Antioxidant properties and toxicity of selected bamboo shoots “iwung” extract: a comparative study

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Abstract. This study aims to compare the nutritional, antioxidant properties, and toxicity of various bamboo shoots extract. Physicochemical, total phenolics content, total flavonoids content, antioxidant activity (IC50) and toxicity were analyzed. Folin-Ciocalteau assay and aluminum chloride assay were used to determine the total phenolics and total flavonoid content, respectively. The antioxidant activity determined by DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging assay. Moreover, the toxicity of bamboo shoots extract was determined by brine shrimp lethality bioassay (LC50). The results showed that bamboo shoots, viz., betung, gombong, buluh, and kuning, are rich in minerals of zinc, iron, and potassium. The ethanolic extract of buluh shoots (BUS) contained the highest total phenolics (27.83 mg GAE/g) followed by KUS > GOS > BES. Bamboo betung shoots extract (BES) is found to have the highest total flavonoids (2.49 mg QE/g) with a ratio of TTF/TP which is 0.17. Meanwhile, the DPPH assay (IC50) showed that IC50 of KUS (347.48 μg/mL) was lowest. Based on these values, KUS is found to have the highest antioxidant activity. Furthermore, the level toxicity of LC50, BES, BUS, and KUS extract is valued as cytotoxic. These results provide useful data about the nutritional, phytochemical, and toxicity of bamboo shoots for functional food purposes.

1. Introduction

In decades, there has been a marked interest in health and diseases related transition of nutrition amongst the urban population. The exploration of functional food or nutraceutical research based on local wisdom and commodities has become a concern. Bamboos are gaining popularity, especially in the utilization of its shoots as healthy and nutritious food [1]. In Southeast Asian cuisines, including Indonesia, there is a long history of bamboo shoots usage as an ingredient in traditional food and medicine. It is usually consumed in various forms as raw, dried, boiled and fermented.

There are a number of bamboo species available in Indonesia, and many of them are used for edible purposes. *Bamboo vulgaris*, *Dendrocalamus asper* (Schult.), *Dendrocalamus longispathus*, *Gigantochloa nigroceliata* are used for an edible purpose in West- and East Java, Indonesia [2,3]. In Java, bamboo shoot is used in a variety of traditional foods such as iwung soup, lumpia, pickles, crackers, and cakes.
Bamboo shoots have good profile minerals, vitamins, dietary fiber, phenol and phytosterol [4]. The consumption of these reported can decrease total serum cholesterol, low-density lipoprotein (LDL), increased fecal volume and bowel movement frequency [5]. However, the bamboo shoots also contain anti-nutrient, cyanogenic glycosides. This compound was responsible for the acridity and peculiar smell in the shoots [6]. Bamboo shoots also reported may interfere with hormone metabolism, reproductive function, and hepatic xenobiotransformation enzymes [7].

Since that edible bamboo shoots species has cyanogenic toxicity, hence it is important to evaluate toxicity and compare of various bamboo shoots extract. Therefore, the aim of this research is to compare the nutritional, antioxidant properties and toxicity of selected bamboo shoots extract for functional food purposes. A relationship between total phenolics content, free radical scavenging activity is also demonstrated.

2. Material and methods

2.1 Materials

Quercetin, gallic acid, Folin-Ciocalteau’s reagent, and 1,1-diphenyl 2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich, Singapore. Artemia salina L. larva (Hobby Artemix) was purchased from Dohse Aquaristik GmbH & CO. Gilsdorf, Germany. Alumunium chloride, sodium hydroxide, ethanol, and sodium carbonate were purchased from Merck. All the reagents were analytical grade.

Fresh young bamboo shoots were collected from Rawalele and Dawuan Kaler Village, Dawuan District Subang-West Java, Indonesia, during the month of January – June 2018 (Fig.1). Identification of botanical was performed by “Herbarium Bogoriense”, Research Center for Biology, Indonesian Institute of Sciences (No. 957/IPH.1.01/If.07/IV/2018).

