis. The results were expressed as milligrams per 100 grams of dry weight (mg/100 g DW).

Determination of antioxidant activity and antiglycation activity

The antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and the ferric reducing antioxidant power (FRAP) assay, following previously published methods (Papayrata et al. 2024). Samples (1.0 g) were extracted with 80% methanol and used for analysis of antioxidant activity. For the DPPH assay, 230 μL of sample solution (50 mg/mL) was mixed with 20 μL of DPPH solution (0.1 mM in methanol) and incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using a microplate reader (Varioskan Lux, Thermo Fisher Scientific, USA). Results were compared with a standard curve prepared daily using Trolox at concentrations ranging from 1 to 100 μg/mL. Antioxidant activity was calculated based on this curve and expressed as micrograms of Trolox equivalent per gram of dry weight sample (μg TE/g DW). The FRAP assay was performed as previously reported. The absorbance of the final mixture was measured using a Varioskan Lux microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) at 593 nm. For the FRAP assay, a standard curve was prepared using $FeSO_4$ at concentrations ranging from 1 to 100 μg/mL, and the FRAP values were expressed as millimoles of ferrous sulfate equivalent per gram of dry weight (mmol $FeSO_4$/g DW) of the sample. Antiglycation activity was assessed using a perier method. Extract (500 μL) was mixed with bovine serum albumin (20 mg/mL), 0.5 M glucose, and 0.02% sodium azide in 100 mM phosphate buffer (pH 7.4), then incubated at 37°C in the dark for 5 days. AGE formation was measured by fluorescence (Ex 330 nm, Em 410 nm; LS 50B, Perkin Elmer). Results were reported as the percentage inhibition (%inhibition) of AGE formation.

Statistical analysis

All data were carried out in three replicates with Standard Deviation (SD). The results were evaluated using one-way ANOVA and the Least Significant Difference (LSD) test, with $p<0.05$ indicating significant differences. The statistical analysis was performed using SPSS software version 29. MetaboAnalyst 6.0 was used to statistically analyze the observed values (Cao et al. 2025). Principal Component Analysis (PCA) was used to identify the important metabolites, and compounds with Variable Importance in Projection (VIP) values higher than 1.5 were deemed relevant. Metabolic clustering and Hierarchical Clustering Analysis (HCA) were performed, with a dendrogram to visualize clustering patterns and evaluate group similarities.

RESULTS AND DISCUSSION

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of five species of Zingiberaceae

The TPC of the five Zingiberaceae species were significantly different ($p<0.05$) (Table 1). *Zingiber chrysostachys* (ZC) had the highest TPC (200.04 mg GAE/100 g DW), significantly higher than the other 4 Zingiberaceae species, followed by *Z. niveum* (ZN; 108.51 mg GAE/100 g DW) and *Z. ligulatum* (ZS; 106.76 mg GAE/100 g DW). *Alpinia galanga* white (AGW) and yellow (AGY) had much lower TPC (66.22 and 45.43 mg

GAE/100 g DW, respectively). Genetic diversity between species in the same family affects the level of phenolic biosynthesis (Sharifi-Rad et al. 2017; Asamenew et al. 2019; Sarfaraz et al. 2021). Yoon et al. (2024) reported that phenolic compounds are a group of secondary metabolites sensitive to geographical factors, including altitude, humidity, and sunlight intensity, which affect plant phenolic biosynthesis processes (Rawat et al. 2017; Hu et al. 2024). The TFC was consistent with the TPC. ZC had the highest TFC (157.16 mg RE/100 g DW), followed by ZN (126.35 mg RE/100 g DW) and ZS (117.36 mg RE/100 g DW), respectively, while AGW and AGY had lower TFC (18.36 and 18.72 mg RE/100 g DW, respectively). The Zingiber species had significantly higher TFC than *Alpinia*, which may be used as a plant defense mechanism under various environmental conditions (Yoon et al. 2024). Flavonoids play a crucial role in protecting against UV radiation and mitigating environmental oxidative stress. Therefore, flavonoids are often found at high levels in plants growing in areas with intense sunlight or highly variable environments (Roy et al. 2022; Patil et al. 2024). *Zingiber chrysostachys* showed high potential as a natural antioxidant source, with the highest TPC and TFC, while *A. galanga* had the lowest TPC and TFC values. Genetic diversity between plant species, as well as the growth environment, plays a crucial role in determining the content of plant compounds.

Phenolic acid contents of five Zingiberaceae species

The phenolic acid contents of five Zingiberaceae species (supplement data Table S1) were significantly different ($p<0.05$). *Zingiber niveum* (ZN) had the highest content (413.95 µg/100 g DW), followed by white *A. galanga* (AGW; 322.10 µg/100 g DW), yellow *A. galanga* (AGY; 293.99 µg/100 g DW), and *Z. ligulatum* (ZS; 204.12 µg/100 g DW), with the lowest value recorded in *Z. chrysostachys* (ZC; 274.63 µg/100 g DW). A high amount of caffeic acid was found in almost all the samples, particularly in ZN (109.60 µg/100 g DW), ZS (102.91 µg/100 g DW), AGW (106.90 µg/100 g DW), and AGY (108.53 µg/100 g DW). Caffeic acid of five Zingiberaceae species is not significantly different and is widely found as a major compound in Zingiberaceae. (Chumroenphat et al. 2019). Conversely, the distribution of phenolic acids in different species was significantly different. Ferulic acid content was high in ZN (99.87 µg/100 g DW) but low in ZS (9.39 µg/100 g DW) and ZC (24.17 µg/100 g DW). p-Coumaric acid was high in ZN (91.93 µg/100 g DW), significantly higher than in the other 4 plant species. Vanillic acid and protocatechuic acid were found to be present in higher concentrations in AGY and AGW compared to the genus *Zingiber*. Differences in the contents of these compounds between species are affected by genetic factors which control the synthesis of enzymes in the phenylpropanoid metabolism pathway and hydroxybenzoic acid, as well as the geographical environment of light intensity and soil properties that affect the expression of genes related to phenolic acid production (Rawat et al. 2017; Sharifi-Rad et al. 2017; Tanweer et al. 2020; Alolga et al. 2022; Hu et al. 2024). The analysis results showed distinct variations in the amount of phenolic acids in each plant species, even within the same family. Five Zingiberaceae species in this study exhibited high levels of caffeic, ferulic, and p-coumaric acids, indicating potential for development as raw materials for the food supplement, medicinal, or cosmetic product industries.

The data were analyzed by Principal Component Analysis (PCA), Hierarchical Clustering Analysis (HCA), a heatmap, and Variable Importance in Projection (VIP) methods to examine the diverse distribution of phenolic acids in the Zingiberaceae family. The PCA results (Figure 1.A) revealed distinct sample clustering along the PC1 and PC2 axes, which explained most of the total variance. ZN and AGW exhibited markedly different phenolic acid profiles compared to the other samples, whereas ZC and AGY clustered closely together, suggesting similarities in their chemical composition. The PCA biplot (Figure 1.B) indicated that caffeic acid, ferulic acid, and p-coumaric acid had a significant influence on the clustering of species. These compounds had high weighting coefficients on the PC1 and PC2 axes, consistent with the values shown in supplement data (Table S1), specifically ZN, which had very high contents of these three compounds. The HCA dendrogram (Figure 1.C) clustered species with similar compositions. AGY and AGW were in the same group, consistent with recent findings that these species had similar phenolic acid compositions, while ZN was in a separate group. The heatmap (Figure 1.D) provided insight into the levels of phenolic acids in each species. The results confirmed that ZN had the highest values of caffeic acid, p-coumaric acid, and ferulic acid; on the other hand, AGY and ZC had significantly lower values of chlorogenic acid and syringic acid, indicating the chemical diversity between the strains. Caffeic acid, ferulic acid, and p-coumaric acid had the highest VIP values (Figure 1.E), suggesting that they were important variables in discriminating species, particularly in the PCA and clustering models.

