

Comparative Phytochemical Profiling (GC-MS and HPLC) and Evaluation of Antioxidant Activities of Wild, *In Vitro* Cultured and Greenhouse Plants of *Kaempferia grandifolia* Saensouk and Jenjitt and *Kaempferia siamensis* Sirirugsa; Rare Plant Species in Thailand

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Abstract

Background: *Kaempferia grandifolia* and *Kaempferia siamensis* are rare plant species in Thailand used for medicinal, food, and ornamental purposes.

Objectives: To compare the phytochemical profiles of leaf and rhizome extracts from wild, *in vitro* culture, and greenhouse plants of *K. grandifolia* and *K. siamensis*.

Materials and Methods: Leaf and rhizome were extracted and analyzed phytochemically by high-performance liquid chromatography (HPLC), gas chromatography mass spectrometry (GC-MS), total phenolic contents (TPC), and total flavonoid contents (TFC). Antioxidant activities were examined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) assays.

Results: The results of HPLC analysis showed that the main compounds found in all plants were p-coumaric acid, ferulic acid, and quercetin. GC-MS results revealed 38 compounds in *K. grandifolia* and 19 compounds in *K. siamensis*. Major constituents in the leaf and rhizome extracts were 9-octadecenamide, (Z)-, hexadecanamide, and octadecanamide. Total phenolic and total flavonoid contents of leaf extracts decreased from wild to *in vitro* cultured and greenhouse plants, respectively, whereas rhizome extract contents increased from wild to greenhouse plants. Antioxidant activities of rhizome extracts showed higher activity than leaf extracts in DPPH and ABTS assays.

Conclusion: The present research is the first report to study the phytochemical profiles and antioxidant activities of these plants. Rhizome extracts of *K. grandifolia* and *K. siamensis* in greenhouse plants presented the highest phenolic and high free radical scavenging activity compared to rhizome extracts of wild plant. The phytochemical profiles of the compounds found in the *K. grandifolia* and *K. siamensis* extracts showed that these plants have potential for future health benefits.

Keywords

K. grandifolia, *K. siamensis*, phytochemical profiles, HPLC, GC-MS

Introduction

Kaempferia grandifolia Saensouk and Jenjitt and *Kaempferia siamensis* Sirirugsa (Zingiberaceae) are both found in Thailand as rare endemic plant species.¹ The genus *Kaempferia* contains cultivated ornamental, food, and medicinal plants,^{2–4} and belongs to the family Zingiberaceae with 60 species found in Thailand. Several *Kaempferia* species are widely used in traditional medicine. Young leaves

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of *K. siamensis* are used as food and utilized as ornamental plants,⁴ while leaves blended with rhizomes of *K. grandifolia* and husked rice are used to treat herpes. Furthermore, the young inflorescence is eaten as food.⁵ The phytochemical study of *Kaempferia* plants revealed the presence of phenolic glycosides, flavonoids, monoterpenoids, diterpenoids, cyclohexane oxide derivatives, and essential oils with various biological activity, antimicrobial, anti-inflammatory, and antioxidant properties.⁶ Therefore, developing tissue culture techniques for mass micropropagation of *Kaempferia* sp. would be useful for the conservation of these medicinal plants. Evaluating the potential biological activities of extracts from tissue-cultured plants would be beneficial as an alternative to natural plants. However, there is no data on the phytochemical content of these plants. Thus, this study investigated the phytochemical differences between samples of wild, *in vitro* cultured, and greenhouse plants. Phytochemical constituents were analyzed by HPLC and GC-MS, while the antioxidant activities of ethanol extracts of *K. grandifolia* and *K. siamensis* plants were compared. Results will be helpful for value addition of rare and endemic plant species in Thailand.

Materials and Methods

Chemicals

Chemicals were obtained as follows: gallic acid (Sigma-Aldrich), sodium carbonate (Na_2CO_3), Folin-Ciocalteu phenol reagent (Sigma-Aldrich), methanol AR grade (Merck), ethanol AR grade (Merck), quercetin (Sigma-Aldrich), aluminum chloride, potassium acetate (Sigma-Aldrich), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich), ascorbic acid (Sigma Aldrich), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Sigma-Aldrich), dipotassium peroxodisulfate ($\text{K}_2\text{S}_2\text{O}_8$) (Sigma-Aldrich), and DMSO (Dimethylsulfoxide) (Sigma-Aldrich). Solvents and reagents are used in HPLC analysis, including acetonitrile and acetic acid (Merck). Phenolic and flavonoid standards, such as quercetin, catechin, gallic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, rutin, and kaempferol (Sigma-Aldrich).

Plant Material

Leaves and rhizomes of *Kaempferia grandifolia* and *K. siamensis*; wild, *in vitro* cultured and greenhouse plants (Figure 1). Wild plants were collected from the Thai National AGRIS Center at Khok Phu Ta Ka, Amphoe Phu Wiang, Khon Kaen Province and Phu Phan National Park, Sakon Nakhon Province. *In vitro* cultured from shoot explants (wild plant) derived from aseptic culture on MS medium without plant growth regulators (PGR) with 30 g/l of sucrose. The medium was adjusted to pH 5.7. Agar (7 g/l) was used to promote shoot induction at eight weeks of culture. Greenhouse

plants from micro shoots on solid MS medium supplemented with 4 mg/l 6-benzylaminopurine (BAP) plus 1 mg/l α -naphthaleneacetic acid (NAA). After eight weeks of culture, the plantlets were transplanted into plastic pots with soil sand (1:1 v/v). The potted plants were maintained for 1 year while growing under greenhouse conditions at Mahasarakham University's Department of Biology, Faculty of Science, Thailand (Figure 1).

Plant Preparation and Extraction

Leaves and rhizomes were obtained from three types of plants: wild, *in vitro* cultured, and greenhouse plants. Plant parts were dried in a hot air oven at 60°C for 24 hour⁷ and then ground to a fine powder. Five grams of plant parts in powder form were extracted by a soxhlet extraction apparatus with 95% ethanol for 4 hour. The extracts were evaporated and stored in dark containers at room temperature until required.

Using the HPLC method and liquid-liquid extraction, the ethanol extract was separated into ethyl acetate and dichloromethane fractions. A vacuum evaporator was used to evaporate each of the extracts and fractions.

Total Phenolic Content Determination

The folin-ciocalteu colorimetric method was modified from Pothitirat et al.⁸ to determine the total phenolic content of the samples. Gallic acid was used as the standard (concentration of 15.625–250 μg). The crude extracts were dissolved in 50% DMSO in methanol. Samples of 200 μl (1 mg/ml) were added to 500 μl of folin-ciocalteu reagent and left for 5 min at room temperature. Then, 800 μl of Na_2CO_3 was added. The solutions were mixed in volumes of 1,500 μl and stored at room temperature for 5 min in the dark. Samples were measured as three replicates for absorbance at 765 nm wavelength. Total phenolic contents were measured by comparison of standard curves. The values were expressed as mg gallic acid equivalent (GAE)/g of extract sample.

