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Metabolite Profiling Differences of Single and Physical Mixture of Moringa Leaves (*Moringa oleifera*) and Red Galangal (*Alpinia purpurata (Vieill) K. Schum*)

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ORIGINAL ARTICLES

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ABSTRACT

Secondary metabolites are bioactive plant constituents that contribute to antioxidant, anti-inflammatory, and anti-microbial activities, making them valuable for functional food and nutraceuticals applications. This study aimed to characterize the secondary metabolites present in powdered Moringa oleifera leaves, red galangal (Alpinia purpurata (Vieill) K. Schum) rhizomes, and their combination. The 85:15 ratio used in the mixed powder was selected based on preliminary material composition considerations. Metabolite identification was conducted using Ultra High Performance Liquid Chromatography Tandem High Resolution Mass Spectrometry (UHPLC-HRMS). Data processing and compound annotation were performed using ChemSpider and mzCloud. A total of 21 metabolites were identified in moringa leaf powder, 16 compounds in red galangal rhizome, and 17 compounds in the combined preparation. Flavonoid were the dominant class in moringa leaves with quercitrin, 4-aminobenzoid acids, and pheophorbide A as the most abundant constituents. In contrast, phenolic compounds, particularly benzoic derivatives were predominant in red galangal, where menadiol, abietin, and sweroside were the major metabolites. The combined formulation retained key constituents from both plants, including cinnamaldehyde, 4-aminobenzoic acid, and pheophorbide A, and additionally showed an increased level of nictoflorin. These findings provide preliminary compositional evidence demostrating the distinct and complementary phytochemical profiles of moringa leaves and red galangal rhizomes. This information may guide future studies into potential functional food or nutraceutical formulations for further validation through bioactivity, bioavailability, and interaction studies.

Key Messages:

- Predominant flavonoid compounds in *Moringa oleifera* leaves powder
- The red galangal rhizome powder and its combination with moringa leaves powder contain a high number of benzenoid and flavonoid content

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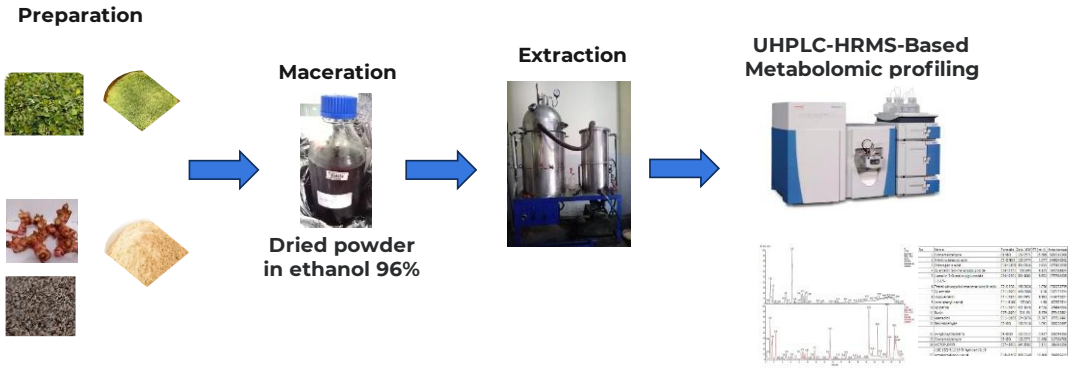
GRAPHICAL ABSTRACT

Metabolite Profiling Differences of Single and Physical Mixture of Moringa Leaves (*Moringa oleifera*) and Red Galangal (*Alpinia purpurata* (Vieill) K. Schum)

This study investigated the secondary metabolites present in *Moringa oleifera* leaf powder, red galangal (*Alpinia purpurata* (Vieill) K. Schum) rhizome powder, and their combination. A total of 21 compounds were identified in *M. oleifera* leaf powder, 16 compounds in red galangal rhizome powder, and 17 secondary metabolites in the combined sample.



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INTRODUCTION

Plant-derived secondary metabolites have gained increasing attention in recent decades due to their diverse biological activities and potential health benefits. These metabolites such as flavonoids, phenolic acid, alkaloids, terpenoids, and other polyphenolic compounds play critical roles in plant defense and adaptation(1). Numerous studies have highlighted their antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, making them valuable both for traditional medicine and for development of functional foods and nutraceutical products(2). With growing consumer interest in natural health-promoting compounds, the identification and characterization of secondary metabolites from locally available plant species are of scientific importance.

