

Ethnobotany, nutritional composition and DPPH radical scavenging of leafy vegetables of wild *Paederia foetida* and *Erechtites hieracifolia*

by Ignatius Srianta

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Ethnobotany, nutritional composition and DPPH radical scavenging of leafy vegetables of wild *Paederia foetida* and *Erechtites hieracifolia*

^{1,2*} Srianta, I., ²Arisasmita, J.H., ³Patria, H.D. and ²Epriliati, I.

¹Center for Food and Nutrition Research, Widya Mandala Surabaya Catholic University, Jalan Dinoyo 42-44, Surabaya 60265, East Java, Indonesia

²Department of Food Technology, Widya Mandala Surabaya Catholic University, Jalan Dinoyo 42-44, Surabaya 60265, East Java, Indonesia

³Mantasa Research Institute, Kompleks Jaya Maspion Permata A6/10, Sidoarjo, East Java, Indonesia

Abstract: Study on ethnobotany, nutritional composition and DPPH radical scavenging of leafy vegetables of wild *Paederia foetida* and *Erechtites hieracifolia* in two villages of Pandaan City, East Java, Indonesia, have been done. A focus group discussion (FGD) and in-depth interview showed that the wild plants are well recognized and commonly consumed by local people. The samples, collected from local forest of the two villages, were analyzed for the proximate composition, dietary fibers, minerals, vitamin C and total phenol contents; and DPPH radical scavenging activity. Both leafy vegetables are potential dietary fiber, Ca, Na, K and Fe sources. Vitamin C content of the leafy vegetable of *Paederia foetida* (271.40 ± 42.65 mg/100g) was much higher than that of *Erechtites hieracifolia* (20.62 ± 1.63 mg/100 g). Both leafy vegetables are potential antioxidant sources with IC₅₀ values of 4.53 mg/mL and 8.46 mg/mL, respectively for methanolic extract of leafy vegetable of wild *Paederia foetida* and *Erechtites hieracifolia*.

Keywords: Ethnobotany, nutritional composition, DPPH radical scavenging, leafy vegetable, wild, *Erechtites hieracifolia*, *Paederia foetida*

Introduction

The lack of awareness, limited nutritional data and the perception that most wild plants are of poor nutritional value caused many wild food plants were neglected by local people, research and operational organizations, and governments (Mc Burney *et al.*, 2004; Padulosi *et al.*, 2006; Misra *et al.*, 2008). Assessment of nutritional composition and health benefit of wild food plants is fundamental for promoting them to be accepted by locals and the larger population; important for community health, agriculture and nutrition education initiatives; important for improving the management and conservation of biodiversity for food and agriculture; and also fundamental to the elements and activities of the global 'Cross-Cutting Initiative on Biodiversity for Food and Nutrition' within the framework of the Convention on Biological Diversity (Toledo and Burlingame, 2006; Adeka *et al.*, 2009; Nesbitt *et al.*, 2010).

Though wild plant species in Indonesia are well reported and documented in Plant Resources of South East Asia (PROSEA) by PROSEA Foundation, research and publication on the nutritional composition and health benefit potential of wild food plants, including wild leafy vegetables, are very limited (Irawan *et al.*, 2006; Mahmud *et al.*, 2009; Andarwulan *et al.*, 2010). Leafy vegetables of

wild *Erechtites hieracifolia* and *Paederia foetida* are traditionally consumed in Indonesia as documented in PROSEA and Tumbuhan Berguna Indonesia (Heyne, 1987; Siemonsma and Piluek, 1994). Diets rich in vegetables are associated with maintenance of human health and prevention of non-communicable diseases such as cancer, heart diseases and chronic diseases of aging, because of their essential nutrients and phytochemicals (Berdanier *et al.*, 2007; Hounsoume *et al.*, 2008; Gupta and Prakash, 2009). According to our field survey, those wild food plants are available in area of Arjuna Mountain in East Java. Local people in the area have been traditionally utilizing and consuming the raw and boiled of the wild leafy vegetables, but they do not cultivate it. The aim of this research was to study the ethnobotany, nutritional composition and DPPH radical scavenging activity of leafy vegetables of wild *Paederia foetida* and *Erechtites hieracifolia* that ancient knowledge indicates diet and medicinal uses.