Table 1. Total phenolic content and total flavonoid content of five Zingiberaceae species

Parameter	ZC	ZN	ZS	AGW	AGY
TPC (mg GAE/100 g DW)	200.04±3.64^{a}	108.51±1.26^{b}	106.76±2.94^{b}	66.22±4.05^{c}	45.43±1.50^{d}
TFC (mg RE/100 g DW)	157.16±0.54^{a}	126.35±0.23^{b}	117.36±0.17^{c}	18.36±0.17^{d}	18.72±0.24^{d}

Note: Values are expressed as mean±SD of triplicate measurements (n: 3). Means with different lowercase superscripts within the same row are significantly different at $p<0.05$. ZC: *Z. chrysostachys*, ZN: *Z. niveum*, ZS: *Z. ligulatum*, AGW: *A. galanga* (white), AGY: *A. galanga* (yellow)

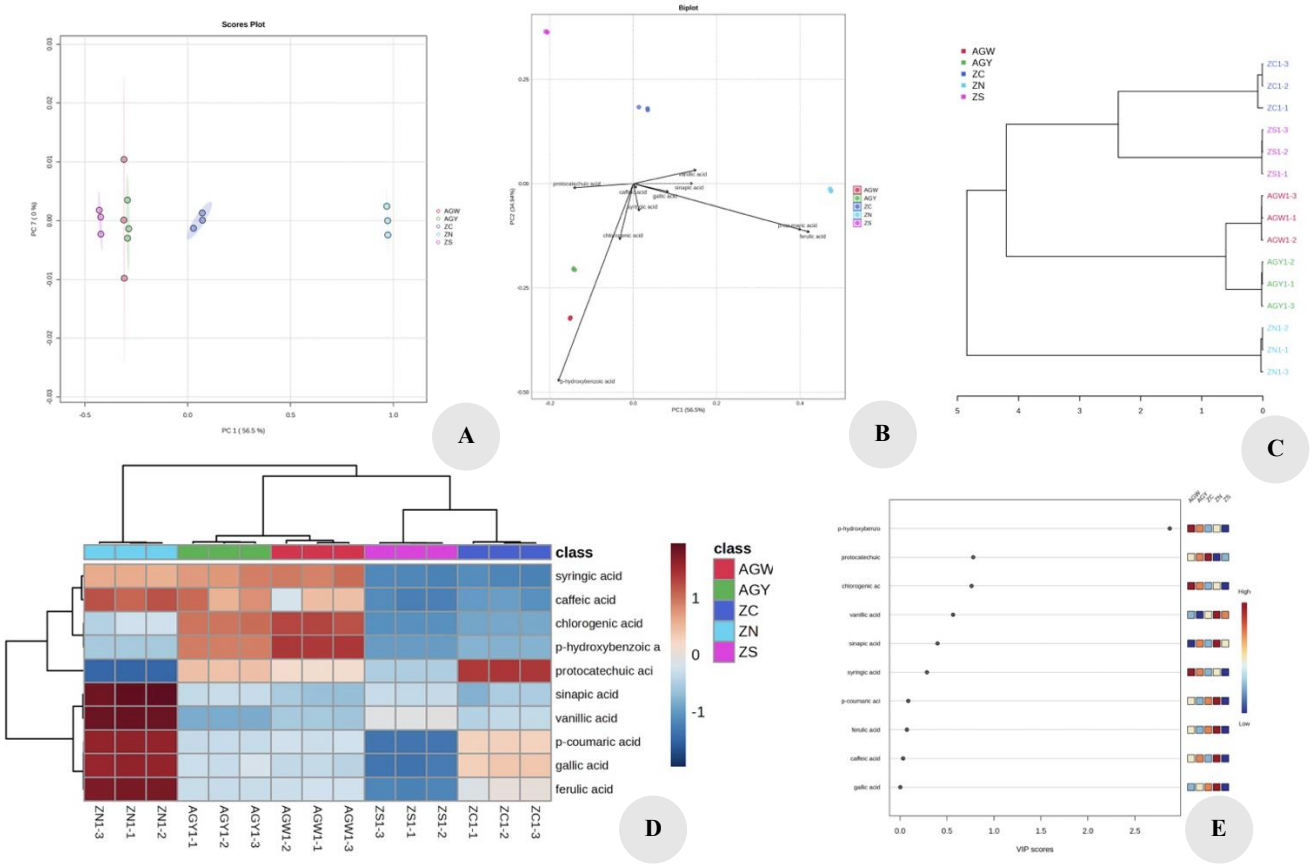

Figure 1. The relationship between phenolic acids in different species of Zingiberaceae. A. PCA scores plot showing group separation based on phenolic acids, B. PCA biplot, C. HCA dendrogram clustering samples by phenolic acid relationship patterns, D. Heatmap illustrating phenolic acid intensity relationships across samples, E. VIP scores identifying key phenolic acids contributing to group differences

Flavonoid compound contents of five Zingiberaceae species

Flavonoid compounds that were determined in five Zingiberaceae were rutin, myricetin, quercetin, apigenin, and kaempferol shown in supplement data (Table S2). Each species contained significantly different types and amounts of flavonoids ($p<0.05$). *Zingiber chrysostachys* (ZC) exhibited the highest total flavonoid compounds (289.15 µg/g DW), followed by *Z. niveum* (ZN) and *Z. ligulatum* (ZS) with total values of 225.76 and 208.56 µg/g DW, respectively. The two *A. galanga* cultivars (AGW and AGY) had the lowest TFC, with AGY being the lowest (88.52 µg/g DW). This difference may be related to the diversity of gene expression levels in flavonoid metabolism pathways between the genera *Zingiber* and *Alpinia* (Yuan et al. 2021; Hasnat et al. 2024). The highest myricetin was in ZC (146.14 µg/g DW), indicating the potential of this species in flavonol biosynthesis. Flavonols with multiple hydroxyl groups, particularly on the B ring of their structure, exhibit excellent antioxidant activity (Chen et al. 2023). ZS had the highest concentration of kaempferol (134.4 µg/g DW), a flavonol with multiple biological roles such as anticancer and anti-inflammatory (Qattan et al. 2022), while rutin was found in abundance in ZN (101.08 µg/g DW), resulting from the conversion of quercetin by the enzyme glycosyltransferase, which increased the structural stability (Yang et al. 2019). Quercetin was found in high levels in ZS (29.61 µg/g DW); it plays a role in preventing several non-communicable chronic ailments such as heart disease, diabetes, and cancer (Deepika and Maurya 2022). Apigenin was a predominant flavone found in ZN (26.05 µg/g DW), indicating that this species synthesized substances in the flavone pathway. ZC was high in total flavonoid compounds, particularly in the accumulation of myricetin, while ZN produced high levels of rutin and apigenin. ZS showed a high accumulation of kaempferol and quercetin, which are important compounds with prominent pharmacological properties. The flavonoid diversity in each species reflected the specificity of metabolism in each plant species.

