

Research Article



# Plant-Derived Exosome-like Nanoparticles from Emprit Ginger (*Zingiber officinale* var. *Amarum*) and Its Potential Metabolite as Functional Food Ingredients

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## ABSTRACT

The Plant-Derived Exosome-like Nanoparticles (PDENs) are nano-sized vesicles secreted by plants that carry various bioactive compounds and have shown promise for use in functional food applications. This study investigated PDENs isolated from emprit ginger (*Zingiber officinale* var. *Amarum*), a local ginger variety renowned for its health-promoting properties. PDENs were extracted from rhizomes harvested at 8, 10, and 12 months, and analyzed for total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity. The best-performing sample based on these parameters was selected for further metabolite profiling using LC-QTOF-MS. A total of 41 compounds were identified from the selected GDEN and ginger extract samples—32 compounds in the GDEN and 24 in the extract. These compounds belong to various groups, including amino acids, flavonoids, phenolics, lipids, terpenoids, vitamins, and others. Compound identification was based on public databases and literature concerning their potential as functional food ingredients. Among these, amino acids were the most abundant group in the GDENs, whereas phenolics were the dominant group in the ginger extract. This study underscores the potential of ginger-derived exosome nanoparticles as a rich source of bioactive compounds, supporting their further exploration and application in the development of functional food products. The balanced metabolite profile observed in GDENs highlights their unique advantages over conventional extracts. These findings reinforce the potential of emprit GDENs as promising candidates for functional food development.



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## 1. Introduction

Using active plant ingredients to maintain human health is an intensively practiced approach. Apart from reducing potential ethical problems associated with

animal sources, bioactive compounds from plants can also be produced in greater quantities on a continuous basis. Research on plant-derived exosome-like nanoparticles (PDENs) has increased rapidly in the last decade. There is still a lot of untapped potential in PDEN, especially when discussing the possibility of its bioactive components as health service providers

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and drug carriers. PDEN research has been conducted on several plants, including grapes (Garaeva *et al.* 2021), broccoli (Deng *et al.* 2017), shiitake mushrooms (Liu *et al.* 2020), ginger (Zhang *et al.* 2016a; Zhang *et al.* 2017a; Man *et al.* 2021; Yin *et al.* 2022), and ginseng (Kim *et al.* 2022).

Ginger (Zingiberaceae) offers various benefits, including antitumor, anti-inflammatory, and antioxidant properties. Ginger rhizomes are widely used in the health sector as herbal products, standardized medicines, and phytopharmaceuticals. Ginger rhizome contains the main chemical components zingiberene, shogaol, and gingerol, which are efficacious as antioxidants and anti-inflammatory agents (Prasad & Tyagi 2015; Mao *et al.* 2019; Nishidono *et al.* 2020; Arcusa *et al.* 2022). These characteristics are associated with several compounds, including polyphenols, flavonoids, terpenoids, and vitamins (Abeyasinghe *et al.* 2021). PDEN, like mammalian exosomes, also contains lipids, proteins, and bioactive compounds, suggesting that it may contain compounds with physiological functions for human health, such as vitamins and amino acids (Hessvik & Lorente 2018; Crescitelli *et al.* 2020). Additionally, it has the potential to serve as a functional food ingredient. Although there are many definitions, functional food is broadly defined as food with additional functions beyond basic nutrition, particularly promoting and maintaining health when consumed regularly as part of a balanced diet (Lenssen *et al.* 2018).

PDENs isolated from ginger rhizomes have been studied. Ginger-derived exosome-like nanoparticles (GDENs) contained several compounds, such as 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol (Nishidono *et al.* 2020; Yin *et al.* 2022). Many of them reported that GDENs have anti-inflammatory benefits (Zhang *et al.* 2016b; Yin *et al.* 2022), effects in preventing organ damage (Zhuang *et al.* 2015), and the ability to regulate gut microbiota (Teng *et al.* 2018). Research on GDEN has not focused on the subtype and its metabolites; therefore, it is necessary to investigate the metabolite content of GDEN in detail from a specific type or variety of ginger. Compared to other types of ginger, emprit ginger has small rhizomes but a sharp aroma, soft fibers, and relatively high antioxidant content (Mahmudati *et al.* 2020). Preliminary studies revealed that among the three varieties of ginger we have in Indonesia, namely gajah ginger (*Z. officinale* var. *Officinarum*), red ginger (*Z. officinale* var. *Rubrum*), and emprit ginger