Moringa oleifera, commonly known as the drumstick tree, is widely recognized for its exceptional nutritional profile and rich phytochemistry. The leaves of moringa contain high levels of flavonoids, phenolics acids, vitamins, and essential mineral, contributing to its use as a traditional remedy for inflammation, oxidative stress, and metabolic disorders (3,4). Previous investigations have reported that flavonoids such as rutin, quercetin and kaempferol, along with phenolic acids like gallic acid are key bioactive constituents responsible for these benefial effects (5). This phytochemical richness has positioned moringa as an important candidate for both dietary supplementation and herbal medicine. Similarly, red galangal is a rhizomatous herb that can be used as a medicinal plant, in addition to its role as a spice that enhances the flavor and aroma of dishes(6). Its rhizome also contains the bioactive compound rutin which exhibit significant immunomodulator, antioxidant, antimicrobial, and anti-inflammatory activities (7). Despite its potential, the phytochemical profile of red galangal remains less extensively documented compared to other zingiberaceae members, underscoring the need for detailed metabolite analysis.

The potential and functional activity of food material can be initially assessed through metabolite composition analysis to determine the presence and abundance of tis bioactive compounds (8). Understanding the secondary metabolite composition of such combination is critical for evaluating its potential as a functional food ingredients or herbal formulations. Moreover, profiling the metabolites of the individual plants and their mixture may reveal unique chemical fingerprints that could guide future product development.

This study addressess the existing research gap by conducting untargeted metabolomic analysis of the individual powders and their combination using UHPLC-HRMS, followed by a comparative evaluation

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of their metabolite profiles. This approach also provides insights into the distinctive contributions of each plant material. The primary novelty of this study lies in the identification of complementary and additive phytochemical profiles, which helps to elucidate the rationale for combining the two materials within a formulation. In this context, the present study aims to identify the secondary metabolites present in powdered moringa leaves, red galangal rhizome and their combination. By employing analytical techniques to screen and characterize these compounds, we seek to provide a comprehensive overview of their phytochemical composition. The findings are expected to support the scientific basis for the traditional use of these plants and to inform future investigation on their biological activities and potential application in functional foods and natural therapeutics.

METHODS

Materials

The study used samples of dried moringa leaves from PT. Moringa Organik Indonesia, Blora Regency, Indonesia. The fresh red galangal rhizomes aged 10 months used in this study were obtained from Jalupang Mulya Village, Leuwidamar District, Lebak Regency, Banten Province. The solvent used for analysis, namely ethanol 96% food grade (Reg. No: 811997990, Calmo Medical), methanol (CAS. No: 67-56-1, Merck KGaA, Germany), formic acid (CAS-No: 64-18-6, Merck KGaA, Germany), acetonitrile (CAS-No: 75-05-8, Merck KGaA, Germany), and distilled water (H_2O) (CAS-No: 7732-18-5, Merck KGaA, Germany).

Preparation and extraction

The dried *Moringa oleifera* leaves were ground using a milling machine and sieved through a 60-mesh screen. For the red galangal rhizomes, the materials were cleaned, sliced, and dried for 6 hours at 70 °C using a cabinet dryer. The dried rhizome was then milled using a stainless-steel disc mill and sieved through a 60-mesh screen. Each plant material, namely moringa leaves and red galangal rhizomes, was weighed to 200 g. For the combination treatment, a mixture consisting of 85% moringa leaves and 15% red galangal rhizomes was prepared. Extraction was performed by the maceration method with slight modification(9). The powdered samples were placed into a glass container, after which food-grade ethanol 96% was added at a ratio of 1:7 (b/v) and allowed to stand for 3 × 24 h in successive stages. The first extraction was performed with a ratio of 1:3 (b/v). The plant residue was re-macerated with fresh solvent, followed by the second and third extractions with a ratio of 1:2 (b/v). The maceration was carried out in a closed vessel, protected from light, with occasional stirring throughout the process. After three days, the extract solution was filtered using Whatman No. 40 filter paper and a vacuum filtration apparatus. The filtrates obtained from each cycle were combined and evaporated to dryness using vacuum evaporator to obtain a thick ethanolic extract.

Metabolites profiling by UHPLC-HRMS

The qualitative analysis of secondary metabolite profiles was carried out using an UHPLC Vanquish system coupled with a Q Exactive Plus Orbitrap high-resolution mass spectrometer (Thermo Scientific) (10). For sample preparation, 5 mg of the paste extract was dissolved in 1 mL of methanol and subsequently filtered through a 0.2 µm nylon membrane. Chromatographic separation was performed on an Accucore C18 column (100 × 2.1 mm, 1.5 µm; Thermo Scientific) operated at a flow rate of 0.2 mL/min. The mobile phase consisted of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid, employing a gradient elution program of 0–1 min (5% B), 1–25 min (5–95% B), 25–28 min (95% B), and 28–33 min (5% B). The column temperature was maintained at 30 °C, and the injection volume was set at 2 µL. Mass spectrometric detection was performed in positive and negative ionization modes, with a mass range of 100–1500 m/z. The MS conditions were as follows: resolution 70,000, AGC target 3×10^6, and a maximum injection time of 100 ms.

