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Flavonoid content and antioxidant activity of vegetables from Indonesia

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Abstract

Extracts from 11 vegetables of Indonesian origin were screened for flavonoid content, total phenolics, and antioxidant activity. The flavonols myricetin, quercetin, and kaempferol and flavones luteolin and apigenin were quantified by HPLC. Flavonoid content in mg/100 g fresh weight (fw) was apparently initially reported for *Cosmos caudatus* H.B.K. (52.19), *Polyscias pinnata* (52.19), *Pluchea indica* Less. (6.39), *Nothopanax scutellarius* (Burm.f.) Merr (5.43), *Talinum triangulare* (Jacq.) Willd. (3.93), *Pilea melastomoides* (Poir.) Bl. (2.27), and *Etlingera elatior* (Jack) R.M.Sm (1.18). The flavonoid content of the vegetables studied were mainly quercetin and kaempferol and ranged from 0.3 to 143 mg/100 g fw, with the highest level found in *Sauropus androgynus* (L) Merr. *C. caudatus* H.B.K. had the greatest total phenols among the vegetables analysed, with 1.52 mg GAE/100 g fw. *P. indica* Less. and *C. caudatus* H.B.K. had the highest antioxidant activity as measured by ferric cyanide reducing power, DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2[']-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) scavenging, and inhibition of linoleic acid oxidation. Therefore, *S. androgynus* (L) Merr, *C. caudatus* H.B.K., and *P. pinnata* were identified as potentially rich sources of dietary flavonoids and antioxidants.

Keywords

Antioxidant; Cosmos caudatus H.B.K; Flavonoid; Phenolic; Pluchea indica Less; Vegetables

1. Introduction

The flavonoid content of some western foods has been reported and archived in the USDA flavonoid database (USDA, 2007). However, less is known about the flavonoid content and non-nutritive bioactivity of foods from developing nations, including Indonesia. A number of west Javanese vegetables are used for both food and traditional medicine. For food, these

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plants are eaten raw or boiled. Medicinally, they may be used raw, boiled, or applied as a poultice. Some plants of Indonesian origin have been screened for their antiinflammatory and antioxidant activity (Choi & Hwang, 2005), yet little is known about their constituents that may contribute to their medicinal functionality. This information is necessary to validate the safety, traditional uses, and to standardise preparations of these plants. Furthermore, this information may be used to establish flavonoid databases for Indonesia or other Southeast Asian countries.

Characterisation of the antioxidant activity of vegetables may also yield more insight into their functionality. Dietary antioxidants are necessary to cope with reactive oxidant species that could damage DNA, RNA, modify proteins, and cause lipid peroxidation of cellular targets. Antioxidants may inhibit the initiation or propagation of oxidation (Velioglu, Mazza, Gao, & Oomah, 1998). Vegetable extracts with high antioxidant activity may also be useful for food preservation. Therefore, the aims of this research were to identify and quantify flavonoid compounds of 11 leafy green vegetables, from west Java, Indonesia, and to screen for antioxidant activity and total phenols.

2. Materials and methods

2.1. Chemicals and reagents

Flavonoids, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), DPPH, ABTS, and *tert*-butylhydroquinone (TBHQ) were purchased from Sigma–Aldrich (St. Louis, MO). Acetonitrile, methanol, ethanol, Folin–Ciocalteau reagent, HCl, KH₂PO₄, potassium ferric cyanide, and trichloroacetic acid (TCA) were obtained from E-Merck (Darmstadt, Germany).

2.2. Sample preparation

Fresh vegetables, free of blemishes or obvious defects (Table 1) were obtained from Bogor, west Java, Indonesia. Kenikir, kemangi leaves, katuk leaves and pohpohan leaves were purchased from a local market in Bogor and the others were harvested from uncultivated or cultivated fields near Bogor Agricultural University, Indonesia. The vegetables were identified by the botanist, Dr. Eko Baroto Waluyo, APU, Indonesian Institute of Science, Research Centre for Biology, Bogor, Indonesia. Samples were cleaned, immediately stored at -20 °C overnight, and then lyophilised for 48 h (FreeZone 6 l Console Freeze Dry System, Labconco, Kansas City, MO). Following lyophilisation, the dried vegetables were crushed to a 30 mesh powder. Dried powder was stored at -20 °C in darkness.