Multi-statistical methods were employed to analyze the patterns and distribution of flavonoid diversity in Zingiberaceae. The PCA score plot (Figure 2.A) displays the distribution of samples along the PC1 and PC2 axes, accounting for 61.03% and 20.42% of the total variance, respectively. *Zingiber chrysostachys* (ZC), *Z. ligulatum* (ZS), and *Z. niveum* (ZN) had significantly different flavonoid expressions from the two *A. galanga* (AGW and AGY) cultivars. The PCA biplot results (Figure 2.B) indicated that myricetin, kaempferol, and rutin had a

significant influence on the species grouping. Myricetin affected the species grouping of ZC, kaempferol, and quercetin, which were related to ZS, whereas apigenin and rutin affected the separation of ZN groups. This result concurred with supplement data (Table S2), indicating that these species contained high levels of specific flavonoids. In contrast, AGW and AGY with low flavonoid contents were grouped in the same area of the PCA biplot, indicating similar composition. The HCA dendrogram (Figure 2.C) grouped samples based on similar flavonoid profiles. The *Alpinia* cultivars, AGY and AGW, were in the same group, consistent with the PCA results. ZS and ZN were in distinct groups, while ZC was prominent in its group, indicating specific flavonoid compositions, mainly myricetin at high levels. The heatmap (Figure 2.D) showed higher-than-average levels of myricetin in ZC, kaempferol, and quercetin in ZS, and rutin and apigenin in ZN, with color codes indicating the concentrations of each sample. The AGY and AGW cultivars had the lowest flavonoid compound contents, particularly kaempferol and quercetin, showcasing the chemical diversity of plants in the same family due to genetic differences and secondary metabolism. The VIP scores (Figure 2.E) showed that myricetin, kaempferol, and rutin had VIP values greater than 1, indicating their importance in discriminating between plant species in this dataset. Myricetin had the highest influence in the discriminant model, consistent with the PCA and heatmap, indicating that this compound was predominant in ZC. Myricetin and kaempferol have been shown to exhibit antioxidant, anti-inflammatory, and cell-protective effects against oxidative stress (Chagas et al. 2022; Deepika and Maurya 2022; Roy et al. 2022; Hasnat et al. 2024).

Curcumin, 6-gingerol, eugenol, and vitamin C content of five Zingiberaceae species

The curcumin, 6-gingerol, eugenol, and vitamin C contents in the five Zingiberaceae species are shown in supplement data (Table S3). Curcumin contents significantly differed among the plant species ($p<0.05$). The highest curcumin concentration was found in yellow *A. galanga* (AGY; 263.42 µg/g DW), which is a primary natural source of curcumin, as previously reported in turmeric (*Curcuma longa* L.), with a concentration range of 1000-3000 µg/g dry weight (Chumroenphat et al. 2021b). This result suggested that AGY showed pharmaceutical potential, followed by AGW (150.42 µg/g DW) and ZN (85.42 µg/g DW), while ZS had the lowest curcumin concentration (4.94 µg/g DW). Curcumin has been quantified in many species of Zingiberaceae, including *Z. officinale*, *Z. mekongense* Gagnep., *Curcuma angustifolia* Roxb., and *A. zerumbet* (Pers.) B.L.Burtt & R.M.Sm., ranging from 3 to 26 µg/g DW. Differences in genetic structure influence the synthesis of curcumin diarylheptanoids, which are abundant in the genus *Curcuma* and certain *Alpinia* species (Sun et al. 2020; Kuzminska et al. 2024).

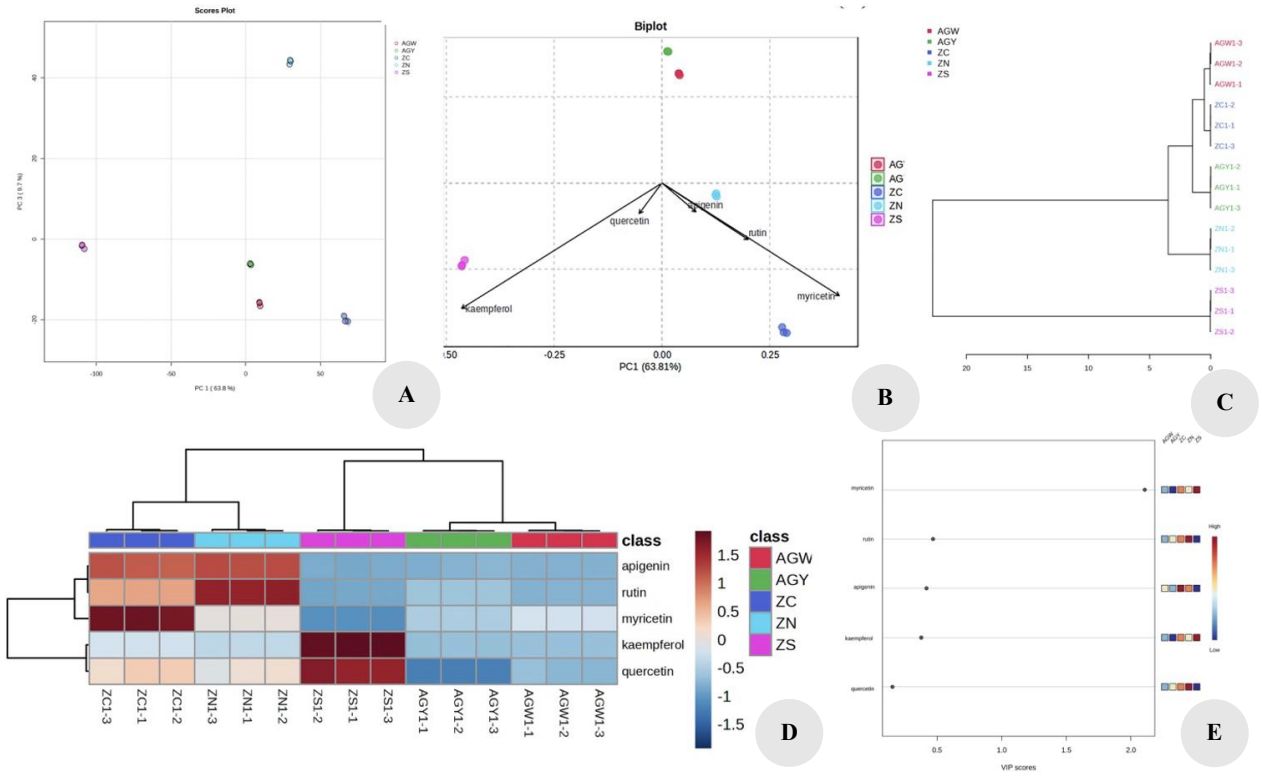

Figure 2. The relationship between flavonoid compounds in different species of Zingiberaceae. A. PCA scores plot showing group separation based on flavonoid compounds, B. PCA biplot, C. HCA dendrogram clustering samples by flavonoid compounds relationship patterns, D. Heatmap illustrating phenolic acid intensity relationships across samples, E. VIP scores identifying key phenolic acids contributing to group differences