Total Flavonoid Content Determination

Total flavonoid contents were determined following the modified method of Pothitirat et al.⁸ Quercetin at a concentration of 15.625–250 $\mu\text{g/ml}$ was used as the standard. Samples of 500 μl (1 mg/ml) were added to 1,500 μl of 95% ethanol and incubated at room temperature for 6 min. Then, 100 μl of 10% aluminum chloride was added to the solutions and mixed. After 5 min, 100 μl of 1 M potassium acetate was added to the solutions and mixed. With distilled water, the volume was adjusted to 5,000 μl and left at room temperature for 30 min in the dark. Samples were measured as three replicates for absorbance at 415 nm. Total flavonoid



Figure 1. Rhizomes and Leaves of *K. grandifolia* (A–C), (A) Wild Plant, (B) Greenhouse Plant, and (C) *In Vitro* Culture; Rhizomes and Leaves of *K. siamensis* (D–F), (D) Wild Plant, (E) Greenhouse Plant, and (F) *In Vitro* Culture.

contents were measured by comparison with standard curves. The values were expressed as mg quercetin equivalent (QE)/g of extract sample.

DPPH Assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was investigated following the method described by Likhitwitayawuid et al.⁹ Ascorbic acid was used as the standard. The DPPH solution was prepared in methanol at a concentration of 100 μ M. Twenty microliters of extracts (1 mg/ml) were added in 180 μ l DPPH solution. The solutions were incubated in a dark condition at room temperature. After 30 min of preparation, absorbance by microplate reader (ASYS UVM 340) was measured at 517 nm. Methanol was used blank (Ac). Extracts were measured as three replicates. The calculation of percentage inhibition was performed by the following equation;

$$\text{Percentage Radical Scavenging} = \frac{[(Ac - As)/Ac]}{100} \times 100 \text{ ABTS Assay}$$

ABTS assay was investigated following the modified method of Payet et al.¹⁰ The reaction between 7 mM ABTS and 2.45 mM

potassium persulfate produced the ABTS⁺ cation radical, which was incubated in the dark at room temperature for 12–16 hours before use. The ABTS solution was diluted with ethanol to obtain an absorbance of 0.700 at 734 nm. Extracts were measured in three replicates. The absorbance of extracts (As), methanol was used as a blank (Ac), and ascorbic acid is used as a standard. The calculation of percentage inhibition was performed by the following equation:

$$\text{Percentage radical scavenging} = \frac{[(Ac - As) / Ac] \times 100.}$$

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Leaf and rhizome extracts were performed by GC-MS adapted from Suphrom et al.¹¹ The injector was set at 250°C and carried out in split mode with a 10:1 split ratio (in 1 μ l). Hewlett Packard HP-5 (5% phenyl methyl siloxane) uses a fused silica capillary column (30 m $\times$ 0.25 mm i.d., 0.25 m film thickness) with helium as the carrier gas at a constant flow rate of 1 ml/min. The GC-MS analysis was detected using GC-MS-QP 2010 Ultra (Shimadzu). The initial oven temperature was 70°C, which was kept for 3 min before being ramped up to 280°C at a rate of 5°C/min and eventually held

Table 1. Total Phenolic Contents, Total Flavonoid Contents, and Free Radical Scavenging Activity (DPPH and ABTS) of *K. grandifolia* and *K. siamensis* Extracts by Ethanol Solvent.

Species	Explant		Total phenolics (mg GAE/g extracts)	Total flavonoids (mg QE/g extracts)	DPPH (%Inhibition)	ABTS (%Inhibition)
<i>K. grandifolia</i>	Wild	Leaves	170.24 ± 8.06 ^a	70.24 ± 0.00 ^a	29.98 ± 0.33 ^b	17.18 ± 1.11 ^{ab}
		Rhizomes	164.38 ± 5.70 ^a	22.91 ± 1.67 ^d	24.77 ± 0.74 ^d	13.09 ± 1.19 ^{ab}
	Greenhouse	Leaves	169.84 ± 5.72 ^a	61.73 ± 0.21 ^b	27.11 ± 0.11 ^c	15.22 ± 3.24 ^{ab}
		Rhizomes	177.65 ± 6.99 ^a	24.88 ± 1.35 ^d	38.10 ± 0.18 ^a	18.48 ± 0.29 ^a
	<i>In vitro</i> culture	Leaves	70.42 ± 5.16 ^b	55.66 ± 0.68 ^c	20.05 ± 0.30 ^e	12.19 ± 1.19 ^b
		Rhizomes	177.65 ± 6.99 ^a	24.88 ± 1.35 ^d	38.10 ± 0.18 ^a	18.48 ± 0.29 ^a
<i>K. siamensis</i>	Wild	Leaves	233.51 ± 1.99 ^a	137.35 ± 8.11 ^a	28.08 ± 1.31 ^a	25.52 ± 0.43 ^a
		Rhizomes	183.00 ± 2.69 ^b	41.28 ± 2.16 ^d	21.52 ± 0.22 ^b	16.00 ± 0.77 ^b
	Greenhouse	Leaves	180.07 ± 1.34 ^b	109.22 ± 4.36 ^b	21.20 ± 1.02 ^b	11.57 ± 1.08 ^c
		Rhizomes	187.59 ± 4.63 ^b	73.20 ± 3.93 ^c	27.79 ± 0.29 ^a	17.49 ± 0.57 ^b
	<i>In vitro</i> culture	Leaves	100.42 ± 4.38 ^c	65.52 ± 1.36 ^c	20.79 ± 0.38 ^b	9.27 ± 0.26 ^d
		Rhizomes	187.59 ± 4.63 ^b	73.20 ± 3.93 ^c	27.79 ± 0.29 ^a	17.49 ± 0.57 ^b

Note: Values are expressed as mean ± SD (n = 3). Different superscripts in the same column indicate significant differences (p < .05). Means with no significant difference are marked with the same letters (a, b, c, d, e) (p > 0.05).

Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt.

for 10 min. The transfer line heater was adjusted to a temperature of 280°C. In a complete scan, the mass scanning range was set to 50–550 amu.