2.3. Moisture analysis

The moisture content of raw vegetables (fresh and freeze-dried/ground) was determined according to a previously published method (AOAC, 1984). The samples were put in an aluminium cup (~5 g sample), then dried in 100 °C oven for 6 h.

2.4. Total phenols

Freeze-dried vegetable samples (50.0 mg) were extracted by shaking with 2.5 ml of 95% aqueous ethanol (v/v). Following centrifugation at 1536g (IEC Centra-8 Centrifuge,

Waltham, MA) for 5 min, aliquots of supernatant were reserved for the total phenolic content and antioxidant assays. The Folin method was used to determine phenolic content of vegetable extracts, as described by Shetty, Curtis, Levin, Witkowsky, and Ang (1995). Total phenols were quantified based on standard curves of 50–300 mg/l gallic acid. Inter-assay and intra-assay CV were 1.8% and 7.0%, respectively.

2.5. DPPH scavenging

DPPH scavenging activity was measured according to the method by Brand-Williams, Cuvelier, and Berset (1995). Extract (100 mg/ml) was added to a 5 ml of 0.1 M DPPH solution in methanol and absorbance at 517 nm was measured following incubation for 30 min at 27 °C. Antioxidant activity was expressed as Trolox equivalents, on the basis of 0.8 mM Trolox scavenging 41% of DPPH radicals. Inter-assay and intra-assay CV were 2.2% and 3.5%, respectively.

2.6. ABTS Trolox equivalent antioxidant capacity (TEAC)

ABTS scavenging activity was determined by the TEAC method according to Yeh and Yen (2003). The TEAC assay is based on the capacity to quench ABTS⁺⁺ radical formation relative to Trolox. Briefly, 0.1 ml of extract representing 0.14–0.51 mg/ml dry weight of plants was incubated for 45 s with 0.9 ml ABTS solution, and ABTS absorbance was measured at 734 nm. Data was expressed as Trolox equivalents, based on standard curves of $0-0.5 \mu$ M Trolox. Inter-assay and intra-assay CV were 0.4% and 3.7%, respectively.

2.7. Ferric reducing power

Reducing power was determined by the method of Oyaizu (1986). Potassium ferric cyanide was added to dry extract (0–500 mg/ml) in 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and incubated at 50 °C for 20 min. After incubation, TCA (100 mg/ml, 2.5 ml) and ferric chloride (10 mg/ml, 0.5 ml) were added to the mixture in a total volume of 5.5 ml and absorbance was read at 700 nm. Reducing power was calculated on the basis of Trolox equivalents, from standard curves of 0–0.5 μ M Trolox. Inter-assay and intra-assay CV were 1.9% and 1.0%, respectively.

2.8. Inhibition of lipid oxidation

The ability of extracts to prevent linoleic acid oxidation was quantified using a TBA (2thiobarbituric acid) method as previously described (Aqil, Ahmad, & Mehmood, 2006). Linoleic acid was oxidised by heating in the presence of ethanolic vegetable extracts. The reaction was composed of 4 mg dried extract in 4 ml ethanol absolute, 4.1 ml linoleic acid in 2.52% ethanol, 8 ml of 0.05 M phosphate buffer at pH 7 and 3.9 ml water in a closed vial and incubated in a 40 °C oven for 6 days. TBA reactive substances (TBARs) were quantified at 532 nm. The result was expressed as percent inhibition of TBARs formation of the control without addition of antioxidant, using a standard curve of 0.5–3.0 μ M tetramethoxypropane. Inter-assay and intra-assay CV were 0.3% and 0.5%, respectively.