(*Z. officinale* var. *Amarum*), the latter demonstrated the highest antioxidant capacity. Based on those results, we conducted further research on emprit GDEN metabolites and tried to explore their beneficial properties as healthy food ingredients. Adding herbal extracts to a food preparation can increase its phytochemical content and biological potential (Nabila *et al.* 2025). However, the incorporation of a new component into a food mixture can alter both its flavor and texture, which is often undesirable. Therefore, ginger nanoparticles containing high-potential food ingredients could be a valuable solution to reduce such unfavorable effects.

## 2. Materials and Methods

### 2.1. Plant Materials

The plant material used in this research was fresh emprit ginger (*Z. officinale* var. *Amarum*) 8, 10, and 12-month rhizomes collected from the field in Sukabumi (West Java, Indonesia). The rhizomes were used as the source of GDEN and served as the control (extract).

### 2.2. PDEN Isolation

Ginger rhizomes were cleaned with tap water and then peeled. The peeled ginger rhizomes were grated with a coarse grater. The grated material was centrifuged at 4000 ×g for 30 minutes at 4°C. The supernatant was filtered through a coffee filter, and then the filtrate was filtered through a 40 µm nylon filter. The last filtrate was then passed through a PES membrane filter, gradually decreasing in pore size from 0.65 µm to 0.22 µm and finally to 0.1 µm, with the help of a vacuum pump. The final filtrate containing PDENs was stored in a refrigerator (4°C).

### 2.3. Antioxidant Analysis

The radical scavenging activity of emprit GDENs was tested using the DPPH method described by Salazar-Aranda *et al.* (2011). Ascorbic acid solution is used as the standard. Antioxidant capacity was measured at a wavelength of 517 nm.

### 2.4. Total Phenolic and Flavonoid Analysis

The total phenolic content (TPC) was determined according to Batubara *et al.* (2020) using a 50% Follin-Ciocalteu reagent and a 7.5% Na<sub>2</sub>CO<sub>3</sub> solution. The absorbance of the mixture was measured at 765 nm. The TPC was expressed as gallic acid equivalent (ppm GAE). Total flavonoid content (TFC) was analyzed according

to Batubara *et al.* (2020). The absorbance was measured at  $\lambda$  415 nm. The total flavonoid content was expressed as quercetin equivalent (mg QE/g).

## 2.5. LC-QTOF-MS Preparation

Ginger rhizomes were cleaned and peeled, then weighed  $\pm 150$  g. The rhizomes were cut into small pieces and dried at 40°C for 24 hours in an oven until they were completely dry. The dry sample was macerated in 96% ethanol (1:7.5). Maceration was carried out for three days on a shaker at 100 rpm. A Whatman No. 1 filter paper was used to separate the macerate. The extract was then extracted with a vacuum rotary evaporator at 45°C and dried in a pressurized dryer. This sample served as the control for the PDENs.

PDENs and ginger extract samples were analyzed using a non-targeted metabolomics approach. The ginger extract was further processed with Oasis HLB SPE and eluted with methanol. qTOF LC-MS/MS analysis was carried out using ultra-performance liquid chromatography (UPLC) (LC: Acquity UPLC H-class system, Waters, USA) and mass spectrometer (Xevo G2-S QToF, Waters, USA). Separation was performed using an Acquity UPLC® HSS column (Waters, USA) C18 (2.1  $\times$  100 mm 1.8  $\mu$ m) at 50°C column temperature. The sample was filtered through a 0.45  $\mu$ m Millipore filter, and a 10  $\mu$ L aliquot was injected with three replicates. LC analysis used a mobile phase of water + 5 mM ammonium formate (A) and acetonitrile + 0.05% formic acid (B), with a flow rate of 0.2 mL min<sup>-1</sup>. The delivery system operated at a constant rate of 200  $\mu$ L min<sup>-1</sup>, and the mobile phase consisted of 70% acetonitrile and 1 mmol of 30% formic acid. MS/MS operation utilized electrospray ionization (ESI) in both positive and negative modes, with a mass range of 50-1200 m/z. The source and desolvation temperatures were set at 100 °C and 350 °C, respectively. In addition, the cone and desolvation gas rates were 0 and 793 L/h, respectively.