HPLC Determination of Phenolic Acids and Flavonoids

Extracts were analyzed by the procedures of Siriamornpun and Kaewseejan.¹² HPLC analysis was performed using chromatographic conditions as follows: column Inertsil ODS-3, C₁₈ (4.6 mm × 250 mm, 5 µm); ingredient elution acetic acid pH 2.74 (solvent A) – acetonitrile (solvent B); and flow rate of 0.8 ml/min. The operating conditions were as follows: column temperature of 38°C, 20 µl injections of sample solution, and UV-diode array detection at 280 nm for phenolic acids and 370 nm for flavonoids. Peak identification of phenolic and flavonoid compounds in the samples was done by comparing their relative retention times (RT) and UV-spectra to those of authentic compounds and was done using external standard procedures. Quercetin, catechin, gallic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, rutin, and kaempferol were used as standards for analysis (Figure 4).

Statistical Analysis

All values were expressed as the mean ± S.D (obtained from three determinations). Pearson's correlation coefficient (r) was calculated using Microsoft Excel 2021. Difference among means was analyzed by one-way ANOVA with significance at p < .05.

Results

Total Phenolic Contents

Total phenolic contents of *K. grandifolia* ranged from 70.42 to 177.65 mg GAE/g of extract. Total phenolic contents of

wild, greenhouse, and *in vitro* culture ranged from 164.38 to 170.24, 169.84 to 177.65, and 70.42 mg GAE/g extracts, respectively. The total phenolic contents of *K. siamensis* ranged from 100.42 to 233.51 mg GAE/g extracts. Total phenolic contents of wild, greenhouse and *in vitro* culture were range (183.00–233.51, 180.07–187.59, and 100.42 mg GAE/g extracts, respectively) (Table 1). Leaf extract of *K. siamensis* wild had the highest total phenolic contents, with 233.51 mg GAE/g extracts. Results showed that phenolic contents of rhizome extracts increased from wild to greenhouse plants.

Total Flavonoid Contents

Total flavonoid contents were calculated from the standard curve of quercetin. Total flavonoid contents of *K. grandifolia* ranged from 22.91 to 70.24 mg QE/g of extracts. Total flavonoid contents of wild, greenhouse, and *in vitro* culture ranged from 22.91 to 70.24, 24.88 to 61.73, and 55.66 mg QE/g extracts, respectively. The total flavonoid contents of *K. siamensis* ranged from 41.28 to 137.35 mg QE/g extracts (Table 1). The total flavonoid contents of wild, greenhouse, and *in vitro* culture ranged from 41.28 to 137.35, 73.20 to 109.22, and 65.52 mg QE/g extracts, respectively). The highest total flavonoid contents were found in leaf extracts from wild plants of *K. siamensis* (137.35 ± 8.11 mg QE/g extracts). Results showed that flavonoid contents of leaf extracts decreased from wild to *in vitro* cultured and greenhouse, respectively.

DPPH and ABTS Assays

The DPPH results indicated that the rhizome extracts from greenhouses of *K. grandifolia* had the highest percentage of radical scavenging with 38.10%, whereas leaf extracts from *in vitro* cultured showed the lowest percentage radical scavenging compared to the other extracts. For the

Table 2. Phytochemicals Detected in Ethanol Extracts of Leaves and Rhizomes from *K. grandifolia*.

No.	Name of the compound	RT	Peak area %				
			Leaves			Rhizome	
			Wild	Greenhouse	In vitro	Wild	Greenhouse
1	Neophytadiene	28.169	0.40	0.39	0.19	—	—
2	Phenanthrene, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,1,4a,7-tetra methyl-, [4aS-(4a α ,4b β ,7 β ,10a β)]-	31.103	—	—	—	0.33	0.33
3	Hexadecane	31.425	—	—	—	0.10	—
4	Hexadecanoic acid, ethyl ester	31.450	0.38	—	—	—	—
5	Kaur-16-ene	31.703	—	—	—	0.08	0.13
6	Retinal, 9-cis-	33.504	—	—	—	0.08	—
7	2,4',6-Trimethyl-1,1'-biphenyl	33.650	—	—	—	0.06	—
8	Neophytadiene	33.726	—	—	0.08	—	—
9	Phytol	33.739	0.55	0.19	—	—	—
10	Androst-5-en-17-one,3-(acetyloxy)-19-hydroxy-, (3b)-	34.067	—	—	—	0.48	—
11	Bicyclo[4.4.0]dec-5-ene, 1,5-dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)	34.162	—	—	—	—	0.15
12	(1R,4aR,4bS,7R,10aR)-1,4a,7-Trimethyl-7-vinyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodeca-hydrophenanthrene-1-carbaldehyde	34.340	—	—	—	2.61	—
13	1-Naphthalenepropanol, α -ethyldecahydro- α ,5,5,8a-tetramethyl-2-methylene, [1S[1 α (R*),4a β ,8a α]]- ^a	34.346	—	—	—	—	2.46
14	(E)-9-Octadecenoic acid ethyl ester	34.738	0.17	—	—	—	—
15	Hexadecanamide	35.110	2.78	8.25	7.72	5.25	6.15
16	Pregnane-3,17,20-triol, (3 α ,5 β ,20S)-	35.552	—	—	—	0.50	0.81
17	Geranyl ethyl ether	35.617	—	—	0.25	—	—
18	Bicyclo[9.3.1]pentadeca-3,7-dien-12-ol, 4,8,12,15,15-pentamethyl-, [1R-(1R*,3E,7E,11R*,12R*)]-	35.934	—	—	—	0.18	0.19
19	5-Hydroxymethyl-1,1,4a-trimethyl-6-methylenedecahydronaphthalen-2-ol	36.098	—	—	—	4.50	6.49
20	Podocarp-7-en-3 β -ol, 13 β -methyl-13-vinyl-	36.697	—	—	—	1.37	1.92
21	Retinol acetate	37.211	—	—	—	0.54	—
22	Pregnane-3,11,17,20,21-pentol, cyclic 17,21-[(1,1-dimethylethyl) boronate	37.402	—	—	—	0.50	—
23	Tetracyclo[16.1.0.0(2,9).0(10,17)]nonadeca-2(9),10(17)-diene, 19,19-diphenyl-	37.519	—	—	—	0.70	—
24	Pentacyclo[9.1.0.0(2,4).0(5,7).0(8,10)]dodecane, 3,3,6,6,9,9,12,12-octamethoxy-	37.706	—	—	—	0.23	—
25	Abietic acid	37.841	—	—	—	0.42	—
26	9-Octadecenamide, (Z)-	38.281	53.68	88.06	88.00	71.49	71.43
27	Octadecanamide	38.686	8.94	3.10	3.04	1.69	1.99
28	Androstane-3,17-diol, 17-methyl-, (3 α ,5 α ,17 β) -	38.921	—	—	—	5.88	6.58
29	Bicyclo[9.3.1]pentadeca-3,7-dien-12-ol,4,8,12,15,15-pentamethyl-, [1R(1R*,3E,7E,11R*,12R*)]-	39.981	—	—	—	0.74	—
30	1-Naphthalenepentanol, decahydro-5-(hydroxymethyl)-5,8a-dimethyl- γ ,2-bis(methylene)-(1 α ,4a β ,5 α ,8a α)	39.982	—	—	—	—	0.96

