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Research

The Morphological Dimension and Antioxidant Composition of Selected Indigenous Flavouring Plants in Bintulu, Sarawak

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ABSTRACT

The use of plants as food flavourings, commonly known as herbs and spices, serves as natural sources of flavour, altering the taste and aroma of dishes with only a small amount. Beyond flavour, these plants also contribute essential antioxidants crucial for human health by inhibiting free radicals that can lead to various diseases. In Bintulu, Sarawak, Malaysia, locals traditionally consume indigenous flavouring plants primarily for their culinary impact, often overlooking the pharmaceutical value these plants may offer. This study aimed to assess the marketable appearance and antioxidant composition of indigenous food flavouring plants in Bintulu. Bunches of *Pangium edule, Premna serratifolia, Pycnarrhena tumefacta, Scorodocarpus borneensis*, and *Syzygium polyanthum* were obtained from the local farmers market and analyzed for morphological dimensions, total phenolic content, free radical scavenging ability (DPPH), and ferric reducing ability (FRAP). The edible portion of the plants ranged from 57.33% to 84.99%, with *P. edule* exhibiting the largest edible blade. Total phenolic content varied from 343.27 to 3245.67 mg GAE/100 g, with *P. serratifolia* having the highest value. *Premna serratifolia* demonstrated the strongest radical scavenging activity, while *S. polyanthum* exhibited the highest ferric reducing ability. All species exhibited high antioxidant composition (IC50 = 0.10 to 27.6 µg/mL, FRAP = 469.88 to 9272.50 mg TE/100 g), indicating potential medicinal utility. Further studies on anti-nutrients like oxalate and phytate are recommended to complement the obtained data. Additionally, an ethnobotanical study is suggested to document the traditional medicinal uses of these plants alongside their role as flavor enhancers in cooking.

Key words: Antioxidant activity, DPPH, FRAP, gallic acid, total phenolic content

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INTRODUCTION

A wide variety of plant species have been utilized by man for their flavouring properties since the dawn of time (Kaur *et al*., 2018). Flavouring plants contain abundant sources of bioactive phytochemicals such as phenolic compounds, carotenoids, sterols, terpenes, alkaloids, glucosinolates, and other sulfur-containing compounds. The majority of these compounds have strong antioxidant activity responsible for therapeutic effects such as anti-inflammatory, anti-allergic, anti-microbial, anti-bacterial and anti-cancer properties (Jun *et al*., 2006; Herrera *et al*., 2020). Among the phenolic components in flavouring plants are phenolic acid, rosmarinic acid, phenolic diterpenes, carnosic acid and its derivative, carnosol (Hedges & Lister, 2007).

Antioxidants are a group of chemical compounds that are naturally present in food which can inhibit or lessen the physiological systemic exposure to free radicals produced by oxidative stress which is harmful to the human body (El Far & Taie, 2009; Mamta *et al*., 2013). In modern times, synthetic antioxidants are available but are strictly regulated as they are suspected of being carcinogenic (Namiki, 1990; Gülçin, 2002: Pokorny, 2007). Antioxidants are major components in flavouring plants that function to control oxidation and subsequently increase shelf-life, provide medicinal benefits, and control phytochemical contents responsible for flavouring.

In Bintulu as in most parts of Sarawak, locals consume

indigenous flavouring plants (IFPs) with little or no regard for their pharmaceutical value. These IFPs are collected from the wild or bought from local markets. Currently, there have been antioxidant studies regarding commercial crops of Sarawak namely *Piper nigrum* and *Solanum lasiocarpum,* however, studies on the antioxidant composition of indigenous flavouring plants are lacking. Information about the antioxidant composition of five selected IFPs in this study may provide a comparison and guideline for other related studies. Therefore, this study aims to record the morphology of the edible portions and antioxidant compositions in five selected IFPs consumed by the locals in Bintulu, Sarawak, Malaysia.