## 2.6. Data Analysis

Quantitative data from phenolics, flavonoids, and antioxidant determinations were analyzed in a CRD by a two-way ANOVA with three replicates using R 4.3.1. Furthermore, Tukey's test was performed ( $\alpha$  = 0.05%) if applicable. Metabolites data analysis used MS-Dial version 3.82 and MS Finder version 3.5.2. The raw data were converted into .abf format using Abf Converter version 4.0.0. and MS FileReader 2.2.62. Compound identification was performed using internal

databases (MSP File) in MS/MS positive and negative modes (<http://prime.psc.riken.jp/compms/msdial/main.html#MSP>) with a 70% score cutoff and adduct types [M+H]<sup>+</sup> and [M-H]<sup>-</sup>. Data were further filtered and selected based on the search for potential compounds as functional food ingredients through several databases, including PubChem (<https://pubchem.ncbi.nlm.nih.gov/>), KNApSack (<http://www.knapsackfamily.com>), IJAH Analytics (<https://ijah.apps.cs.ipb.ac.id/>), FoodB (<https://www.foodb.ca/>), and independent searches from research journals. The distribution of metabolite groups was determined semi-quantitatively based on the frequency of compound occurrence at different retention times.

## 3. Results

### 3.1. Antioxidant Capacity

Figure 1 presents the results of antioxidant capacity equivalent to ascorbic acid against DPPH free radicals from two types of samples.

The antioxidant capacity of the two types of ginger samples at all ages shows relatively high values. Both graphs (Figure 1A and B) indicate that the different ages did not differ significantly in antioxidant capacity; however, the values tended to decrease as the rhizome aged. In GDENs, the average antioxidant capacity was lower than that of the extracts. For GDEN, the highest antioxidant capacity was recorded at 8 months and declined by 12 months. The extract samples consistently showed high antioxidant capacity, ranging from 93.54 to 95.59 ppm AAE.

### 3.2. Total Phenolics Content (TPC) and Total Flavonoids Content (TFC)

The TPC and TFC results obtained from emprit GDENs and the extract are presented in Table 1.

The TPC values of GDENs and the extract had a significant gap. The phenolic content of the three age groups of ginger, as determined by GDEN, revealed that the highest level was found in 10-month-old ginger, while the best value in the extract was obtained from 12-month-old ginger. A reversed condition happened to TFC; ginger extract demonstrated a higher content of flavonoids than the GDENs. In the GDEN sample, TFC was found at a deficient concentration and continued to decrease at 12 months. A very high TFC was observed in the 8-month-old ginger extract and drastically reduced in the 12-month-old ginger.