Data Analysis

The metabolite identification results were analyzed using descriptive quantitative methods and subsequently summarized in tabulated form. The output consisted of chromatographic profiles

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accompanied by a detailed list of detected chemical constituents, including molecular structures, retention times, area sample, and measured m/z. Data processing was performed using online databases including ChemSpider and mzCloud to support compound identification and annotation. Metabolite profiling for each sample was conducted by determining both the total number of metabolites and their relative abundances. The metabolite abundance was calculated by summing the peak areas of all detected metabolites in each sample. The dominant metabolite was identified based on the highest percentage peak area. The 2D structural representations of the identified metabolites were obtained from the PubChem online database.

RESULTS

Table 1 presents the secondary metabolites identified in *Moringa oleifera* leaf powder. The majority of the identified compounds belong to the flavonoid class.

Table 1. Secondary metabolite profile of *Moringa oleifera* leaf powder

RT [min]	Proposed metabolites	Formula	Mol. Weight	Measured m/z	Area sample	Composition (%)	Category
26.67	Pheophorbide A	$C_{35}H_{36}N_4O_5$	592.27	593.27	5543933879.48	19.64	Tetrapyrolles
1.07	4-Aminobenzoic acid	$C_7H_7NO_2$	137.05	138.05	286224817.84	10.14	Benzenoid
9.38	Quercitrin	$C_{21}H_{20}O_{11}$	448.10	447.09	2846673780.92	10.08	Flavonoid
4.70	Chlorogenic acid	$C_{16}H_{18}O_9$	354.09	353.08	2511896298.41	8.90	Phenolic
9.12	Quercetin 3-O-malonylglucoside	$C_{24}H_{22}O_{15}$	550.10	549.08	2367906231.70	8.39	Flavonoid
8.78	Isoquercetin	$C_{21}H_{20}O_{12}$	464.10	463.08	1928376085.81	6.83	Flavonoid
1.09	1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid	$C_7H_{12}O_6$	192.06	191.05	151029116.15	5.35	Organic acid
9.85	Luteolin 7-O-malonylglucoside	$C_{24}H_{22}O_{14}$	534.10	533.09	1434630110.29	5.08	Flavonoid
4.88	Indoleacrylic acid	$C_{11}H_9NO_2$	187.06	188.07	1464339297.61	5.19	Alkaloid
29.61	Echinenone	$C_{40}H_{54}O$	550.41	551.42	817240605.26	2.90	Tetraterpenoid
8.54	Apigetrin	$C_{21}H_{20}O_{10}$	432.10	431.09	655685757.88	2.32	Flavonoid
8.47	Rutin	$C_{27}H_{30}O_{16}$	610.15	611.15	557364543.21	1.97	Flavonoid
12.41	(10E,15Z)-9,12,13-Trihydroxy-10,15-octadecadienoic acid	$C_{18}H_{32}O_5$	328.22	327.21	510917175.82	1.81	Fatty acils
27.73	(3R,4S,9R,11R)-29-(4-Hydroxyphenyl)-4-methyl-3,9,11-nonacosanetriol	$C_{36}H_{66}O_4$	562.50	563.50	485096417.61	1.72	Fatty acils
1.10	Benzaldehyde	C_7H_6O	106.04	107.04	466538203.64	1.65	Benzenoid
9.09	Zingerol	$C_{11}H_{16}O_3$	196.11	197.11	397269992.69	1.41	Benzenoid
2.35	Ethynylbenzene	C_8H_6	102.05	103.05	376791087.94	1.33	Benzenoid
4.70	4-hydroxycoumarin	$C_9H_6O_3$	162.03	163.03	375502804.65	1.33	Coumarin
29.34	24-methylenecholesterol	$C_{28}H_{46}O$	398.35	399.36	386418711.21	1.37	Steroid
18.69	13-KODE	$C_{18}H_{30}O_3$	294.22	293.21	368616892.52	1.31	Fatty acils
9.80	4-[7-(β-D-Glucopyranosyloxy)-4-oxo-4H-chromen-3-yl]phenyl β-D-glucopyranosiduronic acid	$C_{27}H_{28}O_{15}$	592.14	591.13	359946055.77	1.28	Isoflavonoid

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Table 2 shows the compounds detected in red galangal (*Alpinia purpurata*) rhizome powder. Phenolic compounds were identified as the dominant group, particularly those belonging to the benzenoid class.