Materials and Methods

Ethnobotanical study

Paederia foetida and *Erechtites hieracifolia* plants were identified taxonomically by a botanist. Ethnobotany study by focus group discussion (FGD) and in-depth interview was conducted in Jatiarjo and Dayurejo villages, District of Pasuruan,

*Corresponding author.

Email: srianta_wm@yahoo.com

Tel: +62 31 5678478; Fax: +62 31 5610818

Province of East Java, Indonesia, in June 2010. Total respondents of FGD were 50 of local people consist of 27 male and 23 female (range of age: 38-80 years old) with consent form signed and documented. Non probability sampling was used and it was not designed to generalize of survey data (McGuirk and O'Neill, 2005). Respondents, who will be interviewed for in-depth interview, were randomly sampled. The objective of in-depth interview was to get more information about the wild plants. In-depth interview was stopped when consistent information provided by the respondent indicating objectivity and no more new information. There were 15 respondents of 38-80 years old selected to be interviewed.

Plant materials

Leafy vegetable samples of wild *Erechtites hieracifolia* and *Paederia foetida* were collected from forest situated in Arjuna Mountain (7° 45' 55.8" south latitude, 112° 35' 24.72" east longitude; ± 800 meters above sea level, temperature varied between 20-25°C, average rainfall of 2,700 mm a year), in Jatiarjo and Dayurejo villages, Pandaan City, Province of East Java, Indonesia, in July 2010. The collected samples were placed in dry-iced-cooled box immediately after being picked up from the plants, then brought to the laboratory on the same day, then they were immediately sorted for their edible portion (29) analysis. Cooled raw samples were analyzed for the proximate composition (moisture, ash, crude protein and crude fat), dietary fibers (total, soluble and insoluble dietary fibers), minerals (Ca, Na, K and Fe) and vitamin C contents. The samples were freeze-dried for 48 hours in a freeze dryer machine (VD-800F, Taitec, Japan), then extracted by using methanol (analytical grade, Merck) in a soxhlet apparatus at 60°C for 6 hours, prior to analysis of total phenol and DPPH radical scavenging activity.

Proximate analysis

Proximate analysis was carried out using standard methods (AOAC, 1990). Moisture content was determined by using a (16) vacuum drying oven (Mettler) at 940 mbar, 70°C until constant weight. Ash content was determined by using a muffle furnace (Termolyne) at 550°C. Crude protein was determined by using macro Kjeldahl. Crude fat of the samples was determined by Soxhlet extraction with n-hexane at 80°C for 6 hours.

Dietary fiber analysis

Analysis of Soluble Dietary Fiber (SDF) and Insoluble Dietary Fiber (IDF) were conducted by using multi enzymatic-gravimetric method according