![Figure 1. Young bamboo shoots (A) Dendrocalamus asper (Schult.) Backer ex Heyne; (B) Gigantochla psedoarundinacea (Steud.) Widjaja; (C) Gigantochla apus; (D) Bambusa vulgaris var. striata.](image)

2.2 Samples preparation

Fresh bamboo shoots were rinsed, sliced, and steamed for 10 minutes, then dried at 45 °C for 2 days with the tray drier. After those, dried bamboo shoots ground into powder. The powder soaking in
ethanol (1:10) for overnight, then extracted for 24 hours (three times) by maceration. The filtrates were consolidated and dried by vacuum evaporator. Accurately weigh of young bamboo shoots extract (0.06 g) put into a centrifuge tube, then added ethanol (10 mL). The sample was shaken for 10 minutes and then centrifuged. The supernatant was then diluted with ethanol until the 10 ml volumetric flask boundary line and mixed for 10 minutes.

2.3 Procedure analysis

2.3.1 Physicochemical properties
Physicochemical properties, viz., yields, water content, and ash content were determined in accordance with [8]. Minerals, namely, sodium, potassium, magnesium, iron, zinc, and calcium, were determined by atomic absorption spectrometry methods.

2.3.2 Preliminary phytochemical screening
The powdered of bamboo shoot were tested for the presence of saponin, glycosides, terpenoids, flavonoids, tannins, and alkaloids [9-11].

2.3.3 Total phenolics content
Folin-Ciocalteau assay was used to determine the total phenolics content of young bamboo shoots [12]. Young bamboo shoots extract or blank or gallic acid standard solutions (100 µL) were add sodium carbonate (2 mL, 2%) and distilled water (2.8 mL) then left standing for 4 minutes. After that Folin-Ciocalteu (100 µL) was added into the solutions and left standing for 30 minutes in the dark condition. The measurement was conducted on a spectrophotometer (Shimadzu 1800 UV-Vis, Japan) at λ= 760 nm against the blank. Total phenolics content expressed as mg gallic acid equivalent (GAE) in a gram of dry weight of plant extract and calculated from expression (1). Samples were analyzed in triplicates.

\[
\text{Absorbance} = 0.0009 \text{ gallic acid (µg/mL)} + 0.0024
\] (1)

2.3.4 Total flavonoids content
The aluminum chloride assay was used to determine the total flavonoids content of young bamboo shoots [13]. Bamboo shoots extract or blank, or quercetin standard solution (1 mL) were add 2 mL aluminum chloride (2%) in methanol solution, mixed, and left standing for 30 minutes. The measurement was conducted on the spectrophotometer (Shimadzu 1800 UV-Vis, Japan) at λ= 415 nm against the blank. Total flavonoids content expressed as mg quercetin equivalent (QE) in a gram of dry weight of plant extract and calculated from expression (2). Samples were analyzed in triplicates.

\[
\text{Absorbance} = 0.0145 \text{ quercetin (µg/mL)} + 1.558
\] (2)

2.3.5 Antioxidant activity (DPPH) assay
The varying concentration of sample or standard solution or blank (1 mL) was added DPPH methanolic solution (3 mL, 0.004%), then left for 30 minutes in dark condition. The measurement was conducted on a spectrophotometer (Shimadzu 1800 UV-Vis, Japan) at λ= 517 nm against a blank [14]. Data reported as a concentration of antioxidant required for 50% scavenging of DPPH radicals in the specified time period (IC50) and calculated from expression (3). Samples were analyzed in triplicates.

\[
\%\text{Inhibition} = \left( \frac{Ac - As}{Ac} \right)
\] (3)

Where:
Ac= absorbance control or blank,
As= absorbance with sample or standard

2.3.6 Toxicity assay
The brine shrimp lethality (BSLT) method was used to investigate the toxicity of various bamboo shoots extract [15]. Brine shrimp eggs (Hobby Artemix®, Germany), contained salt, in a conical shaped vessel, and filled with sterile distilled water under constant aeration for 48 hours. Ten active
nauplii put into each vial containing brine solution (4.5 mL) using a glass capillary. The young bamboo shoots extract (0.5 mL) were added, then left for 24 hours at room temperature. After that surviving larva (a dead and live larvae in each test) were counted. The experiments were conducted along with control. The different concentrations (12.5 - 1000 µg/mL) of each test substances in a set of three tubes per dosage. The probity analysis used to calculate a compound content capable of killing larvae up to 50% (LC50).