Table 2. Secondary metabolite profile of red galangal (*Alpinia purpurata*) rhizome powder

RT [min]	Proposed metabolites	Formula	Mol. Weight	Measured m/z	Area Sample	Composition (%)	Category
15.39	Menadiol	$C_{11}H_{10}O_2$	174.07	175.07	1970990444.03	18.01	Benzenoid
9.80	Abietin	$C_{16}H_{22}O_8$	342.13	341.12	1914241406.87	17.49	Organic Acid
4.83	Sweroside	$C_{16}H_{22}O_9$	358.13	357.11	1413240559.33	12.91	Glycoside
15.34	Maraniol	$C_{12}H_{12}O_3$	204.08	205.08	753606944.03	6.88	Coumarin
6.99	Benzoic acid	$C_7H_6O_2$	122.04	121.02	708297496.25	6.47	Benzenoid
15.11	Hexahydrocurcumin	$C_{21}H_{26}O_6$	374.17	373.16	701324846.65	6.41	Curcuminoid
15.39	Styrene	C_8H_8	104.06	105.06	502513930.16	4.59	Benzenoid
15.34	Safrole	$C_{10}H_{10}O_2$	162.07	163.07	489843769.00	4.47	Benzenoid
15.00	Hernanol	$C_{22}H_{26}O_7$	402.17	401.16	428372139.17	3.91	Seskuiterpenoid
1.08	4-Aminobenzoic acid	$C_7H_7NO_2$	137.05	138.05	371442001.69	3.39	Benzenoid
16.03	Cinnamyl alcohol	$C_9H_{10}O$	134.07	135.08	326084588.03	2.98	Phenylpropanoid
1.11	Pyrogallol	$C_6H_6O_3$	126.03	127.03	316050729.55	2.89	Benzenoid
11.75	Trans-Cinnamic acid	$C_9H_8O_2$	148.05	147.04	305700156.78	2.79	Phenolic
9.27	Cinnamaldehyde	C_9H_8O	132.06	133.06	295981859.45	2.70	Coumarin
15.35	Ethynylbenzene	C_8H_6	102.05	103.05	231995109.00	2.12	Benzenoid
1.10	Emiglitate	$C_{17}H_{25}NO_7$	355.16	356.16	216632960.99	1.98	Benzenoid

Table 3. Secondary metabolite profile of the combined *Moringa oleifera* leaf and red galangal (*Alpinia purpurata*) rhizome powders

RT [min]	Proposed metabolites	Formula	Mol. Weight	Measured m/z	Area sample	Composition (%)	Category
15.39	Cinnamaldehyde	C_9H_8O	132.06	133.06	3832161568.97	15.73	Coumarin
26.67	Pheophorbide A	$C_{35}H_{36}N_4O_5$	592.27	593.27	3431970313.83	14.09	Tetrapyrolles
1.08	4-Aminobenzoic acid	$C_7H_7NO_2$	137.05	138.05	2448240641.60	10.05	Benzenoid
4.81	Chlorogenic acid	$C_{16}H_{18}O_9$	354.09	353.08	2373635039.01	9.74	Phenolic
9.13	Quercetin 3-O-malonylglucoside	$C_{24}H_{22}O_{15}$	550.09	549.08	1922368823.73	7.89	Flavonoid
9.85	Luteolin 7-O-malonylglucoside	$C_{24}H_{22}O_{14}$	534.10	533.09	1777504638.59	7.30	Flavonoid
1.08	1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid	$C_7H_{12}O_6$	192.06	191.05	1732273738.90	7.11	Organic acid
9.38	Quercitrin	$C_{21}H_{20}O_{11}$	448.10	447.09	1547172223.72	6.35	Flavonoid
8.78	Isoquercetin	$C_{21}H_{20}O_{12}$	464.10	463.08	1418475020.58	5.82	Flavonoid
4.98	Indoleacrylic acid	$C_{11}H_9NO_2$	187.06	188.07	865972813.54	3.55	Alkaloid
8.54	Apigetrin	$C_{21}H_{20}O_{10}$	432.10	431.09	679892066.18	2.79	Flavonoid
8.48	Rutin	$C_{27}H_{30}O_{16}$	610.15	611.15	675425801.00	2.77	Flavonoid