The 6-gingerol, as the principal constituent of ginger, was found in high concentrations of *Z. niveum* (ZN) (118.37 µg/g DW), followed by AGY (73.3 µg/g DW) and AGW (72.98 µg/g DW). The 6-gingerol content in ZN was significantly higher than in the other samples. The ZC and ZS levels were low (<1 µg/g DW), suggesting the limited synthesis of 6-gingerol in some plant species. Eugenol, the major volatile compound in Zingiberaceae, showed the highest content in AGW (17.92 µg/g DW), with AGY and ZS also having a high content of eugenol (15.9 and 15.6 µg/g DW, respectively). These results concurred with previous reports on the essential oil compositions in the rhizomes of Zingiberaceae, which contain terpenoids such as eugenol (Alolga et al. 2022; Guerrini et al. 2023). Vitamin C was highest in ZS (16.29 mg/100 g DW), and higher than previously reported values in *Z. mekongense*, *Z. rubens* Roxb., and *Z. junceum* Gagnep., which ranged from 7 to 12 mg/100 g DW, as well as *Alpinia* species such as *A. zerumbet* and *A. conchigera* (4 mg/100 g DW) (Chumroenphat et al. 2019). *Zingiber officinale* and *C. angustifolia* had higher vitamin C contents than ZS, indicating the wide-ranging vitamin C accumulation among species in the same family. The vitamin C content is also affected by genetic and environmental factors during cultivation, such as sunlight and oxidative stress (Rawat et al. 2017; Alolga et al. 2022; Hu et al. 2024). AGY and ZN had high curcumin and 6-gingerol, respectively, while ZS had high vitamin C and eugenol contents.

The data were analyzed using multivariate statistical techniques to assess the diversity of bioactive compound constituents within the Zingiberaceae family. These include PCA, HCA, a heatmap, and VIP, as shown in Figures 3 A-E. In the PCA score plot (Figure 3.A), the PC1 and PC2 axes explained 87.25% and 12.75% of the total variance, respectively, with a clear separation of the species groups. AGY and AGW were separated from the other plant species in the same direction as the curcumin and 6-gingerol contents, respectively. This result was consistent with the values in supplement data (Table S3), indicating that these species had the potential to synthesize bioactive compounds with high phenolic ketone and phenylpropanoid structures. The PCA biplot results (Figure 3.B) showed that curcumin was highly associated with AGY, while 6-gingerol was associated with AGW. Eugenol and vitamin C were distributed between ZC, ZN, and ZS, reflecting the diversity of compounds in each species, especially ZS, which, despite low curcumin and 6-gingerol, had high levels of eugenol and vitamin C. The HCA clustering (Figure 3.C) revealed that AGY and AGW were in the same group, exhibiting similar bioactive compound profiles. ZN and ZS were in groups separated from the main group, reflecting more specific chemical compositions, with ZC expressed as a separate species. The heatmap (Figure 3.D) showed that AGY had the highest curcumin level among the samples, while AGW had significantly high levels of 6-gingerol. ZS and ZN exhibited high expression in response to eugenol and vitamin C, indicating their potential as antioxidants and anti-inflammatory agents. The clarity of the color groups in the heatmap supported the results of PCA and HCA, showing the specificity of compounds in each species. Curcumin and 6-gingerol had the highest VIP scores (Figure 3.E), indicating that these two compounds can be used as chemical markers to identify species with the potential to produce bioactive compounds for health and cosmeceutical products. The results confirmed that bioactive compound profiles can be used to discriminate species within the Zingiberaceae family accurately. AGY had a high curcumin content, and AGW had a high 6-gingerol content, while ZS and ZN were prominent in eugenol and vitamin C, which are related to antioxidant, anti-inflammatory, and AGE-inhibiting properties (Prasad et al. 2019; Alolga et al. 2022). These species can be further exploited for the development of commercial herbal products.

Biological activities of DPPH and FRAP scavenging and antiglycation of five Zingiberaceae species

The DPPH and FRAP scavenging activity, as well as the antiglycation of five Zingiberaceae species, are shown in supplement data (Table S4), which differed significantly among plant species in the Zingiberaceae family ($p<0.05$). The DPPH radical scavenging activity analysis measures the ability to scavenge free radicals. ZC had the highest DPPH scavenging activity (471.77 µg TE/g DW), followed by ZS (404.79 µg TE/g DW) and ZN (377.76 µg TE/g DW), while AGW and AGY had the lowest values (303.11 µg TE/g DW and 231.07 µg TE/g DW, respectively). This result concurred with the total phenolic and total flavonoid contents in the ZC cultivar, which was the highest in the group, particularly for myricetin and caffeic acid, which have high free radical scavenging properties (Chagas et al. 2022; Roy et al. 2022). The FRAP assay measures the ability to reduce ions. The highest value of FRAP scavenger was found in ZC (104.71 mmol $FeSO_4$/g DW), followed by ZN and ZS, while AGY had the lowest value (17.94 mmol $FeSO_4$/g DW). These results were correlated with the DPPH values, indicating the efficiency of electron release in inhibiting free radical damage, with varying potentials among the different strains. The antiglycation activity measures the ability to impede AGE (advanced glycation end-product) formation, which is related to protein degradation in the body. ZC had the highest inhibition at 98.15%, followed by AGY (94.05%) and ZN (89.77%). Caffeic acid and ferulic acid inhibit AGE formation via the Maillard reaction and free radical scavenging mechanisms (Liu et al. 2018; Cao et al. 2019). Kaempferol and quercetin were in moderate amounts in ZC, directly inhibiting AGE formation via protein oxidation protection mechanisms (Oriakhi et al. 2022; Remigante et al. 2022). ZC contained high levels of various flavonoid components and may exhibit a synergistic effect, increasing the overall biological activity compared to other varieties that contain only one dominant compound. The ZC exhibited high biological activity in terms of DPPH, FRAP, and antiglycation, which is likely attributed to its high total amounts of phenolic and flavonoid compounds, particularly myricetin and rutin, that play crucial roles in antioxidant activity (Roy et al. 2022).