MATERIALS AND METHODS

Morphological observation of edible fresh bunch

Fresh leaves of *P. edule*, *P. serratifolia*, *P. tumefacta*, *S. borneensis*, and *S. polyanthum* were collected from local markets in Bintulu (Figure 1). Information about the flavour and mode of consumption of the IFPs was obtained through a literature search and from interview sessions with the sellers. The morphological dimensions of the edible fresh bunches were evaluated both qualitatively and quantitatively. The qualitative parameters involved leaf shape, leaf apex, leaf margin, and leaf arrangement were identified whereas the quantitative parameters such as fresh bunch weight, edible blade length, edible blade width, petiole length, and petiole diameter were recorded using a Kernin digital calliper and ruler.

Fig. 1. Fresh leaves a bunch of selected IFPs. (a) *P. edule*, (b) *P. serratifolia*, (c) *P. tumefacta*, (d) *S. borneensis*, (e) *S. polyanthum*.

Sample preparation for antioxidant analysis

In the laboratory, collected samples were cleaned and washed to remove any dirt, midrib, and inedible parts. The residual moisture on the fresh samples was dabbed with tissue paper and evaporated at room temperature (±27 °C). The samples were air-dried using the method by Rajurkar and Hande (2011). Samples were exposed in an open area at the Food Processing Lab, Universiti Putra Malaysia Bintulu Campus under ambient temperatures of 30 °C to 31 °C for 8 h per day of sunlight.

Samples were dried for 10 to 15 days approximately depending on the presence of sunlight. Later, the dried samples were ground into fine powder using a heavy-duty blender (Abuye *et al*., 2003). Ground samples were stored in air-tight containers for further analysis and labelled.

Preparation of methanolic extract

About 5 g of the oven-dried grounded samples were weighed and macerated in 50 mL of methanol. Then, the samples were extracted using an orbital shaker at 125 r.p.m. for 48 h. The samples were subsequently centrifuged at 100 rpm for 10 min and filtered using Whatman No. 2 filter paper.

Total Phenolic Content (TPC)

The phenolic content of samples was determined using a modified version of the Folin Ciocalteu's method by Asami *et al*. (2003). The standard gallic acid solution (100 µg/mL) was prepared by weighing 1 mg of gallic acid dissolved in 10 mL methanol in a volumetric flask. A series of gallic acid in concentrations of 20, 40, 60, 80, and 100 µg/mL was prepared into the test tubes. 1 mL of sample aliquots also was pipetted into different test tubes. Afterwards, 2.5 mL of Folin Ciocalteu's reagent was pipetted into the test tubes and vortex for 1 min. Then, the reaction of the chemicals was to wait for 6 min before adding 2 mL of 7% sodium carbonate (Na_2CO_3). The tubes were covered with parafilm and the mixture was incubated for 90 min at room temperature for colour development. The absorbance was measured at 740 nm using a UV-VIS Spectrophotometer (Lambda 25 Perkin Elmer, Germany). The experiment was performed in triplicate. The TPC content in the samples was calculated by equation from the calibration curve and expressed in gallic acid equivalent (mg GAE / 100 g) dry mass.

DPPH radical scavenging activity assay

The total antioxidant activity (TAA) of the sample extracts against DPPH (2,2-diphenyl-2-picrylhyrazyl) radicals was determined following the modified methods of Brand–Williams *et al*. (1995). The 0.1 mM DPPH stock solution was prepared. The sample extracts were diluted into a series of known concentrations with 80% methanol. Then, 1 mL sample extract was added with 3 mL DPPH stock solution. The solutions were incubated for 30 min at room temperature under dark conditions. The absorbance of the mixture was measured at 517 nm using a UV-VIS Spectrophotometer (Lambda 25 PerkinElmer). The concentration of sample required to inhibit 50% of DPPH (IC_{50}) was calculated by plotting the linear equation of sample concentrations against the percentage of inhibition (%). A lower IC_{50} value indicated a higher antioxidant activity. The experiment was performed in triplicate. Inhibition of free radicals by DPPH in percentage was calculated using Equation 1:

$$\text{Inhibition of DPPH (\%)} = \frac{(AB-AA)}{AB} \times 100$$

where:
AB = absorbance of blank
AA = absorbance of the sample

Ferric reducing antioxidant power (FRAP) assay

The determination of ferric reducing antioxidant power of the extracts was conducted using FRAP assay by Benzie and Strain (1996) with some modifications. The FRAP reagent was prepared at 37 °C with 300 mmol L^{-1} acetate buffer (pH 3.6), 10 mmol Tripyridyl-s-Triazine (TPTZ) in a 40 mmol L^{-1} HCl solution and 20 mmol L^{-1} $FeCl_3$ in the ratio of 1:1:10 under dark condition. A series of Trolox in concentrations of 20, 40, 60, 80, and 100 µmol was prepared into the test tubes and (TPTZ) working reagent was used as blank. Then, 0.24 mL sample extract was pipetted into different test tubes. Next, 1.8 mL FRAP reagent was added and the mixture was incubated at 37 °C for 10 min. The absorbance was measured at 593 nm using a UV-VIS Spectrophotometer (Lambda 25 Perkin Elmer, Germany). The experiment was performed in triplicate. The ferric reducing power in the samples was calculated based on the equation from the calibration curve and expressed in TE equivalent (mg TE / 100 g) dry mass.

Statistical analysis

The data on morphology, TPC, DPPH, and FRAP were analyzed using Statistical Analysis Software (SAS 9.3). The mean of each parameter measured was analysed using a one-way analysis of variance (ANOVA). A significant difference between means was compared using Tukey's Range Test at a significant level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Marketing information of selected indigenous flavouring plant

According to Agbugba *et al*. (2011), indigenous plants tend to be harder to market than to produce and most of the small-scale farmers only sell their products locally. From our observations, the selected flavouring plant price sold at local markets ranged from RM 2.00 – RM 5.00 per bunch at different weights (Table 1). The price offered was affordable, especially for those belonging to the lower income group and this greatly influenced the consumer decision to purchase these plants (Chikkamath *et al*., 2012). The IFPs sold contributed to the economic status of rural communities (Det *et al*., 2013). The percentage of the edible portion for each of the plants ranged from 57.33 - 84.99% of the fresh weight of which *S. polyanthum* had the highest edible portion and *P. cordifolia* with the lowest percentage per bunch. The result was comparable with other leafy vegetables reported by Gupta *et al*. (2005) which showed a range of between 37 - 81% edible portion for various leafy vegetables.

Table 1. The weight, edible portion, and price of the selected IFP per bunch purchased in Bintulu local market

IFP	Weight (g)	Edible portion (%)	Price (RM)
Pangium edule	120.65 ± 9.90^{c}	74.03 ± 3.90	5.00
Premna serratifolia	68.14 ± 3.10^{e}	57.33 ± 1.62	2.00
Pycnarrhena tumefacta	80.21 ± 3.48^{d}	73.94 ± 1.46	3.00
Scorodocarpus borneensis	144.02 ± 10.68^{b}	68.00 ± 7.79	5.00
Syzygium polyanthum	191.59 ± 17.41^{a}	84.99 ± 1.89	4.00

From the preliminary interview with the local sellers, only the edible parts: young leaves and shoots were harvested a day before in the late afternoon or early morning before they were brought to the wet market. At the wet market, the local seller would wash the leaves and tie them using rubber bands or traditionally using the Nipah leaf before placing them on the shelves. Some sellers only sold the young leaves and shoot meanwhile other sellers sold the leaves together with the young stem. To reduce wilting, the sellers would sprinkle water or put the leaves under shade which helped to prolong the shelf-life of the vegetables until they were purchased. From the interview also local sellers informed that selected IFPs had different taste, aroma, and various utilisation by the locals. Detailed botanical information, taste and aroma, and mode of consumption of the selected IFP were shown in Table 2.

Morphological characteristics

The morphology of selected IFPs showed varied dimensions (Table 3) which allowed easier identification of the plant species. The leaves of all selected IFPs were simple. The adaxial and abaxial leaf surfaces of all IFPs had similar characteristics which are smooth and glossy. However, young blades of *S. borneensis* showed a colour difference compared to other species. According to Ainul Asyira *et al*. (2016), the red colouration in young blades of *S. borneensis* may be due to the anthocyanin pigment in the leaves. The present study recorded that *P. edule* and *P. tumefacta* had the longest blade length at 11.23 cm and 11.45 cm respectively while *S. polyanthum* recorded the lowest length at 6.94 cm. *Pangium edule* has the widest blade (15.40 cm), petiole length (9.08 cm), and petiole width (0.62 cm).