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RT [min]	Proposed metabolites	Formula	Mol. Weight	Measured m/z	Area sample	Composition (%)	Category
15.40	Menadiol	$C_{11}H_{10}O_2$	174.07	157.07	377516991.07	1.55	Benzenoid
1.09	Benzaldehyde	C_7H_6O	106.04	107.04	366220485.23	1.50	Benzenoid
4.82	4-hydroxycoumarin	$C_9H_6O_3$	162.03	163.03	346793550.15	1.42	Coumarin
7.17	Nictoflorin	$C_{27}H_{30}O_{15}$	594.16	593.15	286391058.75	1.18	Flavonoid
12.41	(10E,15Z)-9,12,13-Trihydroxy-10,15-octadecadienoic acid	$C_{18}H_{32}O_5$	328.22	327.21	284005610.61	1.17	Fatty acils

The venn diagram (Figure 1) shows the distribution of metabolites identified in the individual samples of moringa leaves, red galangal rhizomes, and their physical combined mixture. The diagram highlights both the unique metabolites present exclusively in each sample and the metabolites shared across the three sample groups. The visualization clarifies the compositional differences and overlaps among the samples, providing insight into the distinct and complementary metabolic contributions of each plant material.

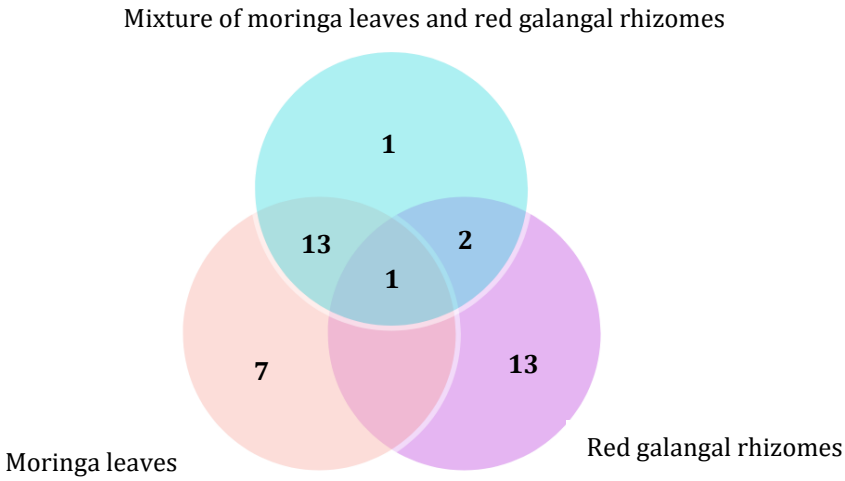

Figure 1 Venn diagram representing metabolites between moringa leaves, red galangal rhizomes, and their physical mixture

Figure 2 presents the distribution of metabolite abundance across three samples. The chart illustrate the relative proportions of major metabolite classes identified in each sample, highlighting both similarities and distincit compositional patterns among the three groups. This comparative visualization provides a clearer understanding of how each plant material contributes to spesific metabolite categories such as benzenoids, phenolic acids, flavonoids, alkaloids, organic acids, fatty acils, terpenoids and how these components are redistributed when the two materials combined. The observed distribution patterns offer important insights into the complementary phytochemical profiles of the individual plants and their mixture.