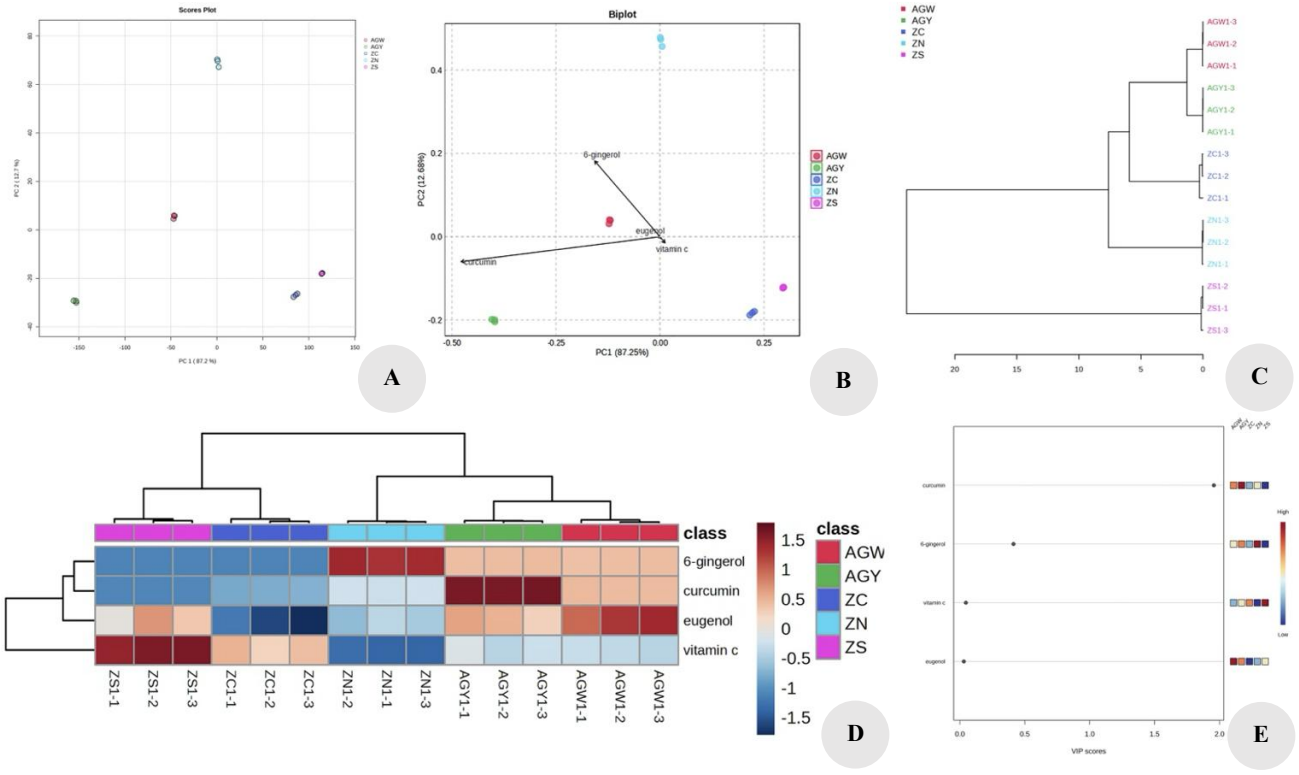

Figure 3. The relationship between curcumin, 6-gingerol, eugenol, and vitamin C in different species of Zingiberaceae. A: PCA score plot showing group separation based on curcumin, 6-gingerol, eugenol and vitamin C, B: PCA biplot, C: HCA dendrogram clustering samples by curcumin, 6-gingerol, eugenol and vitamin C relationship patterns, D: Heatmap illustrating phenolic acid intensity relationships across samples, E: VIP scores identifying curcumin, 6-gingerol, eugenol and vitamin C contributing to group differences

Myricetin features multiple hydroxyl groups in its structure, enabling it to donate hydrogen and efficiently bind to free radicals (Chagas et al. 2022). Among the five Zingiberaceae species, *Z. chrysostachys* (ZC) exhibited the strongest biological activities across all assays. Based on DPPH and FRAP values, the antioxidant activity ranked as follows: ZC>ZS>ZN>AGW>AGY (DPPH) and ZC>ZN>ZS>AGW>AGY (FRAP). For antiglycation activity, the inhibition order was ZC>AGY>ZN>ZS>AGW. These results indicate that ZC consistently displayed greater activity compared with ZN, ZS, AGW, and AGY, including antioxidant and antiglycation activities. *Apinia galanga* cultivars (AGW and AGY) generally presented lower activity, particularly in antioxidant assays.

The antioxidant (DPPH and FRAP assays) and anti-glycation activities, especially in ZC, may be due to the high levels of flavonoids and phenolic acids in the sample, particularly myricetin, which is abundant in this species. Flavonoids, such as myricetin, rutin, and quercetin, can donate hydrogen and stabilize free radicals, thereby enhancing their antioxidant activity (Olszowy-Tomczyk and Wianowska 2023). Together with phenolic acids such as caffeic acid and ferulic acid, these compounds also exhibit strong metal-chelating abilities and hydrogen-donating capacities, contributing to their antioxidant activity (Zheng et al. 2024). In addition, curcumin, 6-gingerol, and eugenol, which are found in various amounts across species, have been reported to exhibit antioxidant and anti-glycation activities, playing a role in reducing the generation of Reactive Oxygen Species (ROS) and inhibiting the formation of Advanced Glycation End-products (AGEs) (Alam et al. 2022). These results are consistent with previous reports in the Zingiberaceae family. Other Zingiberaceae, such as *Z. officinale* and *C. longa*, contain major bioactive compounds, including 6-gingerol, curcumin, and phenolic acids, which contribute significantly to their notable biological activities (Ivanović et al. 2021; Li et al. 2021; Ballester et al. 2023). The data on the anti-glycation and antioxidant properties (DPPH and FRAP) of five Zingiberaceae plants provide insight into the potential of each species. They may support the selection of ideal candidates for the development of pharmaceutical or health food products.

The relationships between antioxidant activity (DPPH, FRAP) and glycation inhibition (AGE), analyzed by multivariate statistical methods including PCA, HCA, a heatmap, and VIP score, are shown in Figure 4. The PCA score plot (Figure 4.A) revealed that the PC1 and PC2 axes explained 53.91% and 24.52% of the total variance, respectively. Notably, ZC showed the highest biological activity among the plant species. The PCA biplot (Figure 4.B) revealed that FRAP, DPPH, and AGE inhibition had high incremental coefficients of sample distribution, specifically ZC, which had the highest values in the group,

indicating good antioxidant and AGE protection potential. This result suggested that the total bioactive compounds in ZC played a vital role in the cell damage prevention mechanism. The HCA results (Figure 4.C) and the heatmap (Figure 4.D) supported the PCA results, with ZC prominently clustered and exhibiting high levels of color intensity in all three variables. AGW and AGY showed lower values than average levels, with significantly reduced AGE values. This result concurred with the data from supplement data (Table S4), showing that ZC had the highest DPPH, FRAP, and % inhibition values among the groups. The VIP scores (Figure 4.E) indicated that AGE inhibition had the highest value, followed by DPPH and FRAP, suggesting that AGE inhibition had the most significant impact on model classification. The results related to the flavonoid components found in ZC, especially myricetin, quercetin, rutin, and kaempferol, which play important roles in inhibiting the Maillard reaction and AGE formation via the free radical reduction mechanism, and also inhibiting the cross-linking of proteins (Prasad et al. 2019; Alolga et al. 2022; Chagas et al. 2022; Roy et al. 2022). ZC showed high antioxidant and AGE inhibition potentials, which were directly related to its flavonoid content, particularly flavonols with multiple hydroxyl groups that can efficiently scavenge free radicals. These results suggest that ZC has potential as a valuable natural source for the development of health products with anti-aging and antioxidant stress activities.

In summary, ZC demonstrated the most potent antioxidant and antiglycation activities, making it a promising candidate for pharmaceutical or nutraceutical development. Future work should focus on isolating compounds and conducting mechanistic studies to support their functional applications.

However, heatmap analysis of phytochemical contents in five Zingiberaceae species revealed apparent differences in the accumulation of bioactive compounds, as shown in Figure 5. In particular, ZC exhibited high levels of several phytochemicals, including vitamin C, protocatechuic acid, kaempferol, quercetin, and curcumin, which correlated with the highest antioxidant (DPPH and FRAP) and anti-glycation activities among the group. ZN and ZS showed moderate levels of flavonoids and phenolic acids, including rutin, apigenin, gallic acid, and ferulic acid, which may contribute to their observed biological activities.