to Asp *et al.* (1983). One gram of (2) each sample was weighed analytically (W), then transferred into a 250 mL Erlenmeyer flask. Twenty-five milliliter of Sodium Phosphate buffer (pH 6.9) was added into the Erlenmeyer flask, then added (11) mg of α -amylase (from hog pancreas, Fluka), incubated in a water bath at 53°C for 1 hour. The mixture was cooled until room temperature (31±1°C), adjusted the pH for 1.5 by using hydrochloric acid 4 N (26) 1 N. One hundred milligrams of Pepsin (Fluka) was added into the mixture, and then incubated at 40°C for 1 hour. The mixture was cooled to room temperature (31±1°C), adjusted the pH to 6.8 by using Sodium Hydroxide 4 N and 1 N. One hundred milligrams of Pancreatin (from porcine pancreas, Sigma), and then incubated at 40°C for 1 hour. pH of the mixture was adjusted for 4.5 by using HCl 1N, filtered with 0.5 gram of celite in a porous crucible with addition of 10 mL distilled water, twice. The residue was washed by using (6) 10 mL ethanol 95% twice and 10 mL acetone twice. The residue was dried in an oven at 105°C until (6) constant weight (D₁). The dried residue was burnt in a muffle furnace at 550°C for 5 hours, moved into oven at 105°C, cooled in a dessicator to room temperature for about 15 minutes, then ash was weighed (I₁). Filtrate: distilled water was added into the filtrate in a 100 mL measuring flask and adjusted the volume until 100 mL. 400 mL of Ethanol 95% (60°C) was added to the diluted filtrate, decanted for 1 hour. The mixture was then filtered with 0.5 gram of celite in a porous crucible. Residue was washed with 10 mL of ethanol 78% twice, 10 mL of ethanol 95% twice, and 10 mL of acetone twice. The residue was dried in an oven at 105°C until (6) constant weight (D₂). The dried residue was burnt in a muffle furnace at 550°C for 5 hours, moved into oven at 105°C, cooled in a dessicator until room temperature for about 15 minutes, then ash was weighed (I₂). Blank of IDF and SDF were performed with the same of each procedure (B₁ dan B₂).

$$\text{IDF (\%)} = \frac{D_1 - I_1 - B_1}{W} \times 100 \%$$

$$\text{SDF (\%)} = \frac{D_2 - I_2 - B_2}{W} \times 100 \%$$

Minerals analysis

Two grams of sample was digested in a digestion flask containing concentrated hydrochloric acid and nitric acid, then 5 mL of hydrochloric acid 4 N was added into the solution, adjusted until 100 mL with distilled water in measuring flask. Calcium, Potassium and Sodium contents were measured by

using a flame photometer (BWB XP, UK) after 2 mL of lanthanum chloride 10% was added to the solution. Determination of Fe content was done according to Riganakos and Veltsistas (2003), by measuring the complex of FeSCN. The complex was formed by oxidation of Fe²⁺ in the solution to Fe³⁺ using saturated K₂S₂O₈ and added by KSCN. An UV-Vis spectrophotometer (Shimadzu UV 1800, Japan) was used to measure the complex formed. K₄Fe(CN)₆ was used as a standard.

Determination of vitamin C content

Vitamin C content was determined according to Suntornsuk *et al.* (2002) by direct titration with iodine. Sample was extracted by using distilled, transferred into a 250 mL Erlenmeyer flask. Twenty-five milliliter of 2 N sulfuric acid was added, mixed, diluted with 50 mL of water and 3 mL of starch T.S. was added as an indicator. The solution was directly titrated with 0.1 N Iodine previously standardized with primary standard Arsenic Trioxide. A blank titration was performed prior titration of each sample. One mL of iodine 0.1 N is equivalent to 8.806 mg ascorbic acid.

Determination of total phenol content

Total phenol content of vegetable and fruit extracts was determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1977) using Gallic Acid as a standard. A 20 µL of the methanolic extract was placed into a 10 mL measuring flask and 5 mL of Folin-Ciocalteu reagent was added to the extract. The measuring flask was allowed to stand at room temperature for 15 minutes. Then, 2.5 mL of 20% (w/v) Na₂CO₃ was added to the mixture. The mixture was adjusted until the volume reaches 10 mL with distilled water. After 30 minutes at room temperature, absorbance was measured at 765 nm versus a blank by using a spectrophotometer (Shimadzu UV 1800, Japan). Total phenol value was expressed as mg Gallic Acid Equivalent/g FW.