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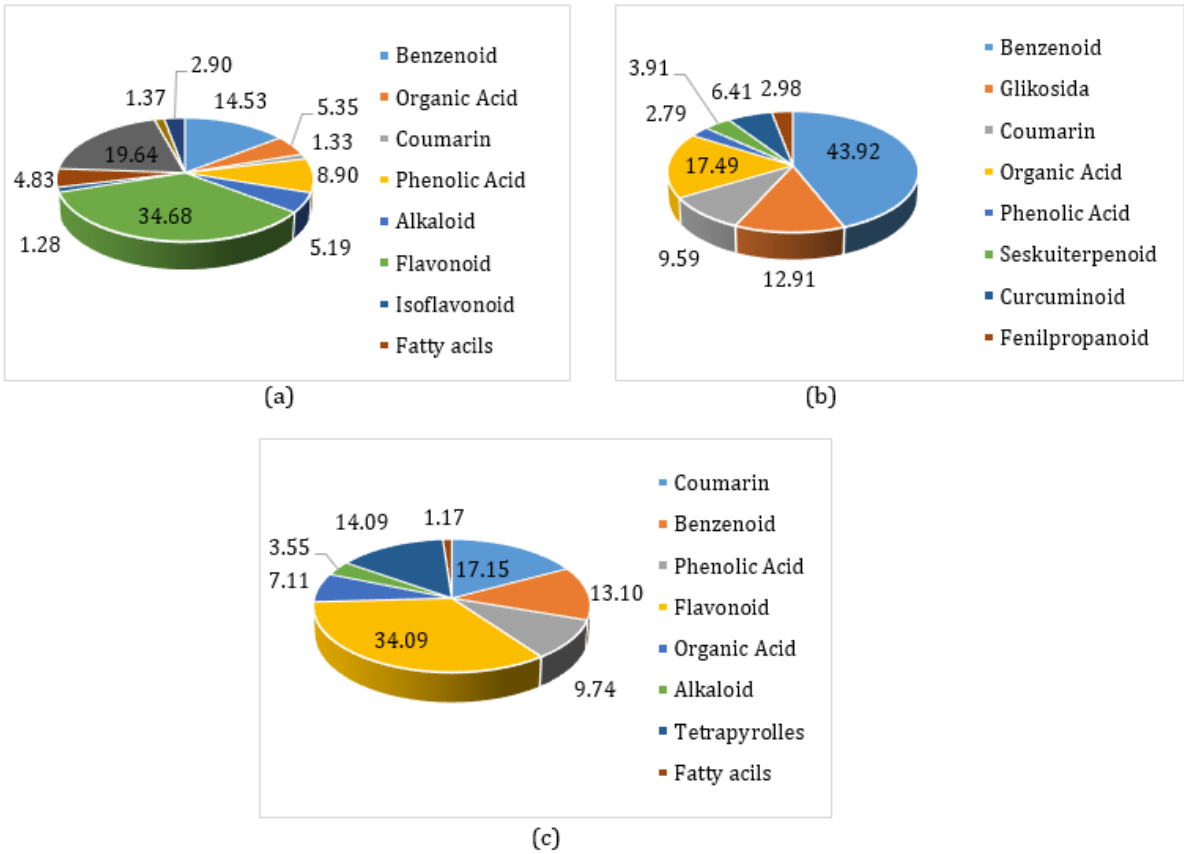
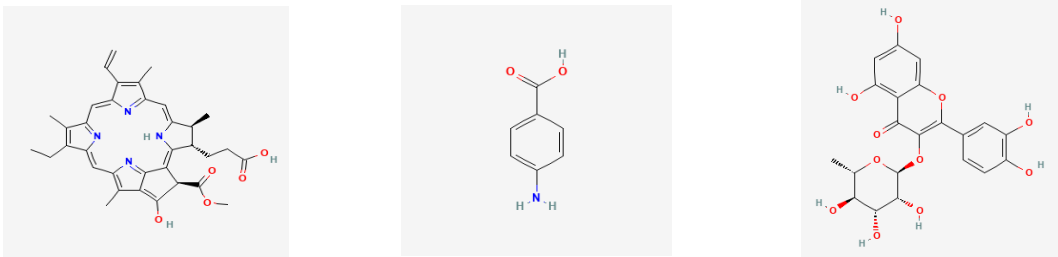


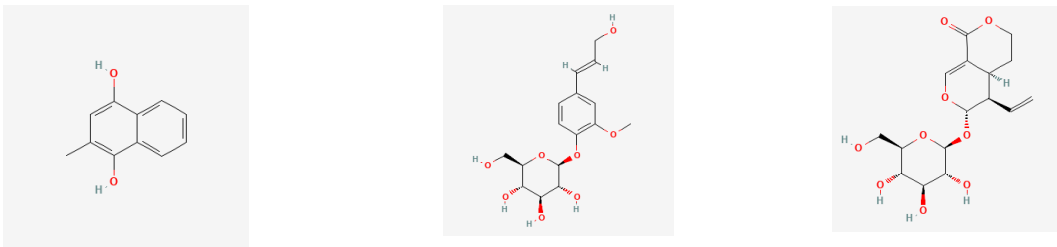
Figure 2 Distribution of metabolite abundance in moringa leaves (a), red galangal rhizomes (b), physical mixture of moringa leaves and red galangal rhizomes (c)

The phytoconstituents and their chemical structures with the highest relative abundance (%) are presented in the figure 3, 4, 5.