(Table 2 continued)

(Table 2 continued)

No.	Name of the compound	RT	Peak area %			
			Leaves			Rhizome
			Wild	Greenhouse	<i>In vitro</i>	Wild Greenhouse
31	Bis(2-ethylhexyl) phthalate	41.261	—	—	0.21	— —
32	Diisooctyl phthalate	41.268	0.09	—	—	0.07 0.06
33	24-Noroleana-3,12-diene	42.033	0.74	—	—	— —
34	Nonanediamide,N,N'-di-benzoyloxy-	43.436	—	—	—	— 0.16
35	Lup-20(29)-en-3-one	43.477	4.85	—	—	— —
36	Benzamide, N-(4-oxo-2-cyclohexen-1-yl)-	43.612	—	—	—	0.27 0.16
37	Lupeol	44.569	11.56	—	—	— —
38	Betulinaldehyde	44.626	11.78	—	—	— —

Table 3. Phytocomponents Detected in the Ethanol Extract of Leaves and Rhizome of *K. siamensis* Plant.

No	Name of the compound	RT	Peak area %			
			Leaves			Rhizome
			Wild	Greenhouse	<i>In vitro</i>	Wild Greenhouse
1	Neophytadiene	28.183	0.27	1.56	0.19	— —
2	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	29.084	—	0.24	—	— —
3	Phenanthrene,7-ethenyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,1,4a,7-tetramethyl-, [4aS-(4a α , 4b β , 7 β , 10a β)]-	31.704	—	—	—	— 0.30
4	Kaur-16-ene	31.711	—	—	—	0.20 —
5	4,6-Dipropyl-nonan-5-one	32.890	—	—	1.68	— —
6	Phytol, acetate	33.725	0.41	—	—	— —
7	Hexadecanamide	35.119	5.81	7.38	6.70	6.16 4.53
8	(1R,4aR,4bS,7R,10aR)-1,4a,7-Trimethyl-7-vinyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydrophenanthrene-1-carbaldehyde	35.267	—	—	—	— 2.16
10	((1R,4aR,4bR,10aR)-7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,4b,5,6,10,10a-decahydrophenanthren-1-yl) methanol	35.818	—	—	—	3.06 4.86
11	Naphthalene, decahydro-1,1,4a-trimethyl-6-methyle-5-(3-methyl-2,4-penta dienyl)-, [4aS-(4a α ,5 α ,8a β)]-	37.037	—	—	—	0.27 0.53
12	1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-a-phenyl-	37.253	—	—	—	0.13 —
13	Bicyclo[9.3.1]pentadeca-3,7-dien-12-ol, 4,8,12,15,15-pentamethyl-, [1R-(1R*,3E,7E,11R*,12R*)]-	37.567	—	—	—	— 2.36
14	Kaur-16-en-18-oic acid, methyl ester, (4 β)-	37.584	—	—	—	1.41 —
15	9-Octadecenamide, (Z)-	38.307	91.06	88.25	88.95	85.41 81.06
16	Octadecanamide	38.693	2.45	2.39	2.48	2.10 1.97
17	1-Phenanthrenecarboxylic acid, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,4a,7-trimethyl-, methyl ester, [1R-(1 α ,4a β , 4b α ,7 α ,10a α)]-	39.561	—	—	—	— 0.86
18	Androst-5-ene-3,19-diol, 3-acetate, (3 β)-	39.576	—	—	—	0.57 —
19	(3E,5E,7E)-6-methyl-8-(2,6,6-trimethyl-1-cyclohexenyl)-3,5,7-octatriene-2-one	40.605	—	—	—	0.34 0.54

