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Short communication

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.

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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 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 anti-inflammatory 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, KH_2PO_4, potassium ferric cyanide, and trichloroacetic acid (TCA) were obtained from E-Merck (Darmstadt, Germany).

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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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 1536*g* (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 $ABTS^{\cdot+}$ 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 μM 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 μM 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 (2-thiobarbituric 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 μM 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 metha-

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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