2.4 Statistical analysis
Data presented in mean±standard deviation. The differences between treatments were analyzed using ANOVA. Significant differences between mean values were determined using Duncan’s Multiple Range Test (α=5%). All statistical analysis was performed using Microsoft Excel 2010 and SPSS ver. 15.00 for Windows.

3. Results and Discussion
3.1. Physicochemical characteristics
Table 1 showed that the yield of various bamboo shoots powder ranged between 5.35% (w/w) and 5.83% (w/w). There is no difference value of water content on various bamboo shoots powder (P>0.05). Water content and ash content of various bamboo shoots powder ranged 4.82 - 5.92% and 7.72 – 8.36 %, respectively. According to [Iqbal et al], reported high ash content value in plant material indicates a large amount of inorganic nutrient. The physicochemical characteristics of various bamboo shoots powder were shown in Table 1.

| Samples        | Gombong | Betung | Buluh | Kuning |
|----------------|---------|--------|-------|--------|
| **Yield (%)**  | 5.83±0.39 | 5.58±0.13 | 5.38±0.32 | 5.35±0.32 |
| **Water content (%)** | 5.92±0.66 | 4.82±0.02 | 5.61±0.55 | 5.19±0.31 |
| **Ash (%)**    | 7.72±0.70 | 8.00±0.43 | 8.36±0.44 | 7.87±0.48 |

**Mineral**:  
- Sodium (µg/kg): 340.00±80.00, 350.00±20.00, 350.00±11.00, 370.00±14.00
- Potassium (mg/kg): 117.19±26.93, 121.87±14.56, 132.62±26.18, 131.21±17.21
- Magnesium (µg/kg): 270.00±80.00, 260.00±11.00, 260.00±8.00, 220.00±7.00
- Zinc (µg/kg): 710.00±40.00, 670.00±40.00, 730.00±20.00, 690.00±20.00
- Iron (µg/kg): 1760.00±89.00, 1880.00±70.00, 2100.00±63.00, 2360.00±76.00
- Calcium (µg/kg): 320.00±90.00, 320.00±40.00, 330.00±10.00, 360.00±90.00

Values are mean±SD of triplicate; different alphabetic in the same rows = significant difference (α=0.05)

Minerals are required for metabolic activities of our body. Bamboo shoots are reported rich quantities of useful minerals, viz., sodium, iron, potassium, and magnesium [17]. Table 1 showed that bamboo gombong shoots (*Giganthochla pseudoarundinacea* (Steud.) Widjaja) powder contained mineral Na (340 µg/kg), K (117 mg/kg), Mg (270 µg/kg), Zn (710 µg/kg), Fe (1760 µg/kg), and Ca (320 µg/kg). Bamboo betung shoots (*Dendrocalamus asper* (Schult.) Backer ex Heyne) powder contained mineral Na (350 µg/kg), K (121 mg/kg), Mg (260 µg/kg), Zn (670 µg/kg), Fe (1880 µg/kg), and Ca (320 µg/kg). Bamboo buluh shoots (*Giganthochla apus*) powder contained mineral Na (350 µg/kg), K (132 mg/kg), Mg (260 µg/kg), Zn (730 µg/kg), Fe (2100 µg/kg), and Ca (330 µg/kg). Moreover, bamboo kuning shoots (*Bambusa vulgaris var. striata*) powder contained mineral Na (370 µg/kg), K (131 mg/kg), Mg (220 µg/kg), Zn (690 µg/kg), Fe (2360 µg/kg), and Ca (360 µg/kg) (Table 1). Analysis of variance (ANOVA) showed that there is no significant difference on mineral content, namely., sodium, potassium, magnesium, zinc, iron, and calcium, of several young bamboo shoots powder (P>0.05). This results agreement with [Nirmala et al.], reported young bamboo shoots high in potassium and also contain mineral elements such as iron, calcium, chromium, manganese, zinc, and
copper. Generally, bamboo shoots are rich in minerals, protein, amino acids, vitamins, and inorganic salts [18].