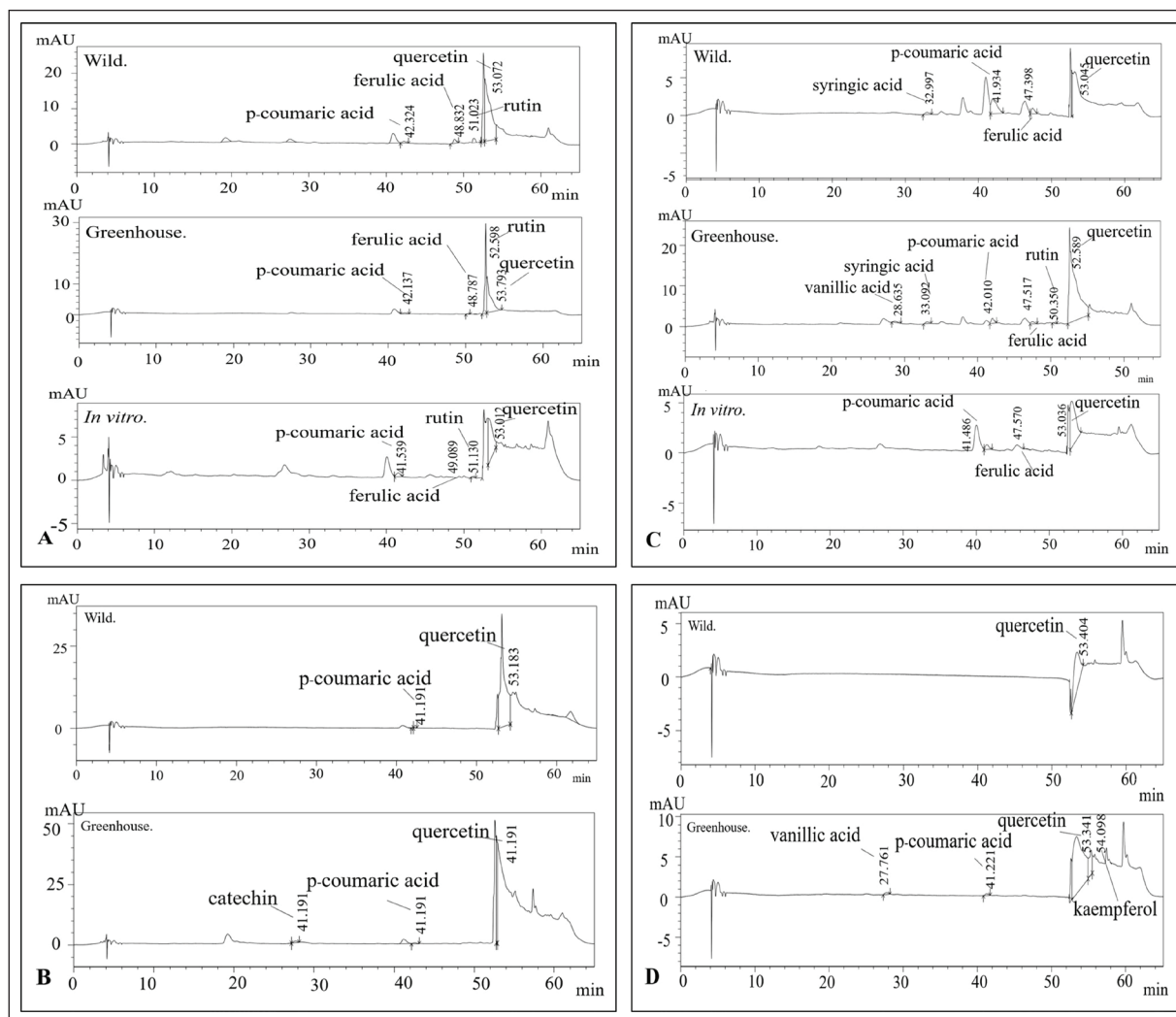


Figure 2. HPLC Chromatogram of *K. grandifolia* and *K. siamensis*, (A) Leaf Extracts of *K. grandifolia*, (B) Rhizome Extracts of *K. grandifolia*, (C) Leaf Extracts of *K. siamensis*, and (D) Rhizome Extracts of *K. siamensis*.

Abbreviation: HPLC, high-performance liquid chromatography.

ABTS assay, results showed that the leaf extracts from wild plants of *K. siamensis* had the highest percentage of radical scavenging with 25.52%, whereas leaf extracts from *in vitro* cultures showed the lowest percentage of radical scavenging (Table 1). The DPPH assays of *K. grandifolia* and *K. siamensis* had higher correlations with TPC ($r = 0.734$ and $r = 0.696$, respectively), while the ABTS assays of *K. grandifolia* and *K. siamensis* had higher correlations with TPC ($r = 0.713$ and $r = 0.876$, respectively).

GC-MS Analysis

The extracts were subjected to GC-MS for identification of the phytochemical constituents. Ethanol extracts of

K. grandifolia revealed the presence of 38 compounds, with 12 compounds identified from wild leaf extracts, 7 compounds from *in vitro* cultured, and 5 compounds from greenhouse plants (Table 2). Many of the compounds from wild plants showed greater similarity to greenhouse plants than *in vitro* cultured plants. Four similar compounds were common to wild, *in vitro*, and greenhouse extracts. Ethanol extracts of *K. siamensis* included 19 compounds. Five compounds were identified from wild leaf extracts, 5 compounds from *in vitro* cultured plants, and 5 compounds from greenhouse plants (Table 3). Rhizome extracts of *K. siamensis* included 10 compounds from wild and 10 compounds from greenhouse extracts, while six similar compounds were found in both extracts. The compounds identified from extracts; neophytadiene, hexadecane, octadecanamide, lupeol, and

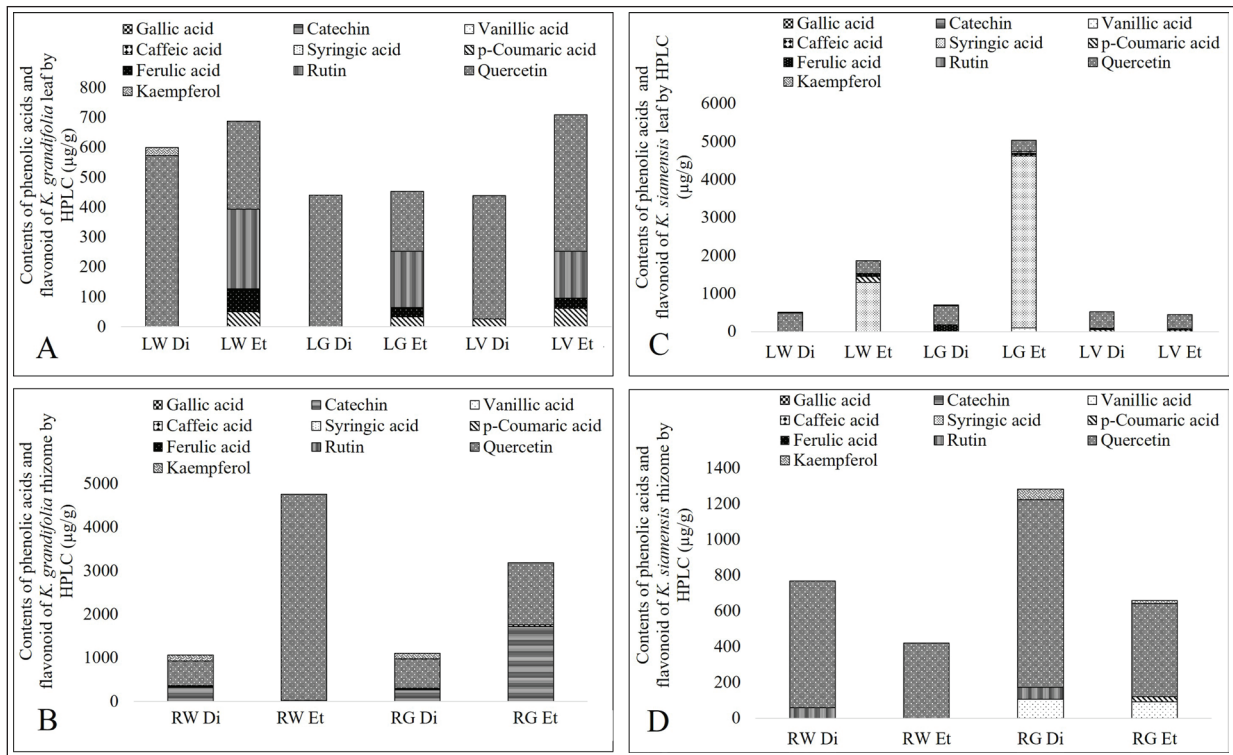


Figure 3. HPLC Analysis of Phytochemical Compounds in Phenolic Acids Contents and Flavonoid Contents Extracts of *K. grandifolia* and *K. siamensis*. (A) Leaf Extracts of *K. grandifolia*, (B) Rhizome Extracts of *K. grandifolia*, (C) Leaf Extracts of *K. siamensis*, and (D) Rhizome Extracts of *K. siamensis*.