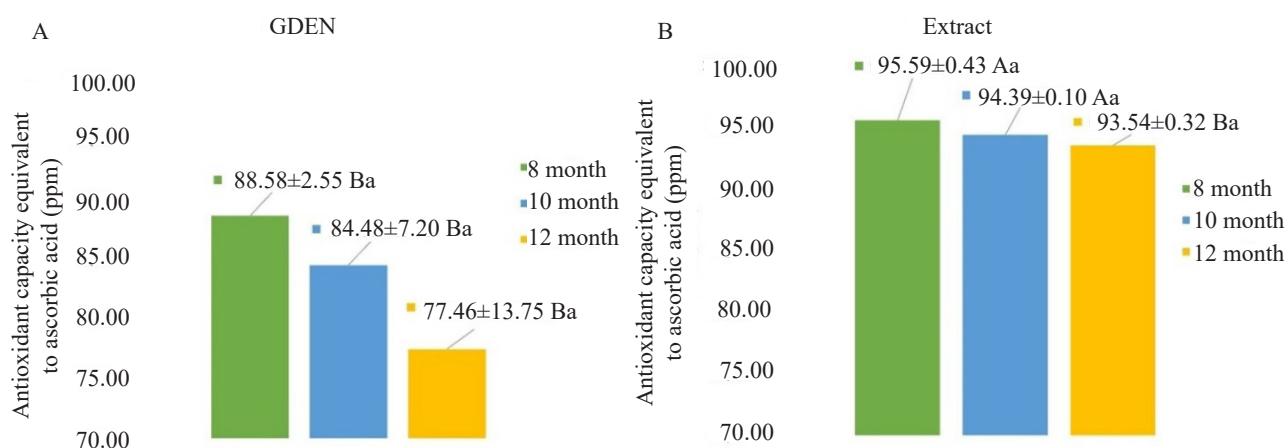


Figure 1. Antioxidant capacity of emprit GDENs (A) and ginger extract (B) in ascorbic acid equivalent (ppm AAE). Uppercase letters (A-B) in both graphs indicate a significant difference between types of sample; Lowercase letter (a) in the same graph indicates no significant difference between the rhizome ages

Table 1. Total phenolics and total flavonoids contents of emprit GDEN and ginger extract

Ginger age (months)	TPC (ppm GAE)		TFC (mg QE g <sup>-1</sup> )	
	GDEN	Extract	GDEN	Extract
8	501.78 ± 14.62 <sup>Ab</sup>	140.92 ± 0.00 <sup>Bb</sup>	7.77 ± 0.25 <sup>Aa</sup>	67.93 ± 0.43 <sup>Ba</sup>
10	576.95 ± 31.19 <sup>Aa</sup>	120.41 ± 2.22 <sup>Bc</sup>	5.43 ± 1.68 <sup>Aab</sup>	61.59 ± 0.10 <sup>Bb</sup>
12	554.65 ± 18.60 <sup>Aab</sup>	390.94 ± 0.87 <sup>Ba</sup>	4.00 ± 0.14 <sup>Ab</sup>	11.72 ± 0.32 <sup>Bc</sup>

TPC was expressed as gallic acid equivalent using a calibration curve:  $y = 0.0091x - 0.0123$ , with  $R^2 = 0.9982$ . TFC was expressed as quercetin equivalent by a calibration curve:  $y = 0.003x - 0.0157$ , with  $R^2 = 0.9935$ . A-B different letters in the same row of each parameter indicate a significant difference between sample types. a-ab-b-c different letters in the same column indicate a significant difference between ginger ages

### 3.3. Metabolite Profile

The richness of potential bioactive metabolites, focusing on compounds with potential as functional food ingredients, contained in GDEN and extract samples can be grouped based on their metabolite classes, as presented in Figure 2.

In GDEN, the most dominant compound group was amino acids (28%), followed by phenolics (23%) and terpenoids (20%). In contrast, the ginger extract displayed a different composition, with phenolics as the predominant compound (55%), followed by amino acids and terpenoids, which were evenly distributed (14% each). The two sample types showed significant differences in the abundance of amino acids, which were present in GDEN but absent in the ginger extract. At the same time, phenolics were the major component in the extract. Additional differences between GDEN and the extract included carbohydrates and vitamins, which were only found in GDEN. However, in small amounts, GDEN also contained nucleotide bases, which were absent in the extract. Alkaloids and nucleosides were rare in GDEN, comprising only a small proportion, a pattern also observed in the extract.

Figure 3 presents the number of metabolite groups detected in GDEN and ginger extract samples. Eight metabolite groups were uniquely found in GDEN, four groups were unique to ginger extract, and both samples shared five groups. This distribution aligns with the compound profiles shown in Figure 2, highlighting the greater diversity of metabolite groups present in GDEN compared to ginger extract.

Table 2 lists the metabolite compounds in GDEN and ginger extract selected from the LC-QTOF-MS data, demonstrating that both samples have the potential to serve as functional food ingredients. The reported pharmacological benefits were obtained from a review of the scientific literature, as outlined in the methods section.