DPPH radical scavenging activity assay

DPPH radical scavenging activity was assayed according to Gupta and Prakash (2009). Different concentrations of methanolic extract were taken in different test tubes and the volume made to 1 mL with methanol. 4 mL of 0.1 mM methanolic solution of DPPH was added. The tubes were shaken vigorously and allowed to stand for 15 minutes at room temperature. A control was prepared as above without any sample and methanol was used for base line correction. Changes in absorbance of samples were measured at 517 nm. DPPH free radical scavenging

activity was expressed as inhibition percentage and was calculated using the following formula:

$$\% \text{ DPPH radical scavenging activity} = \left(\frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \right) \times 100$$

IC₅₀, concentration at which 50% radical scavenging occurred, was calculated.

Data analysis

Mean and standard deviation values were calculated of triplicate analysis.

Results and Discussion

Figure 1 shows the photograph of wild *Erechtites hieracifolia* and *Paederia foetida* growing in Arjuno mountain forest. Figure 2 shows edible portions of the studied plants. Table 1 shows the nutritional composition of leafy vegetables of wild *Paederia foetida* and *Erechtites hieracifolia*. Figure 3 shows DPPH radical scavenging activity of methanolic extract of *Paederia foetida* and *Erechtites hieracifolia* leafy vegetables.

Table 1. Nutritional composition of *Paederia foetida* and *Erechtites hieracifolia*

Component	Contents	
	<i>Paederia foetida</i>	<i>Erechtites hieracifolia</i>
Proximate composition		
Moisture (g/100 g)	82.55±0.12	87.14±1.42
Ash (g/100 g)	4.68±0.13	3.16±0.21
Crude Protein (g/100 g)	2.57±0.07	1.94±0.11
Crude Fat (g/100 g)	0.60±0.09	0.80±0.01
Carbohydrate (g/100 g)	9.60±0.10	6.96±0.44
Dietary fiber		
TDF (g/100 g)	8.47±0.00	6.20±1.71
SDF (g/100 g)	1.01±0.69	5.89±1.61
IDF (g/100 g)	7.46±0.34	0.31±0.05
Mineral		
Calcium (mg/100 g)	200.40±5.63	99.54±1.51
Sodium (mg/100 g)	27.41±0.50	5.30±0.27
Potassium (mg/100 g)	844.39±17.62	529.72±2.70
Iron (mg/100 g)	4.67±0.06	3.04±0.02
Vitamin C (mg/100 g)	271.40±42.65	20.62±1.63
Total phenol (mgGAE/g)	3.15±0.23	1.58±0.11

Note: data was calculated based on FW materials

Ethnobotany

All 50 respondents well recognized both of *Erechtites hieracifolia* and *Paederia foetida*. They usually eat the leaves, young stems and flowers of *Erechtites hieracifolia*, and the leaves of *Paederia foetida*. Wild *Erechtites hieracifolia* grows standing among other bush plants. It has light to dark green toothed oval-shaped leaves, and can be found widely during rainy season. *Erechtites hieracifolia*



Figure 1 Photograph of *Erechites hieracifolia* (a) and *Paederia foetida* (b) growing in Arjuno mountain forest



Figure 2. Photograph of edible portion of *Erechites hieracifolia* (a): leaves, young stems and flowers; and *Paederia foetida* (b): leaves

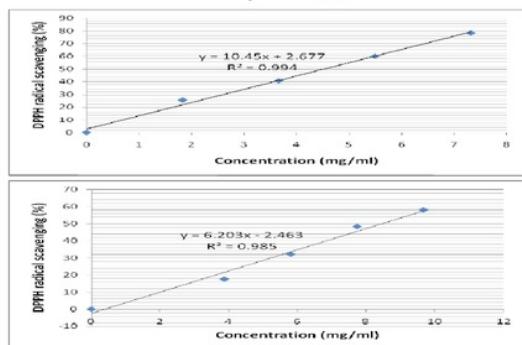


Figure 3. DPPH radical scavenging activity of methanolic extract of (a) *Paederia foetida* and (b) *Erechites hieracifolia* leafy vegetables

can be found with yellowish-orange flowers and magenta red on the top. After the shoot cut, it will produce new leaves and flowers after about 10 days. All respondents said that they like it because of its savory flavor. Several respondents said that it is more delicious than spinach. They eat boiled leaves, young stems and flowers, together with rice and chilli paste (local name “sambal”); or with instant noodle. According to them cooking make the leaves very soft, as documented in Plant Resources of South East Asia (Siemonsma and Piluek, 1994).