Pheophorbide A 4-Aminobenzoic Acid *Quercitrin*

Figure 3. Chemical structure of top 3 secondary metabolite in *Moringa oleifera* leaves powder



Menadiol Abietin Sweroside

Figure 4. Chemical structure of top 3 secondary metabolite in red galangal rhizome powder

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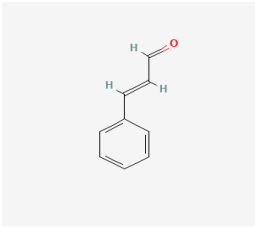
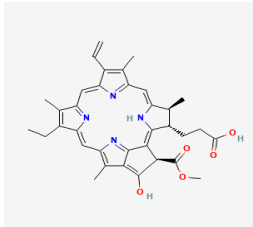
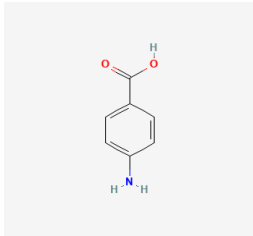

Cinnamaldehyde *Pheophorbide A* 4-Aminobenzoic Acid

Figure 5. Chemical structure of top 3 secondary metabolites in the combined *Moringa oleifera* leaf and red galangal rhizome powder

DISCUSSION

Secondary metabolites are small organic compounds derived from primary metabolic processes in plants and their composition differ among plant species. These metabolites are formed as a result of biosynthetic modifications, notably including methylation, glycosylation, and hydroxylation(11). The major classes of secondary metabolites based on their chemical structures consist of phenolic, terpenoid, and nitrogen-containing compounds(12). The metabolite identification performed on each individual plant or fruit component demonstrates that each possesses a distinct metabolite profile, thereby allowing these components to complement one another when combined (13). Their utilization of secondary metabolites have been widely applied in the pharmaceutical and food sectors due to their biological activities(11).

The study revealed a broad spectrum of secondary metabolites within powdered samples of moringa leaves, red galangal and their combination. In total, 21 compound were identified In the moringa leaf powder, 17 in the red galangal rhizome powder, and 16 in the mixture of the two. These findings demonstrate the phytochemical diversity in each plant as well as in their blend, indicating that both individual source contribute distinct sets of bioactive constituents. The slightly reduced number of metabolites observed in the combined sample, compared with the individual plant powders, may be attributed to overlapping chemical profiles or potential interactions between the plant matrices during processing that could influence the detectability of certain compounds.

The reduction in metabolite abundance in the combination of plant materials may occur as early as the extraction process. Extraction duration is often associated with increased temperature, as prolonged extraction can lead to the degradation of active compounds. Elevated temperatures may disrupt plant tissue structures, thereby facilitating the release of bioactive constituents and enhancing mass transfer. However, high temperatures and extended extraction times may likewise promote the degradation of key metabolites(14). This condition is relevant to the conventional extraction method employed in this study, namely maceration for 3x24 hours, which may result in incomplete extraction (15). Furthermore, the reduction in the diversity of detected metabolites, particularly flavonoid glycosides and their aglycones may be attributed to enzymatic hydrolysis that cleaves glycosidic bonds. This process generates a broader range of metabolites with lower molecular weight (16). Such transformations can influence metabolite consentrations within the extract, whereby compounds present at low concentration are often undetectable through untargeted metabolomics based on LC-MS analysis (17). Fewer metabolites were detected in red galangal compared to the single sample of *Moringa oleifera* and their combination. This difference attributable to the metabolite profiling method employed in this study. The rhizomes of red galangal are known to contain essential oils and volatile non-polar compounds (18). Volatile compounds typically possess low molecular weights (19). Therefore, such low-molecular-weight volatile constituents are more suitably analyzed using Gas Chromatography-Mass Cpectrometry (GC-MS) (20),(21).

The analysis revealed that 4-aminobenzoic acid, also known as para-aminobenzoic acid (PABA) was detected in all three samples. This compound possessess an aromatic structure consisting of a benzene ring with an amino group and a carboxylic acid group. It has been reported to exhibit antioxidant and anti-inflammatory activities, as well as potential roles in the modulation of neurotransmitters (22). Pheophorbide A was also detected in high abundance in both the single moringa leaf powder and the

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combined formulation. This compound has been reported to potentially enhance glucose uptake in β-cells, thereby contributing to the maintenance of glucose homeostasis (23). Flavonoids were identified as the predominant class of secondary metabolites in moringa leaf powder with key constituents including apigetrin, isoquercetin, luteolin 7-O-malonylglucoside, quercetin 3-O-malonylglucoside, quercitrin, and rutin. This finding agrees withs earlier studies reporting that moringa leaves extracted with ethanol, including quinic acid, gallic acid, protocatechuic acid, caffeic acid, *p*-coumaric acid, trans-ferulic acid, quercetin-3-*O*-galactoside, quercetin-3-*O*-rhamnoside, naringin, trans-cinnamic acid, quercetin, naringenin, apigenin, luteolin, and cirsiliol (24).