Abbreviations: HPLC, high-performance liquid chromatography; LW, leaves of wild; LG, leaves of greenhouse; LV, leaves of *in vitro*; RW, rhizome of wild; RG, rhizome of greenhouse.

betulinaldehyde 3,7,11,15-tetramethyl-2-hexadecen-1-ol are normally reported for their antimicrobial, anti-inflammatory, anticancer, antioxidant, antitumor, chemopreventive, lipoxigenase-inhibitor, and pesticidal abilities.^{13–17}

The Quantifications of the Phenolic Acid and Flavonoids by HPLC

The HPLC chromatograms of leaf and rhizome extracts from wild, *in vitro*, and greenhouse-grown *K. grandifolia* and *K. siamensis* plants are shown in Figure 2. The results of the HPLC analysis are presented in Table 4 and Figure 3. Quercetin was found in all extracts; the ethyl acetate rhizome extract from wild plants of *K. grandifolia* had the highest content of quercetin. Vanillic acid was found in the leaf and rhizome of a greenhouse plant of *K. siamensis*, which is not found in the leaves of *in vitro* or wild plants. In rhizome extracts of *K. grandifolia*, catechins were identified. Vanillic acid, syringic acid, p-coumaric acid, ferulic acid, rutin, and quercetin were revealed in leaf extracts from *K. siamensis* greenhouse plants. The quantifications of the phenolic acid and flavonoids based

on calibration curves of authentic standards are presented in Figure 4.

Discussion

The study is the first report on the phytochemical profiles from *K. grandifolia* and *K. siamensis*. Rhizome extracts of *K. grandifolia* in greenhouse plants presented the highest phenolic and high free radical scavenging activity compared to different extracts of wild and *in vitro* cultured. The highest TPC, TFC, DPPH, and ABTS were found in leaf extracts of *K. siamensis*. These results show that wild and greenhouse plants had the highest levels of phenolic, flavonoid contents, and free radical scavenging activity. Wild and greenhouse plants do not have a huge statistical difference, so if plants in the greenhouse will be helpful to replace plants in the wild that is considered the most useful.

The bioactive compounds were identified, and their bioactive applications are listed in Table 5. Bioactive compounds are normally reported for their antioxidant, anti-inflammatory, anticancer, and antimicrobial properties.

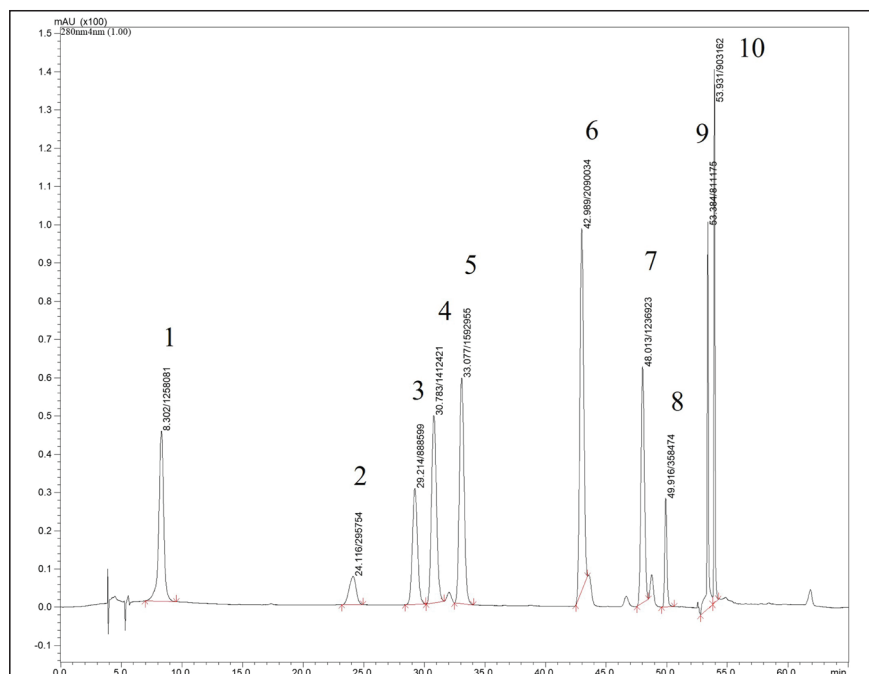


Figure 4. HPLC Chromatogram of Standard. 1: gallic acid; 2: catechin; 3: vanillic acid; 4: caffeic acid; 5: syringic acid; 6: p-coumaric acid; 7: ferulic acid; 8: rutin; 9: quercetin; and 10: kaempferol.

Abbreviation: HPLC, high-performance liquid chromatography.

Table 4. Contents of Phenolic Acids and Flavonoid of *K. grandifolia* and *K. siamensis* by HPLC.

Parameter	Leaves of wild		Leaves of greenhouse		Leaves of <i>in vitro</i>		Rhizome of wild		Rhizome of greenhouse	
	DM	EA	DM	EA	DM	EA	DM	EA	DM	EA
<i>K. grandifolia</i> (µg/g)										
catechin	ND	ND	ND	ND	ND	ND	322.60 ± 12.73 ^c	ND	263.45 ± 5.054 ^b	1714.71 ± 6.17 ^a
p-coumaric acid	ND	49.52 ± 0.76 ^b	ND	32.80 ± 2.87 ^c	25.10 ± 0.23 ^d	61.78 ± 0.57 ^a	ND	22.99 ± 0.01 ^d	ND	48.01 ± 0.79 ^b
ferulic acid	ND	77.56 ± 0.38 ^a	ND	31.34 ± 0.34 ^d	ND	33.33 ± 0.53 ^b	39.57 ± 0.34 ^b	ND	39.65 ± 0.63 ^b	ND
rutin	ND	266.26 ± 2.62 ^a	ND	187.98 ± 0.80 ^b	ND	157.53 ± 0.51 ^c	ND	ND	ND	ND
quercetin	572.25 ± 27.62 ^{cd}	294.23 ± 16.34 ^{ef}	439.96 ± 33.51 ^{de}	200.60 ± 7.26 ^f	413.64 ± 12.54 ^{de}	456.87 ± 7.16 ^{de}	564.48 ± 18.37 ^{cd}	4733.59 ± 19.31 ^a	673.75 ± 5.71 ^c	1423.50 ± 5.10 ^b
kaempferol	27.72 ± 8.30 ^b	ND	ND	ND	ND	ND	134.16 ± 2.13 ^a	ND	130.81 ± 4.37 ^a	ND
<i>K. siamensis</i> (µg/g)										
vanillic acid	ND	ND	ND	94.19 ± 2.45 ^b	ND	ND	ND	ND	107.04 ± 3.58 ^a	91.72 ± 0.77 ^b
syringic acid	ND	1297.60 ± 118.85 ^a	ND	4518.70 ± 90.56 ^b	ND	ND	ND	ND	ND	ND
p-coumaric acid	ND	159.05 ± 5.27 ^a	ND	38.93 ± 2.44 ^c	53.30 ± 0.07 ^b	44.23 ± 0.36 ^c	ND	ND	ND	28.27 ± 0.43 ^d
ferulic acid	ND	70.15 ± 0.55 ^b	174.21 ± 3.34 ^a	34.01 ± 1.37 ^c	33.24 ± 0.48 ^c	29.72 ± 0.13 ^d	ND	ND	ND	ND
rutin	ND	ND	ND	58.41 ± 0.45 ^b	ND	ND	58.30 ± 1.07 ^b	ND	64.53 ± 1.32 ^a	ND
quercetin	491.48 ± 31.00 ^c	339.06 ± 19.24 ^{de}	500.00 ± 29.60 ^c	292.09 ± 12.97 ^e	439.95 ± 20.34 ^{cd}	373.15 ± 13.80 ^{de}	710.04 ± 8.78 ^b	419.78 ± 15.27 ^{cd}	1051.57 ± 90.01 ^a	521.03 ± 9.07 ^c
kaempferol	15.15 ± 7.64 ^b	ND	30.62 ± 1.13 ^{ab}	ND	ND	ND	ND	ND	58.54 ± 32.36 ^a	16.79 ± 6.49 ^b