2.9. Flavonoid analysis

Since flavonoids are ubiquitous in vegetables, the flavonols quercetin, kaempferol, myricetin and flavones apigenin and luteolin were quantified in vegetable extracts according to the method by Hertog, Hollman, and Venema (1992). Lyophilised vegetables (0.5 or 1 g) were extracted for 1 h at 50 °C in 50% aqueous methanol containing 1.2 MHCl and 0.5 g/l TBHQ. The extract was allowed to cool and the solution was made up to 100 ml with methanol. Approximately 2 ml was filtered through a 0.45 µm filter, and 20 µl was injected onto an LC-2040 HPLC (Shimadzu, Kyoto, Japan) equipped with a UV-Vis Hewlett-Packard Series 1100 detector (Agilent Technologies, Inc., Santa Clara, CA) and reversephase Develosil ODS-UG-3 column (4.6 mm i.d. × 75 mm) (Nomura Chemical, Seto, Japan), and eluted with isocratic 25% acetonitrile in 0.025 M KH₂PO₄ at a flow rate 0.9 ml/ min. Flavonoids were quantified on the basis of comparison to standards at 370 nm. The limits of detection (in µg/ml on column) were 0.02 for quercetin, 0.04 for kaempferol, 0.03 for myricetin, 0.19 for apigenin, and 0.04 for luteolin. Standard curve regression equations were Y = 114.2(X) - 25.8 for quercetin, Y = 67.60(X) - 17.8 for kaempferol, Y = 125.9(X)-14.5 for myricetin, Y = 46.19(X) - 8.99 for luteolin, and Y = 16.62(X) - 2.72 for apigenin, where Y was the detector response and X was concentration of standards. For regression equations, R^2 values were 0.999. Inter- and intra-assay CV for quantification ranged from 1.9 to 4.8 and 3.7 to 6.5, respectively for flavonoid standards.

2.10. Statistical analysis

Data are presented as the mean \pm standard deviation of at least triplicate determinations. Statistical significance was by one-way ANOVA, with *P* values 0.05 considered significant. ANOVA and Pearson correlation analysis was by GraphPad prism v 5.01 (Graph-Pad Software, Inc., La Jolla, CA).

3. Results and discussion

Extracts from 11 vegetables from Indonesia were screened for flavonoid content, total phenols, and antioxidant activity since they are frequently used as traditional medicine and as foods (Table 1). Generous flavonoid intake has been implicated in reduction of risk for chronic diseases such stroke, cardiovascular disease, and some cancers. For example, increased consumption of broccoli and spinach, rich in kaempferol, are associated with reduced risk of ovarian cancer (Gates et al., 2007).

3.1. Total phenols

Total phenols ranged from 0.33 to 1.52 mg GAE/g fresh weight (fw) (Table 2). *Cosmos caudatus* H.B.K. and *Sauropus androgynus* (L) Merr had the greatest levels of phenolics with 1.52 and 1.49 mg GAE/g fw, respectively. *S. androgynus* (L) Merr and *Centella asiatica* had 8.71 and 5.82 mg GAE/g dry weight (dw) respectively, which was of a similar magnitude to 11.5 and 12.5 mg GAE/g dw reported by Gupta and Prakash (2009).

3.2. Antioxidant activity of extracts

Pluchea indica Less. and *C. caudatus* H.B.K. extracts inhibited linoleic acid oxidation to the greatest extent and had the greatest DPPH, ABTS, and ferric cyanide antioxidant capacities

relative to other vegetables (Table 2). This is in agreement with a previous report in which *Cosmos caudatas* had greater antioxidant activity than *S. androgynus* (L) Merr and *C. asiatica* in the DPPH and FRAP assays (Wong, Leong, & Koh, 2006). While all extracts had antioxidant activity in the DPPH assay, *S. androgynus* (L) Merr and *Polyscias pinnata* were among the least potent of the extracts, with 7.7 and 7.1 TE/g fw. Relatively high levels of phenolics were observed in *S. androgynus* (L) Merr, so its decreased capacity toward DPPH and ABTS radical scavenging, ferric reduction, and inhibition of lipid peroxidation is notable. *Portulaca oleracea* had the least antioxidant activity in the ABTS and ferric cyanide reducing assays. Therefore, the vegetables in the present study may contribute to dietary antioxidant intake.

Previous work confirms the antioxidant activity of several extracts in the present study. Methanolic extracts of *Talinum triangulare* (Jacq.) Willd. and *P. oleracea* had 79 and 132 µmol TE/g dw respectively in the TEAC assay (Yang et al., 2006). *C. asiatica* and *S. androgynus* (L) Merr extracts also inhibited lipid peroxide formation (Mai, Thu, Tien, & Van Chuyen, 2007). Choi and Hwang (2005) reported *P. indica* Less. had greater DPPH antioxidant activity and total phenols than *C. asiatica*, similar to the present study. In the same study, a similar relationship was reported for ferricyanide reducing activity, in contrast to our results. Yearly and geographical climate differences, soil conditions, and pesticide or herbicide usage may contribute to variations in antioxidant, nutrient, and flavonoid content of vegetables in the present study relative to prior reports.