## 4. Discussion

### 4.1. Phenolics and Flavonoids Related to Antioxidant

Knowledge of plant bioactive compounds has been developing rapidly since the development of phytochemical screening methods to metabolite profiling.



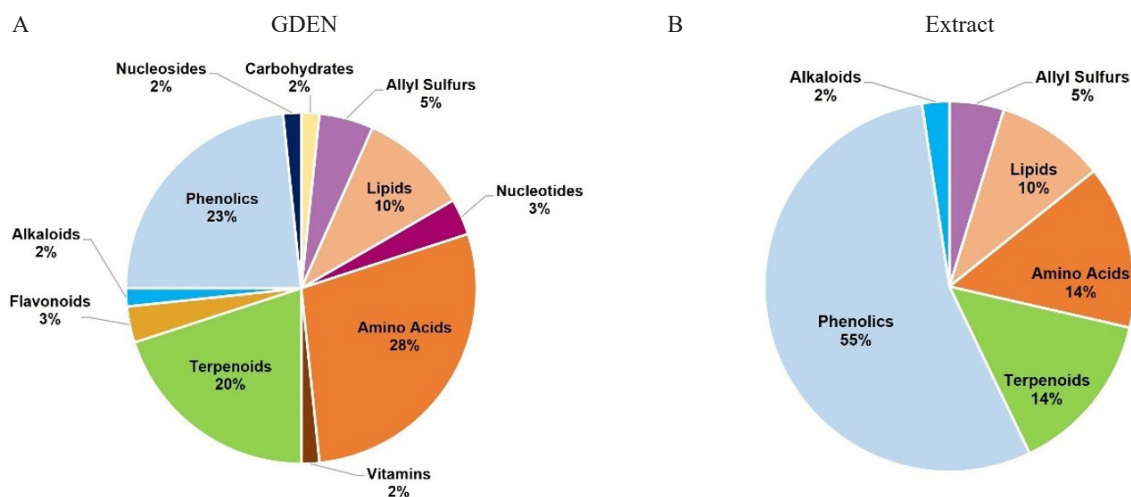


Figure 2. Proportion of metabolite compounds in emprit GDENs (A) and extract (B) categorized potential as functional food ingredients



Figure 3. Number of metabolite group distribution across GDEN and extract samples

Differences in the content of metabolite compounds in ginger and other spices typically occur due to complex factors, including internal factors such as genotype, and external factors from the environment, including harvesting age (Jayasundara & Arampath 2021). In using plants as a functional food and maintaining human health, antioxidants become an essential activity tested on metabolite extracts of medicinal plants. The antioxidant activity may result from the collaboration of multiple compounds, including phenolics and flavonoids. The reduction of antioxidant capacity (Figure 1) suggests a potential reduction in bioactive compounds such as phenolics and flavonoids. The relationship between antioxidant activity and these compounds has been reported in various studies (Fernandes *et al.* 2016; Umar *et al.* 2023). PDEN has been shown to contain



































various bioactive compounds with beneficial health and pharmaceutical effects. Recently, Farid *et al.* (2025) demonstrated the potential of papaya-derived exosome-like nanoparticles in skin photoprotective assays, highlighting their antioxidant capacity.

The highest TPC value of GDEN was found in 10-month-old ginger, whereas in the extract, the highest value was observed at 12 months of age. These results indicate that GDEN from 10-month-old ginger contains relatively high phenolic content, which may be associated with good antioxidant activity. According to Wahid *et al.* (2023), the optimal harvest age for ginger rhizomes to achieve maximum metabolite content is 7–8 months. On the other hand, an increase in TPC was observed in the 12-month-old ginger extract. Several factors, including the type and concentration of the solvent, extraction

Table 2. Potential compounds in GDEN and ginger extract samples and their biological activities/health benefits