Note: The data is presented as a mean standard deviation ($n = 3$). Significant differences ($p < .05$) are indicated by different superscripts in the same column. Means with no significant difference are marked with the same letters (a, b, c, d, e) ($p > 0.05$).

Abbreviations: ND, not detected; DM, dichloromethane; EA, ethyl acetate.

Table 5. Bioactivity of Phytocomponents Identified in the Extract of Leaves and Rhizomes of *K. grandifolia* and *K. siamensis*.

Compound name	Medicinal activity
Neophytadiene	Antipyretic, analgesic, anti-inflammatory, antimicrobial, antioxidant ¹³
Hexadecane	Antianginal, ovulation inhibitor ¹⁸
Hexadecanoic acid, ethyl ester	Antioxidant, hemolytic, hypocholesterolemic, nematocide, antiandrogenic ¹⁴
Phytol	Antimicrobial, anti-inflammatory, antioxidant, cytotoxic, antinociceptive ¹⁵
Hexadecanamide	Analgesic, anti-inflammatory ¹⁶
9-Octadecenamide, (Z)-	Antimicrobial, anti-inflammatory ¹⁷
Octadecanamide	Antibacterial ¹⁹
Lupeol	Anti-inflammatory, oral carcinogenesis ^{20,21}
p-Coumaric acid	Antioxidant, anti-inflammatory, anti-platelet aggregation, analgesic, anticancer and neuroprotective properties ²²
Ferulic acid	Antioxidant, anticancer, anti-inflammatory and antibacterial properties ²³
Rutin	Antiviral, antimicrobial, anti-inflammatory, anticancer, antidiabetic ²⁴
Vanillic acid	anticancer, antiobesity, antidiabetic, antibacterial, anti-inflammatory and antioxidant activity ²⁵
Kaempferol	Antioxidant cardioprotective, neuroprotective and anti-inflammatory ^{26,27}
Quercetin	Antioxidant and anti-inflammatory ²⁷

GC-MS analysis of leaf and rhizome extracts in wild, greenhouse, and *in vitro* cultured extracts of *K. grandifolia* and *K. siamensis* revealed 9-octadecenamide, (Z)-, hexadecanamide, and octadecanamide as the major constituents. 9-octadecenamide, (Z)- is a fatty acid amide known as oleamide. Oleamide, which is found in many species of Zingiberaceae plants, such as the root and fruit of *Zingiber vuquangensis*, *Zingiber castaneum*, and *Curcuma cochinchinensis*.²⁸ It is reported that these compounds have bioactivity such as antioxidant, anti-inflammatory, and antimicrobial.²⁸ HPLC analysis found that the main compounds found in all extracts were p-coumaric acid, ferulic acid, and quercetin, while quercetin content in rhizome extracts from wild plants was higher than that of greenhouse plants. Vanillic acid is not found in the wild plants of these plants, but it is found in greenhouse plants. It possesses anticancer, antiobesity, antidiabetic, antibacterial, anti-inflammatory, and antioxidant activity.²⁵ Krishnakuma et al.¹⁸ studied phytochemical compounds from *Kaempferia rotunda*. They reported that the rhizome extracts contain quercetin, ferulic acid, p-coumaric acid, gallic acid, myricetin, caffeic acid, kaempferol, and syringic acid. The fractions of *K. grandifolia* had higher contents of the phytochemical compounds than *K. siamensis* fractions. Different plant parts, different conditions of plant, and types of extraction solvent are important factors that impact the phytochemical compounds and bioactivity of plant extracts. *K. grandifolia* and *K. siamensis* phytochemical components were shown to have considerable antioxidant activity, whereas greenhouse extracts had potential antioxidant activity.

Conclusion

Extracts of *Kaempferia* from greenhouse plants and tissue-cultured plants were found to be similar to wild plants and exhibited good medicinal activity. When compared to wild

plants, rhizome extracts from greenhouse plants have higher phenolic and antioxidant activity. Biological activities such as anti-inflammatory activity, anti-microbial activity, and anti-cancer activity should be further investigated. This study may be helpful to increase the value of commercial production of ornamental plants, cosmetics, and supplementary foods and lead to future developments in industrial pharmacy.

Summary

According to HPLC results, the main compounds found in all plants were p-coumaric acid, ferulic acid, and quercetin.

The GC-MS results revealed 38 compounds in *K. grandifolia* and 19 in *K. siamensis*. Major constituents in the leaf and rhizome extracts were 9-octadecenamide, (Z)-, hexadecanamide, and octadecanamide.

Total phenolic and total flavonoid contents of leaf extracts decreased from wild to *in vitro* cultured and greenhouse plants, respectively, whereas rhizome extract contents increased from wild to greenhouse plants.

Antioxidant activities of rhizome extracts showed higher activity than leaf extracts in DPPH and ABTS assays.

Abbreviations

GC-MS, gas chromatography-mass spectrometry; HPLC, high-performance liquid chromatography; *K.*, *Kaempferia*; QE, quercetin equivalent; Rt, retention time.

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

Ethical approval is not applicable for this article.

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Informed Consent

Not applicable, because this article does not contain any studies with human or animal subjects.