3.3. Correlations

Consistent with literature, total phenols values were highly correlated with DPPH, ABTS and ferric cyanide reducing power antioxidant values with *R* values of 0.77, 0.79, 0.85, respectively (P < 0.01). Flavonoid content was not correlated with antioxidant activity in the DPPH, ABTS, and reducing power assays. Flavonoid content was negatively correlated with inhibition of lipid oxidation (R = -0.78, P = 0.005). This affect could also be associated with metal or other pro-oxidant constituents in the extracts. ABTS, DPPH, and ferric reducing power antioxidant measures were highly correlated (R > 0.9000, P < 0.001) with each other, with different rank orders of antioxidant capacities. These results highlight the contribution of phenolics to *in vitro* antioxidant activity of vegetables and the need to analyse a multiplicity of antioxidant assays to rank antioxidant activity.

3.4. Flavonoid content

The flavonoid content of 11 vegetables from west Java, Indonesia varied from 0.3 to 143 mg/100 g fw (4.0–832 mg/100 g dw) (Table 3). *S. androgynus* (L) Merr, *C. caudatus* H.B.K., and *P. pinnata* had 1.5-fold or more flavonoids than the remaining vegetables. Quercetin and kaempferol comprised 60% or more of the flavonoids in the vegetables analysed, while myricetin, luteolin, and apigenin were less abundant. The values in the present study are in range of the USDA flavonoid values for western green leafy vegetables such as 312, 6, and 3 mg/100 g fw for parsley, spinach, and iceberg lettuce respectively (USDA, 2007).

The present study is apparently the first report of quantitative flavonoid profiles for seven vegetables, including *P. indica* Less., *T. triangulare* (Jacq.) Willd., *Pilea melastomoides*

(Poir.) Bl., *C. caudatus* H.B.K., *Nothopanax scutellarius* (Burm.f.) Merr, *Etlingera elatior* (Jack) R.M.Sm, and *P. pinnata*. Flavonoid from *P. indica* Less. was 81% quercetin, with lesser amounts of myricetin and kaempferol. *T. triangulare* (Jacq.) Willd. flavonoid was 90% kaempferol, with lesser amounts of quercetin. *P. melastomoides* (Poir.) Bl., *N. scutellarius* (Burm.f.) Merr, *E. elatior* (Jack) R.M.Sm, and *P. indica* Less. had flavonoid content ranging from 1.18 to 6.39 mg/100 g fw. *C. caudatus* H.B.K. and *P. pinnata* could significantly contribute to dietary flavonoid intake when consumed, as they had 50% more than the USDA value for kale, a high-flavonoid western vegetable (USDA, 2007).

The levels of flavonoids reported here are in range of previous analysis. Spina and others (2008) reported that wild and cultivated purslane had 0.46–0.54 mg quercetin/100 g dw. The same work also detected luteolin at 1.0–4.3 mg/100 g dw in purslane, although we did not (LOD of 0.04 mg/100 g). Viera, Grayer, and Paton (2003) reported *Ocimum americanum L*. cultivars had 10–749 mg flavonoids/100 g dw. Miean and Mohamed (2001) reported S. androgynus had 78.5 mg/100 g dw flavonoids, mainly as quercetin and kaempferol, although we observed nearly 10-fold greater content in the same species. This difference may be attributed to differences in extraction and hydrolysis times and temperatures (1 h at 50 °C compared to 2 h at 90 °C), or the aforementioned preharvest factors such as climate, geography, or agronomic practices.

When consumed regularly, these vegetables may contribute a significant amount of flavonoid to the Indonesian diet, supplying approximately 0.08–36 mg for a 25 g (~1 cup) serving. The flavonoid contents of west Javanese vegetables are in the range with western green leafy vegetables, and all but three of the vegetables in the present study have higher flavonoid content than the 1– 3 mg flavonoid/100 g fw reported for western lettuce varieties (USDA, 2007). Generous intakes of flavonoid are correlated to health benefits such as lower risk of chronic conditions such as cardiovascular disease, stroke, and some cancers (Cutler et al., 2008; Geleijnse, Launer, Hofman, Pols, & Witteman, 1999; Keli, Hertog, Feskens, & Kromhout, 1996). Flavonoid fractions from *Chromolaena odorata* were antipyretic in rats (Owoyele et al., 2008). Similarly, flavonoid-containing vegetables such as *S. androgynus* (L) Merr and *P. indica* (Less.) less have antipyretic uses in Indonesian traditional medicine. Therefore, the analysis of potential flavonoid intake and antioxidant activity from these vegetables warrants further investigation to the mechanism(s) of action of the Indonesian traditional medicinal uses of these vegetables.