Antioxidant composition of selected IFPs

The results for total phenolic content, DPPH, and FRAP are presented in Table 4. For total phenolic content, all selected IFPs showed significantly different concentrations. Our study revealed that the concentration of phenolic varied widely from 343.27 to 3245.67 mg GAE/ 100 g where *P. serratifolia* showed the highest concentrations whereas the lowest value was recorded in *S. borneensis*. The total phenolic compounds in *P. serratifolia* were found to be significantly high in comparison to a previous study by Simamora *et al*. (2020) (212 mg GAE/ 100 g) but lower than the findings of Chua *et al* (2015) (5900 mg GAE/ 100 g). Phenolic compounds can be found abundantly in plant food and beverages which play vital parts in pabulum and healthcare (Lin *et al*., 2016). In biological systems, phenolic substances show free radical inhibition, peroxide breakdown, metal inactivation, or oxygen scavenging and reduce the burden of oxidative illness (Babbar *et al*., 2015). Phenolic substances are effective electron donors because the hydroxyl groups can directly support antioxidant activity by promoting the scavenging of free radicals (Bendary *et al*., 2013). Thus, phenolic content showed a positive correlation with the DPPH assay (Aryal, 2019). Various studies have also reported that phenolic chemicals play a crucial role in the defence responses in humans, including anti-ageing, anti-inflammatory, antioxidant, and anti-proliferative.

Table 2. Botanical information, taste and aroma, and mode of consumption of the selected IFPs.

Scientific name	Family	Common name	Taste and aroma	Mode of consumption
Pangium edule Reinw.	Achariaceae	Kepayang	Savoury taste with a slightly earthy aroma	Young leaves are used as food wrappers for seasoned fish dishes called "pais". Young leaves are also used in the fermentation of fish or meat dishes called "pekasam" or "kasam" (Yusli *et al*., 2021).
Premna serratifolia L.	Lamiaceae	Singkil, tebawan, bebuas, buas-buas	Sweet taste with a slightly pungent aroma.	Young leaves are chopped and stir-fried as vegetables. The leaves are used as the primary ingredient in a Sarawak traditional dish called "bubur pedas" (Yusli *et al*., 2021).
Pycnarrhena tumefacta Miers	Menispermaceae	Tubu, mekai, sengkubak, itun kelaleh	Savoury taste (umami)	The leaves are used as MSG substitute in any dishes (Maharani *et al*., 2020; Rohmah *et al*., 2021; Yusli *et al*., 2021)
Scorodocarpus borneensis Becc.	Olacaceae	Kesindu'	Garlicky and slightly sweet taste	Young leaves are used as garlic substitutes (This study; Lim, 2012; Yusli *et al*., 2021).
Syzygium polyanthum (Wight) Walp.	Myrtaceae	Bungkang, daun salam	Savoury, slightly sour taste and citrusy aroma.	Added into soup or during marination of "pansuh" dish (This study, Yusli *et al*., 2021)

Table 3. Morphological dimension on the edible portion of selected IFP

Morphology parameter	*P. edule*	*P. serratifolia*	*P. tumefacta*	*S. borneensis*	*S. polyanthum*
Leaf type	Simple	Simple	Simple	Simple	Simple
Leaf arrangement	Spiral	Opposite	Alternate	Alternate	Opposite
Leaf shape	Lobed	Elliptic	Lanceolate – elliptic	Oblong - elliptic	Elliptic
Leaf margin	Palmate lobed	Entire - undulate	Entire	Entire	Entire
Leaf surface (texture)	Smooth and glossy	Smooth and glossy	Smooth	Smooth and glossy	Smooth
Leaf surface (colour)	Dark green	Dark green	Dark green	Reddish (young blade) Light green (older blade)	Dark green
Blade length	11.23 ± 0.31^{a}	10.50 ± 1.17^{b}	11.45 ± 0.30^{a}	9.97 ± 1.10^{c}	6.94 ± 0.77^{d}
Blade width	15.40 ± 0.45^{a}	6.49 ± 0.67^{b}	5.56 ± 0.18^{c}	3.08 ± 0.41^{d}	3.32 ± 0.66^{d}
Petiole length	9.08 ± 0.36^{a}	2.68 ± 0.32^{b}	2.73 ± 0.11^{b}	1.72 ± 0.16^{c}	1.48 ± 0.27^{c}
Petiole width	0.62 ± 0.02^{a}	0.20 ± 0.02^{c}	0.12 ± 0.01^{d}	0.30 ± 0.04^{b}	0.24 ± 0.13^{b}

The result represented as mean ±S.E. where different letters within the same row indicated significant differences at $p \leq 0.05$ using Tukey Studentized Range Test

Table 4. Total phenolic content, IC_{50} (DPPH), and ferric-reducing ability in selected IFPs.