3.2 Antioxidant properties
Phytochemical screening showed that the ethanolic extract of several young bamboo shoots is rich in tannins, terpenoids, and glycosides. The results of phytochemical screening are given in Table 2.

| Table 2. Phytochemical screening of various bamboo shoots | Gombong | Betung | Buluh | Kuning |
|--------------------------------------------------------|---------|--------|-------|--------|
| Constituent                                            | Mayer   | Terpenoids | Saponin | Tannins | Flavonoids | Glycosides |
| Gombong                                                 | -       | +      | -      | +++     | +         | +         |
| Betung                                                 | -       | +      | -      | ++      | -         | +         |
| Buluh                                                   | -       | -      | -      | -       | -         | -         |
| Kuning                                                  | -       | -      | -      | -       | -         | -         |

(+)= present; (-)= absent

Table 2 shows the numerous phenolic compounds collected in various extracts of bamboo shoots. The phytochemical screening showed that the ethanolic extract of bamboo shoots did not contain alkaloids, saponin, and some flavonoids. These results were in agreement with [Zhang et al.], which reported that the ethanolic extract of bamboo stem shoots (P. nigra var henonis (Mitford Rendle)) is rich in triterpenoids compounds.

The quantitative analysis results reinforce the results of phytochemical screening previously, in which the highest total phenolics content found in bamboo buluh shoots extract (BUS) (see Table 3). The total phenolic contents on various bamboo shoots extract depended on the type of ecology, i.e., bamboo species, time of harvesting, climates, and soil. Different of bamboo species and ecology to growth contained different types of phytochemical compounds [20].

| Table 3. Antioxidant properties of ethanolic extract of several young bamboo shoots | Total phenolics (mg GAE/g) | Total Flavonoids (mg QE/g) | TF/TP Antioxidant activity |
|---------------------------------------------------------------------------------|---------------------------|----------------------------|---------------------------|
| Samples                                                                         |                           |                            | IC50 (µg/mL)              |
| Gombong shoots (GOS)                                                            | 16.47±0.11^c              | 1.11±0.12^bc               | 0.07                      | 1378.46^c |
| Betung shoots (BES)                                                             | 14.62±1.12^d              | 2.49±0.07^a                | 0.17                      | 2489.60^d |
| Buluh shoots (BUS)                                                              | 27.83±0.77^a              | 0.92±0.12^d                | 0.03                      | 418.48^b  |
| Kuning shoots (KUS)                                                             | 26.96±6.42^ab             | 1.11±0.42^bc               | 0.04                      | 347.48^a  |

Values are mean±SD of triplicate; GAE = gallic acid equivalent; QE = quercetin equivalent; a>b>c>d, same alphabetic in the same column = no difference (α=5%).

Table 3 shows that the total phenolic content of ethanolic extract of bamboo shoots ranged from 14.62 – 27.83 mg GAE/g dry weight. This indicated that several of bamboo shoots extract has a different phenolic total content. The bamboo buluh shoots extract (BUS) had the highest total phenolic content value (27.83 mg GAE/g) followed by KUS> GOS> BES (P<0.05). Meanwhile, The bamboo betung shoots extract (BES) is found to have the highest total flavonoids (2.49 mg QE/g) with a ratio of (TF/TP) which is 0.17, followed by GOS and KUS> BUS (P<0.05) (see Table 3). This result is in agreement with [Singhal], which reported that the total phenolics and total flavonoid content of Bambusa vulgaris are 29.0 mg GAE/100g and 49.7 mg CE/100 g, respectively.