Group and compounds	N E	Biological activities/ health benefits
<i>Carbohydrates</i> Phytic acid		Antioxidant (Canan <i>et al.</i> 2012), antidiabetes (Kim <i>et al.</i> 2010), food preservative (Zhang <i>et al.</i> 2013)
<i>Lipids</i> Falcarindiol		Regulate lipid metabolism (Andersen <i>et al.</i> 2020), anticancer (Park <i>et al.</i> 2021)
<i>Amino Acids</i> L-Arginine		Immune enhancer (Geiger <i>et al.</i> 2016), protein synthesis's building block (Kim <i>et al.</i> 2014)
Phenylalanine		Production neurotransmitter, increases intestinal health (Zhang <i>et al.</i> 2023)
L-Tryptophan		Precursor of serotonin (Von Ah <i>et al.</i> 2012), neural function (Xie <i>et al.</i> 2023)
L-Proline		Protein structure, maintenance of cellular homeostasis (Hollinshead <i>et al.</i> 2018)
L-Histidine		Antioxidant (Xu <i>et al.</i> 2017), dietary supplement (Flores <i>et al.</i> 2023)
Homoarginine		Reduce cardiovascular disease (Nitz <i>et al.</i> 2022)
Melatonin		Regulate circadian rhythm (Daugaard <i>et al.</i> 2017), antioxidant, antiaging (Sae-Teaw <i>et al.</i> 2012; Pérez-Llamas <i>et al.</i> 2020)
Methionine		Antioxidant, builds important protein (Park <i>et al.</i> 2018), regulates lipid metabolism (Wu <i>et al.</i> 2020), increases immunity (Hosseini <i>et al.</i> 2012)
Isoleucine		Reducing lipid accumulation (Ma <i>et al.</i> 2020a)
Methionine-Enkephalin		Inhibits colorectal cancer (Wang <i>et al.</i> 2022)
L-Valine		Energy production (Holeček 2021; Yu <i>et al.</i> 2021)
Quinic acid		Antimicrobial (Ercan & Dogru 2022), dietary supplement (Dong <i>et al.</i> 2022)
Tyrosine		Precursor of dopamine and norepinephrine (Frings <i>et al.</i> 2020)
Betaine		Antioxidant, antiinflammation, neuroprotective (Li <i>et al.</i> 2022; Hashim <i>et al.</i> 2024)
<i>Nucleosides</i> Adenosine		Neuromodulator (Sebastião & Ribero 2015; dos Santos <i>et al.</i> 2024)
<i>Nucleotides</i> Adenine		DNA structure, neuromodulator (Yin <i>et al.</i> 2024)
Guanine		DNA structure, neuromodulator (Chojnowski <i>et al.</i> 2021)
<i>Vitamins</i> Pyridoxine		Antioxidative, enhances immune response (Khan & Khan 2021), maintenance of the nervous system (Calderón-Ospina & Nava-Mesa 2020)
<i>Allyl Sulfurs</i> Diallyl Sulfide		Antidiabetic, antihyperlipidemic (Habibi <i>et al.</i> 2024), antibacterial, antioxidant, antiinflammatory (Eren <i>et al.</i> 2023)
<i>Alkaloids</i> Piperidine		Anticancer (Mitra <i>et al.</i> 2022)
Conessine		Antibacterial, antiviral (Zhou <i>et al.</i> 2023), anti-malarial (Dua <i>et al.</i> 2013)
<i>Terpenoids</i> Linalool		Antioxidant, anti-inflammatory, antimicrobial, antifungal (Ola & Sofolahan 2021; dos Santos <i>et al.</i> 2021), flavoring agent (Liang <i>et al.</i> 2023)