Wild *Paederia foetida* is climbing plant epiphyte on other plants such as bush plants or wooden tree. It is vine crop with light to dark green heart-shaped leaves. It can be bloom with small white flowers. It is easy to grow and could inhibit growth of other plants. It produces disliked smell (like the smell of fart) after cutting. It is an important plant for the local people for food and traditional medicine. For traditional medicine, *Paederia foetida* was used raw or after being pounded and applied to the abdomen to cure bloating, as documented in Tumbuhan Berguna Indonesia (Heyne, 1987). Other uses are for increasing

appetite and for protecting the children against disease after they recover from illness (improving the immunity). They usually eat boiled leaves with rice and “sambal”. According to them, boiling can degrade the off-odor components producing different and less powerful odor. This decreased odor is also significantly proven in cooked *Paederia foetida* daily sold by street vendors in villages. They also usually cook them to make a dish namely *bothok*. Prior to processing, the leaves are flushed with hot water then squeezing it until wilted to remove odor. Currently, local people hardly find the plants because they do not cultivate it and have limited knowledge on nutrition and processing of both leafy vegetables since local people usually eat boiled leafy vegetables. They also think that the plants have no commercial value. In the villages surveyed area, only one women resident of 80 years old keeps cultivating the plant in her place.

Nutritional composition

Moisture content of leafy vegetables of wild *Erechites hieracifolia* and *Paederia foetida* were 87.14% and 82.55%, respectively, thus the dry matter are 12.86% and 17.45%. Its high moisture content indicated that both leafy vegetables are perishable food materials. The moisture contents are in a range of moisture content of vegetables commonly consumed in Indonesia i.e. from 60% to 94.8% (Mahmud *et al.*, 2009) and also in a range of moisture content of traditional vegetables commonly consumed in Central Kalimantan, Indonesia i.e. 66.98% to 94.37% (Irawan *et al.*, 2006). The dry matter contents of both leafy vegetables are in a range of the amount of dry matter in most vegetables which is between 10 and 20% (Belitz *et al.*, 2009).

Based on the nutritional composition data, 100 g of fresh leafy vegetable of wild *Paederia foetida* contains crude protein, fat, carbohydrate and energy respectively of about 5%; 1%, 3% and 3% of RDIs for adults and children over 4 years of age. Whereas 100g of leafy vegetable of wild *Erechites hieracifolia* contains crude protein, fat, carbohydrate and energy respectively of 4%; 1%; 2% and 2% of RDIs for adults and children over 4 years of age. Crude protein of leafy vegetables of wild *Erechites hieracifolia* and *Paederia foetida* are comparable to the commonly cultivated vegetables i.e. lettuce, parsley, cabbage and spinach (Mahmud *et al.*, 2009) and to the vegetables traditionally consumed by local people in Central Kalimantan, Indonesia (Irawan *et al.*, 2006). Proteins in the diet provide the essential and non essential amino acids that are used to synthesize protein of structural material for the human body and as enzymes, hormones and antibodies. The lipid content

of vegetables is generally low (0.1-0.9%) (Belitz *et al.*, 2009). Crude fat of *Erechtites hieracifolia* and *Paederia foetida* leafy vegetables were in the range of crude fat of commonly cultivated vegetables in Indonesia (Mahmud *et al.*, 2009) and traditional vegetables commonly consumed by local people in central Kalimantan, Indonesia (Irawan *et al.*, 2006). The nutritive/physiological importance of lipids is based on their role as fuel molecules (37 kJ/g) and a source of essential fatty acids and vitamins.