Antioxidant properties of various bamboo shoots extract were evaluated by DPPH radical assay. Table 3 shows that IC50 of the ethanolic extract of bamboo kuning shoots (KUS) was 347.48 µg/mL followed by BUS (418.48 µg/mL)< GOS (1378 µg/mL)< BES (2489 µg/mL). Based on antioxidant analysis (DPPH assay) found that KUS had the highest antioxidant activity, shown by the lowest IC50 value (P<0.05). However, this activity is still below the standard of gallic acid or ascorbic acid. The phenolics content related to the free radical scavenging activities. According to [Zhang and Hamauzu],
the antioxidant activity of phenolic compounds allows scavenging both electrophiles and active oxygen species.

Fig. 2 shows that the total phenolics content has a strong correlation with antioxidant activities of \((R^2) 0.725\). These results suggest that higher total phenol content will give stronger antioxidant activities.

Fig. 2 also indicates that 72% of the antioxidant properties of various bamboo shoots extract is contributed by the phenolic compounds and the remaining 28% comes from other secondary metabolisms, such as vitamins and others. This result is in agreement with another researcher that reported a close relationship between total phenolics and antioxidant activity on different plants [23-25].

![Figure 2. The relationship between total phenolic content and antioxidant activity of ethanolic extract of several young bamboo shoots.](image)

### Table 4. Toxicity of ethanolic extract of various bamboo shoots

| Samples          | LC50 (µg/mL) | Toxicity |
|------------------|--------------|----------|
| Gombong shoots (GOS) | 1088.39      | Non-toxic |
| Betung shoots (BES)    | 738.08       | Toxic    |
| Buluh shoots (BUS)     | 999.31       | Toxic    |
| Kuning shoots (KUS)    | 773.29       | Toxic    |

Table 4 showed the ethanolic extract of betung shoots, buluh shoots, and kuning shoots having a value of LC50<1000 µg/mL (toxic), but not for gombong shoots extract (LC50 value more than 1000 µg/mL). The LC50 values of ethanolic extract for gombong shoots, betung shoots, buluh shoots, and kuning shoots were 1088.39 µg/mL, 738.08 µg/mL, 999.31 µg/mL, and 773.29 µg/mL, respectively. BES, BUS, and KUS showed lower LC50 values and significant toxicity against brine shrimp larvae of *Artemia salina*. It means that BES, BUS, and KUS might have a numerous of phenolic compounds or cyanogenic glycosides. However, this amount compared to the others reported, the ethanolic extract of BES, BUS and KUS were considered high values of toxicity, except bamboo gombong shoots. The diversity of bamboo species and part of the plants available for medicinal use, but some of that may interfere with hormone metabolism and reproductive function [7]. According to [Stewart and Moorthy], the toxic effect of the young bamboo shoots extract appears to be due to free hydrocyanic acid liberated, possibly one of its cyanogenetic glucosides by a specific enzyme contained in it. These results can be an indicator of potential cytotoxic compounds.

3.3 Toxicity

In this screening, the toxicity of ethanolic extract of various bamboo shoots evaluated by brine shrimp lethality test (LC50) is summarized in Table 4.
4. Conclusion
Generally, bamboo shoots are rich in minerals, protein, amino acids, vitamins, inorganic salts, and bioactive compounds that provide health benefits. Bamboo shoots powder, viz., buluh, betung, gombong and kuning have a good nutritional characteristics. The ethanolic extract of buluh shoots (BUS) contained the highest total phenolics (27.83 mg GAE/g) followed by KUS > GOS > BES. Bamboo betung shoots extract (BES) is found to have the highest total flavonoids (2.49 mg QE/g) with a ratio of TTF/TP) which is 0.17. Meanwhile, the DPPH assay (IC50) showed that IC50 of KUS (347.48 μg/mL) was lowest. Correlation the total phenolics content has a strong with antioxidant activities ($R^2$: 0.72). Based on these values, KUS is found to have the highest antioxidant activity. Furthermore, the level toxicity of LC50, BES, BUS, and KUS extract is valued as cytotoxic.