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Abbreviations

ABTS	2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid

DPPH 2,2-diphenyl-1-picrylhydrazyl

dw	dry weight
fw	fresh weight
GAE	gallic acid equivalents
HPLC	high performance liquid chromatography
MDA	malonyldialdehyde
TBA	2-thiobarbituric acid
TBHQ	tert-butylhydroquinone
TCA	trichloroacetic acid
TE	Trolox equivalents
TEAC	Trolox equivalent antioxidant capacity

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Table 1

Names and traditional uses of vegetables from west Java, Indonesia.

Scientific name	Indonesian name	Traditional uses/effects
Sauropus androgynus (L) Merr	Katuk	Reduces fever, stimulate lactation, hoarse voice (raw) (Yuniarti (2008))
Cosmos caudatus H.B.K	Kenikir	Improves circulation, bone strength (Shui, Leong, & Wong, 2005)
Polyscias pinnata	Kedondong cina	Reduces body odour, eyewash, reduces appetite, nausea (Poedjayanto, 2008)
Centella asiatica	Antanan	Eaten to reduce bleeding, tonic for post-partum women, poultice for wound healing, cough, fever (Harada, Mulyati, & Muzakkir, 2006; Yuniarti, 2008)
Ocimum americanum L.	Kemangi	For headache, fever, cold, douche, canker sores, inflammation in ears, lactation stimulant, constipation (Poedjayanto, 2008)
Pluchea indica Less.	Beluntas	Reduces fever, bad breath, body odour, sore muscles, menstruation, lower abdominal pain, stomach cramps (Yuniarti, 2008)
Nothopanax scutellarius (Burm.f.) Merr	Mangkokan	For swollen breast, lactation aid (topical), wound, urination (topical), hair loss (Yuniarti, 2008)
Talinum triangulare (Jacq.) Willd.	Daun ginseng	Increases stamina and an immunostimulant (Fenny, Andreanus, & Immaculata, 1996)
Pilea melastomoides (Poir.) Bl.	Pohpohan	-
Etlingera elatior (Jack) R.M.Sm	Kecombrang	Reduces the odour of fish, inhibits pathogenic bacteria and moulds on food (Naufalin, 2005)
Portulaca oleracea	Krokot (purslane)	For dysentery, diarrhoea, inflammation, appendix, breast inflammation, constipation, haemorrhoids, worms (Poedjayanto, 2008)

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Total phenols and antioxidant activity of extracts from vegetables of Indonesian origin.^A

Vegetable (% moisture)	Total phenols (mg GAE/g fw)	DPPH (µmol TE/g fw)	ABTS (µmol TE/g fw)	Total phenols (mg GAE/g fw) DPPH (μ mol TE/g fw) ABTS (μ mol TE/g fw) Ferric reducing (μ mol TE/g Inhibition of lipid fw) fw)	Inhibition of lipid peroxidation $(\%)^B$
Sauropus androgynus (L) Merr (82.9)	$1.49 \pm 0.15a$	7.72 ± 0.88^{g}	$1.81 \pm 0.08^{\circ}$	$70.6 \pm 1.0^{\circ}$	$84.7\pm0.2^{\rm f}$
Cosmos caudatus H.B.K. (86.7)	1.52 ± 0.11^{a}	112 ± 3^{a}	4.71 ± 0.19^{a}	172 ± 1^{a}	98.1 ± 0.4^{ab}
Polyscias pinnata (85.4)	0.790 ± 0.111^{b}	7.07 ± 0.83^{g}	$1.73\pm0.03^{ m c}$	$50.4 \pm 0.7^{\mathrm{e}}$	97.0 ± 0.3^{bcd}
Centella asiatica (92.0)	$0.463\pm0.018^{\rm d}$	13.8 ± 0.39^{ef}	$1.18\pm0.02^{\mathrm{e}}$	$38.3 \pm 0.5^{\rm h}$	$92.8\pm1.1^{\rm e}$
Ocimum americanum L. (89.7)	0.812 ± 0.119^{b}	$23.8\pm1.3^{\rm c}$	$1.94\pm0.05^{ m c}$	$47.9\pm0.7^{\rm f}$	97.0 ± 0.2^{bcd}
Pluchea indica Less. (91.9)	0.831 ± 0.129^{b}	$96.4 \pm 15.2^{\mathrm{b}}$	3.75 ± 0.16^{b}	81.1 ± 0.6^{b}	98.5 ± 0.4^{a}
Nothopanax scutellarius (Burm.f.) Merr (85.9)	0.943 ± 0.142^{b}	$15.2\pm0.4^{\rm c}$	$1.80\pm0.01^{ m c}$	$54.2 \pm 1.0^{\mathrm{d}}$	$97.6\pm0.6^{\mathrm{abc}}$
Talinum triangulare (Jacq.) Willd. (92.0)	0.489 ± 0.100^{cd}	7.4 ± 0.2^{g}	$1.03\pm0.02^{\mathrm{e}}$	$28.3\pm0.5^{\rm i}$	97.1 ± 0.4^{bcd}
Pilea melastomoides (Poir.) Bl. (91.6)	$0.701\pm0.134^{\rm bc}$	$11.5\pm0.6^{\mathrm{f}}$	$1.20\pm0.07^{\rm de}$	40.6 ± 0.2^{g}	97.2 ± 1.0^{abcd}
Etlingera elatior (Jack) R.M.Sm (89.9)	$0.806 \pm 0.096^{\rm b}$	$19.5\pm0.6^{ m d}$	$1.40\pm0.05^{ m d}$	$37.2 \pm 0.9^{\text{h}}$	96.5 ± 0.2^{cd}
Portulaca oleracea (92.5)	$0.334\pm0.023^{\rm e}$	5.93 ± 0.21^{g}	$0.567\pm0.014^{\rm f}$	24.6 ± 0.4^{j}	$96.0\pm0.4^{ m d}$