Table 2. Continued

Group and compounds	N E	Biological activities/ health benefits
Costunolide	 	Anti-inflammatory, antidiabetes (Jin <i>et al.</i> 2023), anticancer, anti-neuroinflammation (Liu <i>et al.</i> 2020), antiviral, antifungal (Kim & Choi 2019)
Sabinene	 	Antioxidant, anti-inflammatory, anticancer, antimicrobial, antifungal (Sharma <i>et al.</i> 2019), flavoring agent (Popa <i>et al.</i> 2021)
Geranic acid	 	Food flavoring agent (Sanekata <i>et al.</i> 2018)
Alpha-pinene	 	Antifungal, antimicrobial, flavour (Salehi <i>et al.</i> 2019), antiinflammatory (Koshnazar <i>et al.</i> 2020), antioxidative (Bouzenna <i>et al.</i> 2017), neuroprotective (Lee <i>et al.</i> 2017), gastroprotective (Pinheiro <i>et al.</i> 2015)
Flavonoids Isorhamnetin	 	Antioxidant, cardioprotective (Gong <i>et al.</i> 2020)
Phenolics Myristicin	 	Antioxidant, anti-inflammatory, antimicrobial (Seneme <i>et al.</i> 2021), promoting glucose uptake (Yoshioka <i>et al.</i> 2022)
6-Shogaol	 	Antiinflammatory, antioxidant (Wang <i>et al.</i> 2017; Dugasani <i>et al.</i> 2010), anticancer (Ma <i>et al.</i> 2020b; Pei <i>et al.</i> 2021)
6-Gingerol	 	Antioxidant, anti-inflammatory (Dugasani <i>et al.</i> 2010), anticancer (Zhang <i>et al.</i> 2017b)
Tricoumaroyl spermidine	 	Antiaging, antioxidant, dietary supplement (Madeo <i>et al.</i> 2019; Madeo <i>et al.</i> 2020; Qiao <i>et al.</i> 2024)
Coniferaldehyde	 	Modulate lipid and glucose metabolism (Gai <i>et al.</i> 2020), anti-inflammatory, apoptotic (Kim <i>et al.</i> 2016)
Dicafeoyl coumaroyl spermidine	 	Antiaging, dietary supplement (Madeo <i>et al.</i> 2019; Madeo <i>et al.</i> 2020; Qiao <i>et al.</i> 2024)
10-Gingerol	 	Antioxidative (Dugasani <i>et al.</i> 2010), anticancer (Zhang <i>et al.</i> 2017b), antiinflammatory (Ho <i>et al.</i> 2013)
Vanillin	 	Food flavor, antibiotic (Bezerra <i>et al.</i> 2017), neuroprotective (Salau <i>et al.</i> 2020), antimicrobial (Ngarmsak <i>et al.</i> 2006), antioxidative, antiinflammatory (Bezerra-Filho <i>et al.</i> 2019), anticancer (Bezerra <i>et al.</i> 2016)
Cinnamic acid	 	Antioxidant (Sova 2012), antiinflammatory (de Cássia da Silveira <i>et al.</i> 2014), anticancer (Anantharaju <i>et al.</i> 2016), hypoglycemic agent (Alam <i>et al.</i> 2016)
Hydroxycinnamic acid	 	Antioxidant (Sova 2012), antiinflammatory (de Cássia da Silveira <i>et al.</i> 2014), anticancer (Anantharaju <i>et al.</i> 2016), hypoglycemic agent (Alam <i>et al.</i> 2016)
4-Hydroxybenzaldehyde	 	Improve insulin resistance, anti-obesity (Park <i>et al.</i> 2010; Yu <i>et al.</i> 2010)
Benzaldehyde	 	Flavoring agent (Yu <i>et al.</i> 2020), anticancer (Kciuk <i>et al.</i> 2023), antibiotic (Neto <i>et al.</i> 2021)

N = GDEN; E = Extract

 Gray-shaded cell = compound detected in the sample

 White/unshaded cell = compound not detected in the sample

method, pH, and temperature, also play important roles in determining phenolic content (Al Juhaيمي *et al.* 2018).

Table 1 shows a decrease in flavonoid concentration with age increase in both samples. The highest TFC value was observed in the 8-month-old ginger extract, which then decreased sharply at 12 months. A similar pattern was also observed in GDEN. Compared to GDEN, the TFC in ginger extract was significantly higher. Various drying processes applied to the raw material before extraction can also affect its essential oil content, including increases or decreases in TPC and TFC (An *et al.* 2016).

The average antioxidant capacity of ginger extract was higher than that of the GDEN samples. It was suggested that the processing applied to ginger rhizomes can also be a factor in determining the amount and variation of metabolites (Ghafoor *et al.* 2020). According to Chumroenpat *et al.* (2011), the highest antioxidant capacity in ginger is obtained at mild drying temperatures (60°C), suggesting that the optimal levels of bioactive compounds in ginger likely emerged at that condition. Based on the consistent results between antioxidant capacity and flavonoid content, 8-month-old ginger rhizomes were selected for metabolite profile analysis.