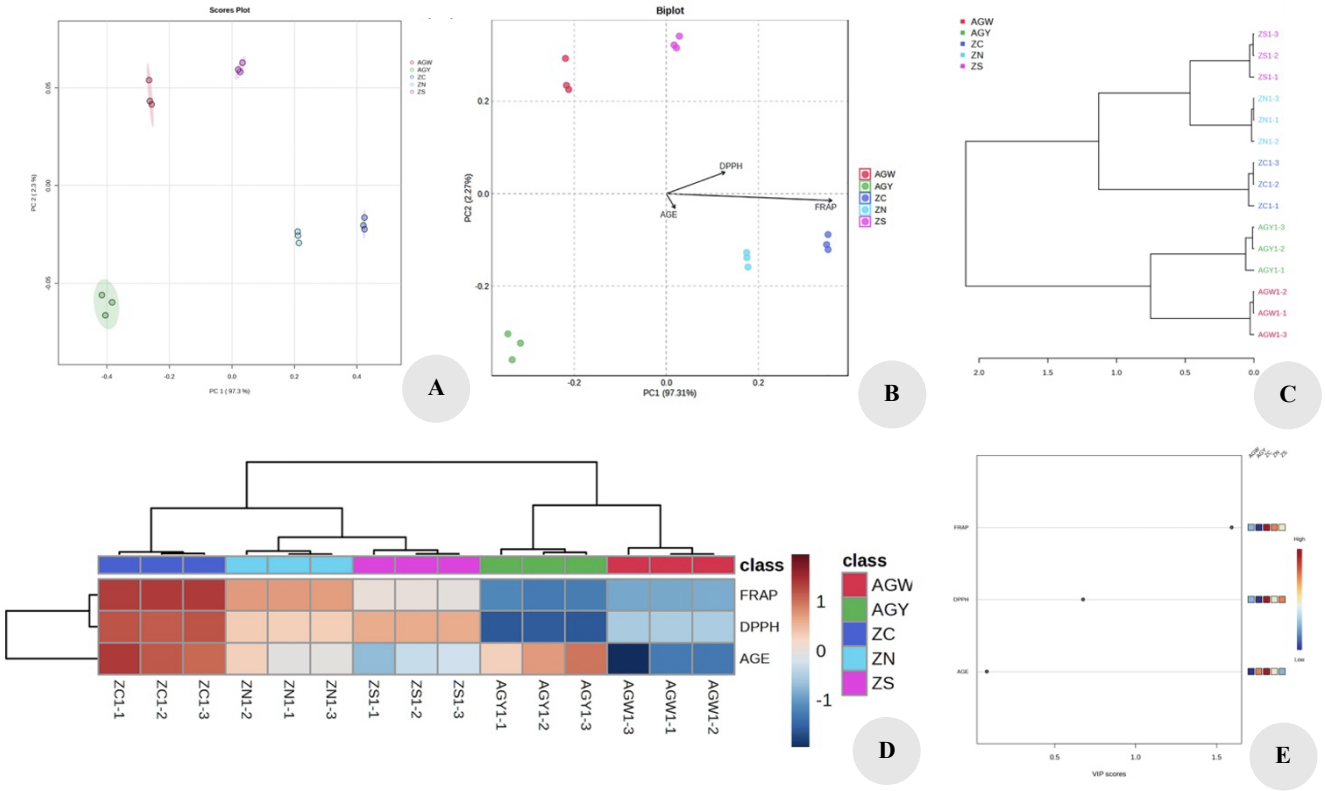

Figure 4. The relationship between biological activity, as assessed by the DPPH and FRAP assays, and antiglycation in the five species of Zingiberaceae. A. PCA scores plot showing group separation based on biological activity with DPPH, FRAP assay and antiglycation, B. PCA biplot, C. HCA dendrogram clustering samples by biological activity with DPPH, FRAP assay and antiglycation relationship patterns with variations of Zingiberaceae plant, D. A heatmap illustrating phenolic acids intensity relationships across samples, E. VIP scores identifying key biological activity by the DPPH and FRAP assays and antiglycation contributing to group differences

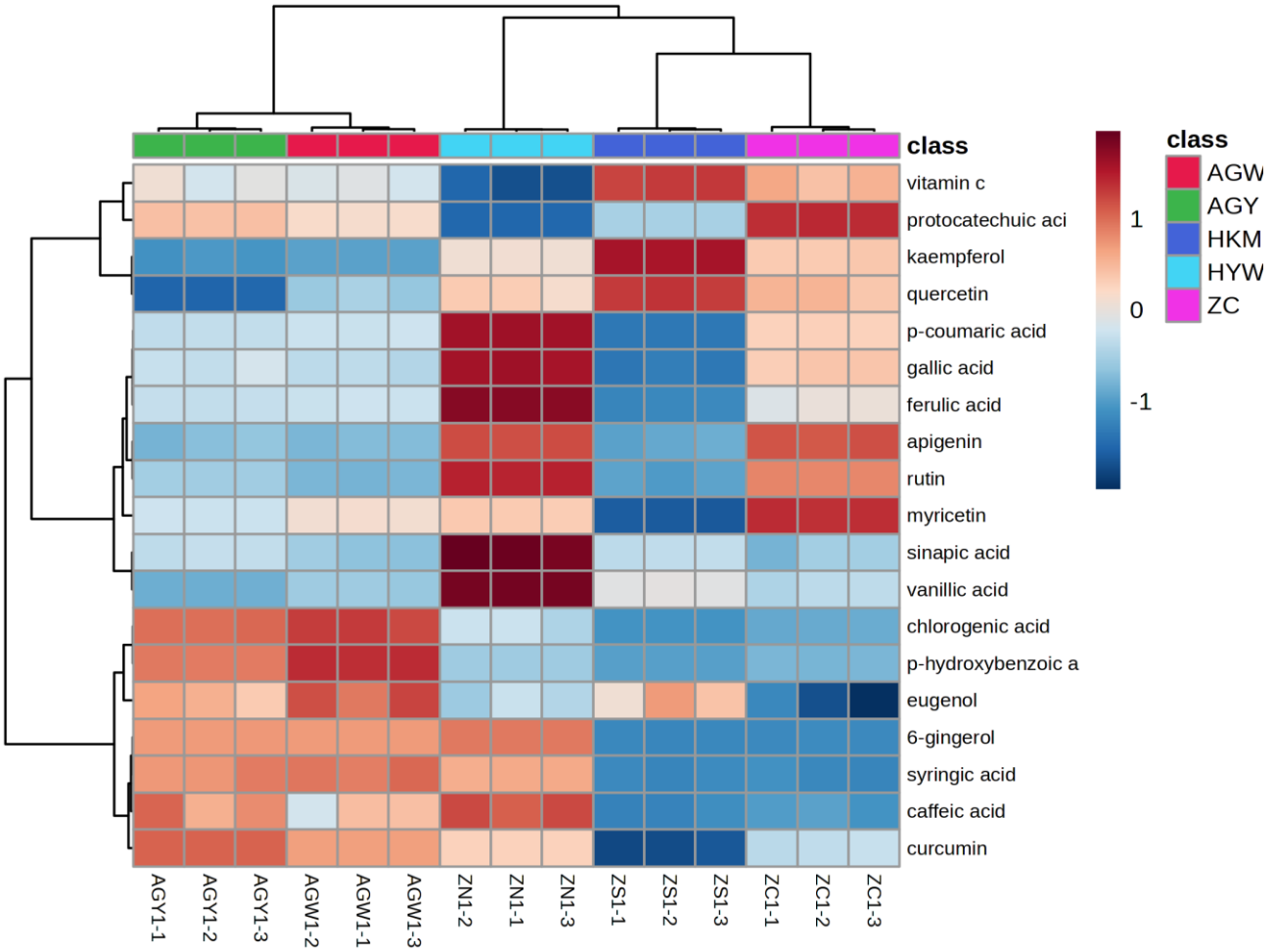

Figure 5. The heatmap illustrates the relative abundance of phytochemical compounds across five Zingiberaceae samples. The color gradient represents normalized Z-scores, indicating the intensity variation of individual phenolic acids, flavonoids, and signature bioactive compounds among species. Abbreviations: AGW: *A. galanga* (white cultivar), AGY: *A. galanga* (yellow cultivar), ZN: *Z. niveum*, ZS: *Z. ligulatum*, ZC: *Z. chrysostachys*

In contrast, *A. galanga*, both white (AGW) and yellow (AGY) varieties, showed low levels of active compounds, particularly AGW, which contained very low levels of curcumin and 6-gingerol. However, AGY had moderate levels of apigenin and rutin, which may be associated with its moderate anti-glycation activity. These results present the relationship between phytochemical composition and biological activity, and support the potential of ZC and ZN for further development as health food products. Further studies on their mechanisms of action, compound purification, safety, and efficacy in biological systems are urgently needed.