Carbohydrate represents one of the basic nutrients and is quantitatively the most important source of energy, even the non digestible carbohydrates acting as bulk material that is important in a balanced daily nutrition. Based on the results, both leafy vegetables are potentially good source of dietary fiber. It is recommended to consume them for sufficient dietary fibers.

Ash content is total mineral index of food material. Ash content of *Erechtites hieracifolia* and *Paederia foetida* were 3.16% and 4.68%, respectively, indicating that the leafy vegetables are potential as good sources of minerals. Calculation based on the minerals analysis data and RDIs for adults and children over 4 years of age, 100 g of *Paederia foetida* potential to supply about 20%; 1%; 24% and 26% of RDIs of Ca, Na, K and Fe, respectively, whereas 100g of *Erechtites hieracifolia* provide about 10%; 0.2%; 15% and 17% of RDIs of Ca, Na, K and Fe, respectively. Na and K are three of six main elements which are essential for human beings in amounts >50 mg/day, while Fe is a trace element which is essential to make hemoglobin and myoglobin, and a number of enzymes peroxidase, catalase, hydroxylase and flavin enzymes. However, excessive intake of sodium should be avoided because it can lead to hypertension (Berdanier *et al.*, 2007; Hounsome *et al.*, 2008; Belitz *et al.*, 2009). Minerals from the studied plants are apparently promising to fulfill the nutritional needs that usually lost in modern diets such as fast foods, generally favorite foods for kids.

Based on the vitamin C analysis data, 100g of *Paederia foetida* and *Erechtites hieracifolia* have potentiality to supply vitamin C about 452% and 34% RDIs for adults and children over 4 years of age. Vitamin C (ascorbic acid, L-3-keto-threo-hexuronic acid- γ -lactone) serves in both oxidation and reduction reactions, depending on the environmental conditions where the vitamin C exists, therefore at high concentration it may act as either an antioxidant or a prooxidant. Total phenol values of leafy vegetables of wild *Paederia foetida* (18.06 mg GAE/g DW) and *Erechtites hieracifolia* (12.32 mg GAE/g DW) were higher than that of vegetables traditionally used for

food and medicine by west Javanese, with the highest total phenol value of 8.71 mg GAE/g DW of *Cosmos caudatus* H.B.K (Andarwulan *et al.*, 2010).

DPPH radical scavenging activity

Figure 3 shows higher capacity to scavenge DPPH radical of extract of leafy vegetable of wild *Paederia foetida* than *Erechtites hieracifolia*, with IC₅₀ values of 4.53 mg/mL and 8.46 mg/mL respectively for *Paederia foetida* and *Erechtites hieracifolia*. It might be due to higher total phenol value of *Paederia foetida*, as Andarwulan *et al.* (2010) reported that total phenol values of vegetables were highly correlated with DPPH radical scavenging capacity. IC₅₀ value of *Paederia foetida* and *Erechtites hieracifolia* was lower than that of Indian green leafy vegetables reported by Gupta and Prakash (2009) i.e. *Murraya koenigii* (9.62mg/mL), *Trigonella foenum graecum* (27.69 mg/mL), *Centella asiatica* (19.89 mg/mL), and *Amaranthus* sp (27.27 mg/mL), which indicate that antioxidant capacity of both leafy vegetables were higher than those of Indian leafy vegetables. High antioxidant capacity of leafy vegetable of *Paederia foetida* might be a reason for this plant was commonly used for traditional medicine by local people. Several studies showed that antioxidants are important in biological systems by counteracting oxidative stress that causes several human diseases such as atherosclerosis, diabetes mellitus, chronic inflammation, neurodegenerative disorders, and certain types of cancer (Karadag *et al.*, 2009).

Conclusions

The wild plants are valuable and promising biological resources for the human life. Both wild plants are potential to supply nutrients and phytochemicals for local people's daily life, which is comparable with the commercial and commonly consumed leafy vegetables in Indonesia. The nutritional potency might be used to encourage local people to take care for the two important wild plants resources.

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