#### 4.2. Distribution of Metabolite

Figure 2 emphasizes the richness of compound variety in GDEN compared to the extract. The comparison of metabolite composition in the GDEN and ginger extract samples shows differing patterns of dominant compound groups, which may reflect differences in extraction methods and, consequently, their functional potential. GDEN maintained protein components (amino acids, nucleosides, nucleotides) more than the extract, indicating that GDEN plays a role in carrying various important bioactive compounds involved in plant cellular functions (Zhang *et al.* 2016b). The high presence of amino acids may also support GDEN's potential as a stable and functional delivery system for bioactive molecules. This indicates that GDEN is capable of retaining water-soluble functional molecules, which tend to be lost during rhizome extraction with ethanol. The centrifugation process in PDEN isolation separated the supernatant from large components and concentrated small components, such as proteins and their amino acids (Li *et al.* 2017; Suharta *et al.* 2021). The presence of carbohydrates in PDENs is possible because they are carried over during the initial filtration process before centrifugation. It is common in PDEN isolation; however, less purity can occur when large molecules, such as proteins and carbohydrates, remain (Zarovni

*et al.* 2015). In contrast, ginger extract keeps more phenolic compounds. The ethanol maceration method used for the extract sample is more efficient in dissolving phenolic compounds (Evitasari & Susanti 2021), but is less optimal for retaining hydrophilic compounds and small proteins.

The distribution and abundance of metabolites uniquely or jointly present in GDEN and extract samples (Figure 3) indicate that GDEN contains a greater variety of metabolites, primarily composed of primary metabolites such as amino acids, nucleotides, and vitamins (Table 2). In contrast, the extract is primarily composed of secondary metabolites, including phenolics and terpenoids. This greater diversity of metabolite groups in GDEN may result from its nanoparticle nature, which selectively encapsulates or enriches a broader range of small molecules compared to the crude extract. Such encapsulation processes can capture not only secondary metabolites but also small primary metabolites that are often lost or diluted in conventional extraction. Additionally, the nearly balanced distribution of primary and secondary metabolites in GDEN suggests multifunctional potential, both as a nutritional source and in terms of physiological activity. The broader variety of metabolite groups in GDEN compared to the extract further supports GDEN's advantage in carrying a more complex spectrum of bioactive compounds, making it potentially useful as a natural "carrier" for food or pharmaceutical applications (Mu *et al.* 2014).

Table 2 lists several compounds with potential as functional food ingredients in GDEN and emprit ginger extract, including those with physiologically significant health effects. Some differences between GDEN and extract can be seen in the most dominant compounds. Among both samples, phenolic compounds appear as the dominant group. In both samples, the presence of shogaol, gingerol, and sabinene not only contributes to the free radical scavenging effect but also serves as the main constituents that give the typical spiciness of Zingiber. Furthermore, a key characteristic of functional foods is their ability to elicit physiological effects in the body while also being sensorially acceptable (Baker *et al.* 2022). Phenolics also exhibit anti-inflammatory activity, contributing to the modulation of the body's immune system (Pázmándi *et al.* 2024). The presence of phenolics in these samples offers great potential as functional food ingredients with broad health effects. Additionally, some phenolics such as vanillin and spermidine may contribute to improving the quality of the human diet, particularly in relation to regulating body metabolism and reducing inflammation.



Beyond phenolics, amino acids are also dominant components found in GDEN, serving as essential protein-building compounds for the body. These amino acids can provide benefits to the body's metabolism, including contributing to tissue repair and regulating various other bodily functions. The physiological activities of amino acids, especially those found in GDEN, also have potential anti-inflammatory effects and may help regulate the body's metabolic balance (Pasini *et al.* 2023). Based on the findings of this study, *Zingiber officinale* var. *Amarum* GDEN demonstrates great potential as a functional food ingredient that could offer significant health benefits to the human body, including the regulation of blood sugar levels, cholesterol levels, and the reduction of inflammation.

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