The relationship between chemical compounds and the biological activity of Zingiberaceae species

Pearson's correlation analysis examines the relationships between chemically active compounds, including phenolic acids, flavonoid compounds, and bioactive compounds (such as curcumin, 6-gingerol, eugenol, and vitamin C, and biological activity, specifically DPPH radical scavenging activity, FRAP, and AGE inhibition. The results were displayed in a heatmap format, as shown in Figures 6 A-C. The correlation between phenolic acids and biological activity (Figure 6.A) revealed that caffeic acid, ferulic acid, and p-coumaric acid exhibited high positive correlations with FRAP and AGE inhibition (dark red), particularly caffeic acid, which showed the highest correlation with FRAP. These hydroxycinnamic acids have a structure that can efficiently donate hydrogen to free radicals (Chen et al. 2020), whereas syringic and gallic acids had low or negative correlations. Figure 6.B shows the correlation between flavonoids and biological activity. Myricetin, kaempferol, and quercetin showed strong correlations with DPPH scavenging activity and AGE inhibition. Notably, myricetin showed a high correlation with all three bioactivity assays (DPPH scavenging, FRAP, and AGE inhibition). These findings are consistent with previous reports highlighting the potent antioxidant and anti-glycation activities of flavonols.

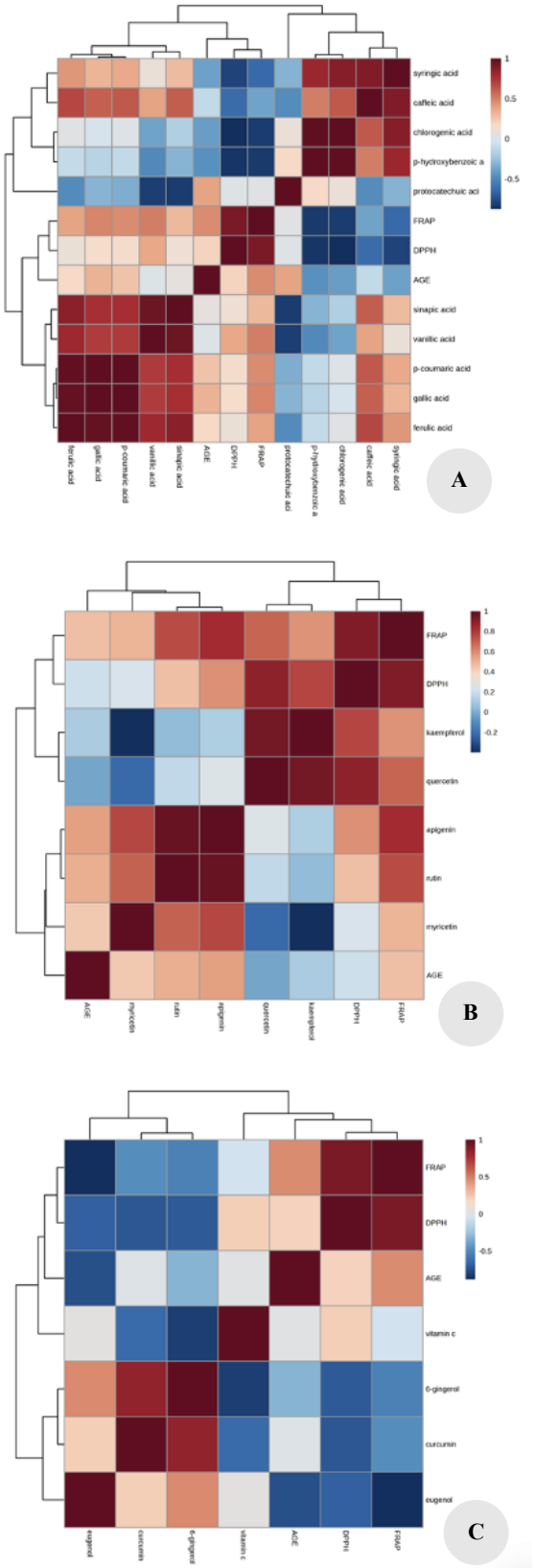

Figure 6. A. The relationship between bioactive compounds and biological activity shows the correlation between phenolic acids and biological activity, B. The correlation between flavonoid compounds and biological activity, C. The correlation between bioactive compounds (vitamin C, 6-gingerol, curcumin, and eugenol) and biological activity

The activities of these compounds are mainly attributed to the donation of a hydrogen atom or electron to scavenge DPPH radicals, electron transfer in the FRAP assay, and inhibition of AGE formation by trapping reactive carbonyl species. Moreover, their structural similarities suggest potential synergistic effects that may enhance their combined antioxidant and anti-glycation activities (Liu et al. 2018; Chagas et al. 2022; Roy et al. 2022). Apigenin and rutin exhibited moderate correlations, while species of Zingiberaceae with low flavonoid content, such as AGY, also showed low bioactivity. Curcumin and eugenol, the predominant bioactive compounds, had moderate positive correlations with DPPH scavenging activity and AGE, especially in the AGW and AGY (Figure 6.C). Vitamin C and 6-gingerol exhibited relatively low correlations compared to the phenolic/flavonoids group, indicating that the observed bioactivity of the entire plant extract resulted from the synergistic effects of phenolic and flavonoid compounds rather than the single bioactivities (Vaou et al. 2022). The correlation analysis results indicated that flavonols, including myricetin, kaempferol, and quercetin, had the highest bioactivity, followed by caffeic acid and ferulic acid. Bioactive compounds, such as curcumin and 6-gingerol, despite their molecular activity, have a less pronounced influence on biological activities in the plant. It suggested that a wide range of structurally active compounds, especially flavonoids and phenolic acids, is an important factor promoting biological properties in Zingiberaceae plants. In addition, variations in chemical compounds contribute to the differences observed in biological activities, particularly antioxidant activities assessed by DPPH and FRAP assays, as well as antiglycation properties.