Species	TPC (mg GAE/ 100 g)	IC_{50} (µg/ mL)	FRAP (mg TE / 100 g)
Pangium edule	569.33 ± 10.43^{c}	1.1 ± 0.00^{c}	1968.95 ± 11.02^{d}
Premna serratifolia	3245.67 ± 11.92^{a}	0.10 ± 0.00^{c}	7377.83 ± 4.41^{b}
Pycnarrhena tumefacta	602.33 ± 6.36^{c}	0.34 ± 0.00^{b}	3384.62 ± 5.90^{c}
Scorodocarpus borneensis	343.27 ± 7.52^{d}	27.6 ± 0.11^{a}	469.88 ± 7.53^{e}
Syzygium polyanthum	2182.33 ± 9.77^{b}	0.17 ± 0.00^{bc}	9272.50 ± 7.23^{a}

Data presented as mean ± S.E where different letters indicate significant differences within the column at $p \leq 0.05$ using the Tukey Studentized Range Test.

DPPH scavenging assay is a method of measuring the antioxidant activity which is widely used to test the activity of natural compounds in both food and biological systems (Kedare & Singh, 2011). It is based on the principle of antioxidants reducing the violet DPPH radical via a hydrogen atom transfer process, resulting in stable pale-yellow DPPH molecules (Sirivibulkovit *et al*., 2018). Determination of IC_{50} value for each selected IFP was determined to perform the doses of extract which were able to reduce the intensity up to 50% of free radical absorption. This study recorded varied IC_{50} values ranging from 0.10 to 27.6 µg/ mL for the selected IFPs extract. *Premna serratifolia* showed the lowest value whereas *S. borneensis* showed the highest value. According to our findings, the IC_{50} for each extract was less than 100 µg/mL which indicated that all the selected species were active as antioxidants due to the IC_{50} being lower than 50 ppm (Ruchiyat, 2013). A lower value of IC_{50} also indicated the higher effectiveness of extracts in reducing free radical absorption (Berrouet *et al*., 2020). However, the results obtained in this study produced differing values compared to those of previous studies. This may be due to the different abiotic conditions in each study.

FRAP assay works by reducing Fe^{3+} to Fe^{2+} in the presence of TPTZ, resulting in a blue-coloured ferrous tripyridyl triazine complex (Henderson *et al*., 2015; Lubaina *et al*, 2016). According to Halvorsen *et al*. (2002), in contrast to other assays evaluating the suppression of free radicals, the FRAP test is the only one that directly assesses antioxidants (or reductants) in a sample. The value obtained from the FRAP assay ranged from 469.88 to 9272.50 mg TE/ 100 g where *Syzygium polyanthum* was found to have the highest value and *S. borneensis* had the lowest value. The data from the FRAP assay reflects the concentration of antioxidants that donate electrons when ferric iron (Fe^{3+}) is reduced to ferrous ion (Fe^{2+}). Therefore, the selected IFPs showed a high ability to reduce ferric and possessed high concentrations of antioxidants which were beneficial for human consumption.

CONCLUSION

The information regarding the morphology of the vegetative parts of the plants studied here can be used as a reference for future generations to help distinguish between species. The current findings showed that all five selected indigenous flavouring plants contained very high antioxidant content based on the analysis conducted. From the result, it is suggested that these species be earmarked for further studies regarding their potential use as medicine. Conversely, studies on anti-nutrients such as oxalate and phytate should also be conducted to complement the data obtained. An ethnobotanical study can also be conducted to document the use of these plants as traditional medicine.

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ETHICAL STATEMENT

Not applicable

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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