References

1. Saensouk S. Endemic and rare plants of ginger family in Thailand. *KKU Res J* 2011; 16(3): 306–330.
2. Woerdenbag HJ, Windono T, Bos R, et al. Composition of the essential oils of *Kaempferia rotunda* L. and *Kaempferia angustifolia* Roscoe rhizomes from Indonesia. *Flavour Fragr J* 2004; 19(2): 145–148. DOI: 10.1002/ffj.1284.
3. Yeap YSY, Kassim NK, Ng RC, et al. Antioxidant properties of ginger (*Kaempferia angustifolia* Rosc.) and its chemical markers. *Int J Food Prop* 2017; 20(Suppl 1): 1158–1172. DOI: 10.1080/10942912.2017.1286508.
4. Saensouk S, Saensouk P, Pasorn P, et al. Diversity and traditional uses of Zingiberaceae in Nakhon Phanom Province, Thailand. *Res Knowl* 2018; 4(1): 47–55.
5. Saensouk S and Jenjittikul T. *Kaempferia grandifolia* sp. nov. (Zingiberaceae) a new species from Thailand. *Nord J Bot* 2001; 21(2): 139–142. DOI: 10.1111/j.1756-1051.2001.tb01349.x.
6. Khanh Pham N, Tuan Nguyen H, and Binh Nguyen Q. A review on the ethnomedicinal uses, phytochemistry and pharmacology of plant species belonging to *Kaempferia* genus (Zingiberaceae). *Pharm Sci Asia* 2021; 48(1): 1–24. DOI: 10.29090/psa.2021.01.19.070.
7. Chumroenphat T and Saensouk S. Taxonomy, phytochemical and bioactive compounds and potential use as material with different drying methods of *Alpinia latilabris* Ridl. new record from Thailand. *Not Bot Horti Agrob* 2022; 50(1). DOI: 10.15835/nbha50112619.
8. Pothitirat W, Chomnawang MT, Supabphol R, et al. Comparison of bioactive compounds content, free radical scavenging and anti-acne inducing bacteria activities of extracts from the mangosteen fruit rind at two stages of maturity. *Fitoterapia* 2009; 80(7): 44–47. DOI: 10.1016/j.fitote.2009.06.005, PMID 19524646.
9. Likhitwitayawuid K, Klongsiriwet C, Jongbunprasert V, et al. Flavones with free radical scavenging activity from *Goniothalamus tenuifolius*. *Arch Pharm Res* 2006; 29(3): 199–202. DOI: 10.1007/BF02969393, PMID 16596991.
10. Payet B, Shum Cheong Sing ASC, and Smadja J. Assessment of antioxidant activity of cane brown sugars by ABTS and DPPH radical scavenging assays: determination of their polyphenolic and volatile constituents. *J Agric Food Chem* 2005; 53(26): 10074–10079. DOI: 10.1021/jf0517703, PMID 16366697.
11. Suphrom N, Sonyota W, Insumronga K, et al. GC-MS analysis and *in vitro* anti-androgenic activity of *Kaempferia rotunda* Linn extract. *NUJST* 2017; 4(25): 34–43.
12. Siriamornpun S and Kaewseejan N. Quality, bioactive compounds and antioxidant capacity of selected climacteric fruits with relation to their maturity. *Scientia Horticulturae* 2017; 221: 33–42. DOI: 10.1016/j.scienta.2017.04.020.
13. Raman VB, Samuel LA, Saradhi MP, et al. Antibacterial, antioxidant activity and GC-MS analysis of *Eupatorium odoratum*. *Asian J Pharm Clin Res* 2012; 5(2): 99–106.
14. Jegajeevanram P, Alhaji NMI, and Velavan S. Identification of insecticidal components of *Mango ginger* rhizome and *Tagetes erecta* flower extracts by GC-MS analysis. *IJCPA* 2014; 1(4): 203–207.
15. Islam MT, Ali ES, Uddin SJ, et al. Phytol: A review of biomedical activities. *Food Chem Toxicol* 2018; 121: 82–94. DOI: 10.1016/j.fct.2018.08.032, PMID 30130593.
16. Al-Rubaye AF, Kaizal AF, and Hameed IH. Phytochemical screening of methanolic leaves extract of *Malva sylvestris*. *Phyto* 2017; 9(4): 537–552. DOI: 10.25258/phyto.v9i4.8127.
17. Salah AI, Ali HAM, and Imad HH. Spectral analysis and anti-bacterial activity of methanolic fruit extract of *Citrullus colocynthis* using gas chromatography-mass spectrometry. *Afr J Biotechnol* 2015; 14(46): 3131–3158. DOI: 10.5897/AJB2015.14957.
18. Kumaresan S, Senthilkumar V, Stephen A, et al. GC-MS analysis and pass-assisted prediction of biological activity spectra of extract of *Phomopsis* sp. isolated from *Andrographis paniculata*. *World J Pharm Res* 2015; 4(1): 1035–1053.
19. Bharose A and Gajera H. Antifungal activity and metabolites study of *Bacillus* strain against aflatoxin producing *aspergillus*. *J Appl Microbiol Biochem* 2018; 02(2): 1–8. DOI: 10.21767/2576-1412.100024.
20. Wal A, Rai A, Wal P, et al. Biological activities of lupeol. *Syst Rev Pharm* 2011; 2(2): 96–103. DOI: 10.4103/0975-8453.86298.
21. Saleem M. Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene. *Cancer Lett* 2009; 285(2): 109–115. DOI: 10.1016/j.canlet.2009.04.033, PMID 19464787.
22. Pei K, Ou J, Huang J, et al. p-Coumaric acid and its conjugates: dietary sources, pharmacokinetic properties and biological activities. *J Sci Food Agric* 2016; 96(9): 2952–2962. DOI: 10.1002/jsfa.7578, PMID 26692250.
23. Stompór-Gorący M and Machaczka M. Recent advances in biological activity, new formulations and prodrugs of ferulic acid. *Int J Mol Sci* 2021; 22(23): 12889. DOI: 10.3390/ijms222312889, PMID 34884693.
24. Gullón B, Lú-Chau TA, Moreira MT, et al. Rutin: a review on extraction, identification and purification methods, biological activities and approaches to enhance its bioavailability. *Trends Food Sci Technol* 2017; 67: 220–235. DOI: 10.1016/j.tifs.2017.07.008.

25. Kaur J, Gulati M, Singh SK, et al. Discovering multifaceted role of vanillic acid beyond flavours: nutraceutical and therapeutic potential. *Trends Food Sci Technol* 2022; 122: 187–200. DOI: 10.1016/j.tifs.2022.02.023.
26. Bangar SP, Chaudhary V, Sharma N, et al. Kaempferol: A flavonoid with wider biological activities and its applications. *Crit Rev Food Sci Nutr* 2022: 1–25. DOI: 10.1080/10408398.2022.2067121, PMID 35468008.
27. Dabeek WM and Marra MV. Dietary quercetin and kaempferol: bioavailability and potential cardiovascular-related bioactivity in humans. *Nutrients* 2019; 11(10): 2288. DOI: 10.3390/nu11102288, PMID 31557798.
28. Naumoska K, Jug U, Metličar V, et al. Oleamide, a bioactive compound, unwittingly introduced into the human body through some plastic food/beverages and medicine containers. *Foods* 2020; 9(5): 1–18. DOI: 10.3390/foods9050549, PMID 32369935.