Data is mean \pm SD of three determinations; values bearing different letters within columns are significantly different by Tukey's HSD (P = 0.05).

 B_{200} ppm dry weight basis.

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Vegetable	Quercetin	Kaempterol	Myricetin	Luteolin	Apigenin	sum of flavonoids ^B
	Concentratio	Concentration (mg/100 g fw ⁴)				
Sauropus androgynus (L) Merr	$4.50\pm0.22d$	138 ± 5.8^{a}	<0.00002	<0.006	<0.03	143 ± 6^{a}
Cosmos caudatus H.B.K.	51.3 ± 4.1^{a}	0.903 ± 0.048^{g}	<0.00002	<0.005	<0.02	52.2 ± 4.1^{b}
Polyscias pinnata	28.5 ± 1.9^{b}	$23.7\pm1.38^{\rm b}$	<0.00002	<0.006	<0.03	52.2 ± 3.3^{b}
Centella asiatica	$12.3\pm0.4^{\rm c}$	$8.56\pm0.38^{\rm c}$	$2.08\pm0.00~(\times10^{-3})^{\rm b}$	<0.003	<0.02	$20.9\pm0.7^{\rm c}$
Ocimum americanum L.	$1.89\pm0.10^{\rm f}$	$2.47\pm0.18^{\text{e}}$	<0.00003	2.12 ± 0.05^{a}	0.737 ± 0.044	7.22 ± 0.36^{d}
Pluchea indica Less.	$5.21\pm0.26^{\rm d}$	$0.283\pm0.018^{\rm h}$	$13.8\pm0.5\;(\times10^{-3})^{a}$	<0.003	<0.02	6.39 ± 0.27^{d}
Nothopanax scutellarius (Burm.f.) Merr	$3.69\pm0.09^{\mathrm{e}}$	$1.74\pm0.07^{\rm f}$	<0.00002	<0.006	<0.03	5.42 ± 0.15^{e}
Talinum triangulare (Jacq.) Willd.	$0.41\pm0.03^{\rm h}$	$3.52\pm0.16^{\rm d}$	<0.00004	<0.003	<0.02	$3.93\pm0.17^{\rm f}$
Pilea melastomoides (Poir.) Bl.	$1.75\pm0.20^{\rm f}$	$0.252\pm0.027^{\rm h}$	<0.00004	$0.266\pm0.016^{\rm b}$	<0.02	2.27 ± 0.21^{g}
Etlingera elatior (Jack) R.M.Sm	1.18 ± 0.06^{g}	<0.004	<0.00003	<0.004	<0.02	$1.18\pm0.06^{\rm h}$
Portulaca oleracea	$0.30\pm0.02^{\rm i}$	<0.002	<0.00004	<0.002	<0.01	$0.30\pm0.02^{\rm i}$

by Tukey's HSD (P 0.05). Moisture content was same as Table 2.