In conclusion, the Zingiberaceae plants exhibited a high diversity of phenolics, flavonoids, and other bioactive compounds. Among the five species examined, ZC demonstrated the most promising potential for pharmaceutical and functional food applications, exhibiting consistently high antioxidant (DPPH and FRAP) and anti-glycation activities, likely attributable to its abundance of phenolic acids, flavonoids, curcumin, 6-gingerol, and eugenol. Moreover, ZS also displayed notable bioactivity, suggesting its suitability as a functional food ingredient with antioxidative properties. In addition, ZN presented moderate but consistent effects in both assays, indicating its potential role in supporting metabolic health. The yellow cultivar (AGY) showed relatively modest antioxidant activity but demonstrated moderate anti-glycation potential, which may be relevant to the prevention of glycation-associated pathologies. Although AGW exhibited relatively low antioxidant and anti-glycation activities, its distinct phytochemical profile suggests potential for alternative bioactivities, such as antidiabetic or anti-inflammatory effects, warranting further investigation. It underscores the importance of biodiversity-oriented studies that link phytochemical variation to ecological and ethnobotanical contexts. Future research should focus on isolating key active compounds, elucidating their mechanisms of action, and validating their efficacy through in vitro and in vivo models, while also

considering broader perspectives on species conservation and sustainable utilization.

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Table S1. Phenolic acid contents of the five Zingiberaceae species

Parameter	Content (µg/100 g DW)				
	ZC	ZN	ZS	AGW	AGY
Gallic acid	15.13±0.10^{e}	18.53±0.07^{f}	11.48±0.07^{e}	13.41±0.09^{g}	13.67±0.17^{e}
Protocatechuic acid	46.98±0.32^{b}	11.57±0.04^{h}	18.81±0.07^{b}	25.87±0.23^{c}	29.37±0.07^{c}
p-Hydroxybenzoic acid	11.15±0.10^{g}	13.74±0.06^{g}	9.36±0.01^{f}	84.76±0.57^{b}	54.26±0.16^{b}
Chlorogenic acid	8.65±0.03^{j}	10.00±0.29^{i}	8.22±0.01^{h}	14.99±0.20^{f}	13.99±0.08^{e}
Vanillic acid	12.23±0.19^{f}	24.29±0.08^{d}	13.50±0.08^{c}	11.56±0.09^{i}	10.62±0.04^{h}
Caffeic acid	103.43±0.17^{a}	109.60±0.22^{a}	102.91±0.02^{a}	106.90±1.04^{a}	108.53±0.69^{a}
Syringic acid	9.49±.0.07^{i}	11.91±0.02^{h}	9.47±0.04^{f}	12.48±0.11^{h}	12.22±0.16^{f}
p-Coumaric acid	32.49±0.13^{c}	91.93±0.12^{c}	9.14±0.03^{g}	21.28±0.25^{d}	20.28±0.14^{d}
Ferulic acid	24.17±1.58^{d}	99.87±0.58^{b}	9.39±0.16^{f}	19.95±0.22^{e}	19.14±0.09^{d}
Sinapic acid	10.91±0.49^{h}	22.52±0.35^{e}	11.84±0.09^{d}	10.90±0.23^{j}	11.90±0.06^{g}
Total phenolic acids	274.63±0.32^{D}	413.95±0.19^{A}	204.12±0.06^{E}	322.10±0.30^{B}	293.99±0.17^{C}

Note: Values are expressed as mean ± SD of triplicate measurements (n = 3). Means with different lowercase superscripts within the same column are significantly different at $p < 0.05$. Means with different uppercase superscripts within the same row are significantly different at $p < 0.05$. ZC: *Z. chrysostachys*, ZN: *Z. niveum*, ZS: *Z. ligulatum*, AGW: *A. galanga* (white), AGY: *A. galanga* (yellow)

Table S2. Flavonoid compound contents of the five Zingiberaceae species

Parameter	Content (µg/g DW)				
	ZC	ZN	ZS	AGW	AGY
Rutin	72.49±0.36^{b}	101.08±0.56^{a}	27.32±0.64^{c}	30.17±0.26^{b}	34.45±0.07^{b}
Myricetin	146.14±1.70^{a}	63.17±0.51^{b}	13.14±0.14^{d}	52.36±0.62^{a}	39.06±0.36^{a}
Quercetin	18.45±0.92^{d}	16.51±1.00^{e}	29.61±0.53^{b}	10.30±0.43^{c}	6.04±0.07^{c}
Apigenin	25.15±0.63^{c}	26.05±0.14^{c}	4.05±0.18^{e}	4.66±0.09^{e}	4.78±0.35^{d}
Kaempferol	26.91±0.66^{c}	18.95±0.23^{d}	134.43±1.56^{a}	4.80±0.02^{d}	4.19±0.17^{d}
Total flavonoid compounds	289.15±0.85^{A}	225.76±0.49^{B}	208.56±0.61^{C}	102.29±0.28^{D}	88.52±0.20^{E}

Note: Values are expressed as mean ± SD of triplicate measurements (n = 3). Means with different lowercase superscripts within the same column are significantly different at $p < 0.05$. Means with different uppercase superscripts within the same row are significantly different at $p < 0.05$. ZC: *Z. chrysostachys*, ZN: *Z. niveum*, ZS: *Z. ligulatum*, AGW: *A. galanga* (white), AGY: *A. galanga* (yellow)

Table S3. Curcumin, 6-gingerol, eugenol and vitamin C bioactive compound contents in the five Zingiberaceae species

Parameter	ZC	ZN	ZS	AGW	AGY
Curcumin (µg/g DW)	35.19±2.21^{d}	85.42±0.28^{c}	4.94±0.47^{e}	150.42±0.76^{b}	263.42±1.37^{a}
6-Gingerol (µg/g DW)	0.98±0.01^{c}	118.37±1.86^{a}	0.92±0.01^{c}	72.98±0.60^{b}	73.36±0.79^{b}
Eugenol (µg/g DW)	10.51±0.81^{d}	13.25±0.39^{c}	15.58±0.97^{b}	17.92±0.61^{a}	15.91±0.49^{b}
Vitamin C (mg/100 g DW)	11.94±0.53^{b}	4.24±0.20^{d}	16.29±0.33^{a}	8.42±0.22^{c}	8.76±0.56^{bc}

Note: Values are expressed as mean ± SD of triplicate measurements (n = 3). Means with different lowercase superscripts within the same row are significantly different at $p < 0.05$. ZC: *Z. chrysostachys*, ZN: *Z. niveum*, ZS: *Z. ligulatum*, AGW: *A. galanga* (white), AGY: *A. galanga* (yellow)

Table S4. Biological activities of DPPH, FRAP and antiglycation of the five Zingiberaceae species

Parameter	ZC	ZN	ZS	AGW	AGY
DPPH (µg TE /g DW)	471.77±3.07^{a}	377.76±1.60^{c}	404.79±0.52^{b}	303.11±0.80^{d}	231.07±0.91^{e}
DPPH (IC_{50}: µg TE /g DW)	251.04±0.10^{a}	247.34±0.08^{c}	248.62±0.02^{b}	242.90±0.06^{d}	236.16±0.11^{e}
FRAP (mmol $FeSO_4$ /g DW)	104.71±0.78^{a}	67.82±0.23^{b}	42.17±0.78^{c}	23.16±0.22^{d}	17.94±0.78^{e}
Antiglycation (%inhibition)	98.15±1.27^{a}	89.77±1.37^{c}	86.18±1.69^{d}	79.02±2.32^{e}	94.05±2.59^{b}

Note: Values are expressed as mean ± SD of triplicate measurements (n = 3). Means with different lowercase superscripts within the same row are significantly different at $p < 0.05$. ZC: *Z. chrysostachys*, ZN: *Z. niveum*, ZS: *Z. ligulatum*, AGW: *A. galanga* (white), AGY: *A. galanga* (yellow)