The detected comoounds in red galangal rhizomes were predominantly classified as benzenoid, including menadiol, benzoic acid, styrene, safrole, 4-aminobenzoic acid, pyrogallol, ethynylbenzene, and emiglitate. Pyrogallol also known as 1,2,3-trihydroxybenzene which is part of polyphenolic molecule may contribute to mitigating conditions linked to oxidative stress (25). Phenolic compounds were also found in greater abundance in the powdered red galangal rhizome formulations and its combination with the moringa leaves. The benzenoid and phenolic profile revealed the presence of compound such as cinnamaldehyde, trans-cinnamic acid, sweroside, safrole, pyrogallol, abietin, maraniol, hexahydrocurcumin, menadiol, styrene, emiglitate. Previous study showed that metabolite analysis using the GC–MS method revealed that red galangal (*Alpinia purpurata*) contains 1,8-cineole, bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-(1S), β-pinene, 3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- (CAS), and cis-ocimene (26). Essential oil extract of this plant has been extensively investigated in previous studies using the GC–MS method. Several constituents identified in this essential oil include α-pinene, β-pinene, trans-caryophyllene, camphene, limonene, bornyl acetate, and β-selinene(27). Other major components found in red galangal rhizome include 4-acetoxy-3-methoxystene, 7,7-dimethyl-8-methyleneoctahydro-1H-3a,6-methanoazulene-3-carboxylic acid, 3-allylguaiacol, cis-13-octadecenal, and palmitic acid (28). Their pronounces presence in red galangal highlights its potential as an important natural source of phenolic antioxidants (29).

The combined preparation exhibited a metabolite composition that reflects a complementary chemical profile with flavonoids from moringa. Their persistence in the combined preparations indicates that the bioactive potential of moringa remains significant even when mixed with red galangal. One notable compound found to be elevated in the combined preparation is nictoflorin, which is likewise classified as a flavonoid. This compound possesses anti-inflammatory properties that can suppress the NF-κB signaling pathway in the treatment of ulcerative colitis (30). The detection of secondary metabolites in plant materials is strongly influenced by the solvent employed. Since MS analysis is selective toward the polarity and volatility of analytes, solvent selection is crucial. Polar metabolites are more effectively extracted with hydrophilic solvents, such as water–alcohol mixtures, whereas non-polar metabolites require hydrophobic solvents (31).

The identification of secondary metabolites, particularly phenolic content can support the evaluation of plant's antioxidant properties, which may be further developed into a product (32). One nutraceutical formulation, the galohgor nutraceutical formula has been utilized as a health supplement and antidiabtic agent. Although its antioxidant activity is relatively weak based on in vitro studies, in vivo studies have shown that this formulation is capable od reducing MDA levels(33). Other studies have reported that phenolic compounds such quercetin and gallic acid in kombucha telang flower may contribute to its antioxidant potential and resulting in a strong antioxidanat category (34). Variations in bioactive compounds including flavonoid, ohenolic acids, anthocyanins, and tannins have an important role in modulating antioxidant interactions(35).

This study has several limitations that should be acknowledged. First, the absence of quantitative analysis restricts the interpretation of metabolite abundance as the results only describe the presence of compounds rather than their actual concentrations. Second, potential matrix suppression effectsmay have reduced ionization efficiency and contributed to understimation or non-detection of certain metabolites. Third, the use of a single extraction solvent may not have been sufficient to comprehensively extract metabolites with diverse polarity levels. Future research should consider integrating quantitative or semi-quantitative approaches, such as targeted LC-MS/MS to provide more precise information on metabolite

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concentration. The use of multiple extraction solvents is recommended to enhance metabolite coverage across different polarity classes. Subsequent studies should evaluate the biological relevance of metabolite changes, including potential synergistic or antagonistic effects, to provide deeper insight into the functional implications of plant combinations.

CONCLUSION

The contrasting dominance of flavonoids in moringa and phenolics in red galangal underscores the distinct phytochemical signatures of two species. The powdered *Moringa oleifera* leaves contained 21 secondary metabolites, whereas the red galangal (*Alpinia purpurata*) rhizome powder contained 16. The combination of the two materials yielded 17 secondary metabolites. In moringa leaf powder, flavonoids were the predominant class with quercitrin, 4-aminobenzoid acids, and pheophorbide A. identified as the three most abundant compounds. In contrast, red galangal powder was characterized by a predominance of compounds with menadiol, abietin, and sweroside as the major constituents. The combined preparation contained key metabolites such as cinnamaldehyde, 4-aminobenzoic acid, and pheophorbide A. These complementary metabolite profiles support both the traditional and emerging applications of these plants in herbal medicine and functional food formulation. To substantiate these findings, future reseatch should investigate how interaction these compounds influence bioavailability and biological efficacy. It will be essential for validating the health-promoting suggested by the current phytochemical analysis and for guiding the development of optimized plant-based products.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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