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6. References
[1] Nirmala C, David E, and Sharma M L 2007 Changes in nutrient components during ageing of emerging juvenile bamboo shoots Int. J. Food Sci. Nut., 58 pp. 612-618
[2] Burkill I H 1935 A Dictionary of the Economic Products of the Malay Peninsula. Crown Agents for the Colonies, London, England
[3] Kencana D, Antara N S, and Widia W 2012 Team UNUD-USAID-TPC project. praktek baik budi daya bambu rebeung tabah (Gigantochloa Nigrociliata BUSE-KURZ)
[4] Nirmala C, Bisht M S, and Lahisram M 2014 Bioactive compounds in bamboo shoots: health benefits and prospects for developing functional foods. Int. J. of Food Sci and Tech. 49 pp. 1425–1431
[5] Park E J and Jhon D Y 2009 Effects of bamboo shoot consumption on lipid profiles and bowel function in healthy young women. Nutrition 25 pp. 723-728
[6] Rawat K, Nirmala C, and Bisht M S 2015 Processing techniques for reduction of cyanogenic glycosides from bamboo shoots. 10th World Bamboo Congress, Korea
[7] Panee J 2015 Review: Potential medicinal application and toxicity evaluation of extracts from bamboo plants J. Med. Plants Res. 9(23) pp. 681-692.
[8] [AOAC] Association of Official Analytical Chemistry 1990 Official Methods of Analysis. Association of Official Analytical Chemist. Arlington, Virginia (US): AOAC
[9] Harborne J B 1973 Phytochemical methods Chapman and Hall, Ltd London. pp. 49-188
[10] Sofowara A 1993 Medicinal plants and traditional medicine in Africa Spectrum Books Ltd, Ibadan, Nigeria p.289
[11] Trease G E and Evans W C 1989 Pharmacognsny. 11th ed. Brailliirir Tiridel Can Macmillian publishers
[12] Iwansyah A C and Yusoff M M 2012 Physicochemical, minerals and antioxidant properties of Labisia pumila var. alata of selected geographic origins. J. of Agr. Sci. (AGRIVITA) 34 pp. 94-104
[13] Meda A, Lamien C E, Romito M, Miliogo J, and Nacoulina O G 2005 Determination of the total phenolic, flavonoid, and proline content in burkina fasan money, as well as their radical scavenging activity Food Chemistry 91 pp. 571-577
[14] Kumaran R J and Kuranakaran 2006 Antioxidant and free radical scavenging activity of an aqueous extract of Coleus aromaticus Food Chemistry 97 pp.109-114
[15] Meyer B, Ferrigni N, Putnam J, Jacobsen L, Nichols D E, and McLaughlin J L 1982 Brine shrimp: a convenient general bioassay for active plant constituents. J. Medicinal Plant Research, 45 pp.31-34
[16] Iqbal S, Younas U, Sirajudin, Chan K W, Sarfraz R A, and Uddin M K 2012 Proximate composition and antioxidant potential of leaves from three varieties of mulberry (Morus Sp): a comparative study. Int. J. Mol. Sci. 13 pp. 6651-6664
[17] Nongdam P and Tikendra L 2014 Review: the nutritional facts of bamboo shoots and their usage as important traditional foods of north-east India. International Scholarly Research Notices pp.1-17
[18] Goyal A K and Brahma B K 2014 Antioxidant and nutraceutical potential of bamboo: an overview. International Journal of Fundamental and Applied Sciences 3(1) pp. 2-10
[19] Zhang Y, Wu X, Ren Y, Fu J, and Zhang Y 2004 Safety evaluation of a triterpenoid-rich extract from bamboo shavings. Food Chem. Toxicol 42 pp. 1867-1875
[20] Maisuthisakul P 2012 Phenolic constituents and antioxidant properties of some thai plants, phytochemicals - a global perspective of their role in nutrition and health, Dr Venketeshwer Rao (ed.)
[21] Singhal P 2016 Effect of processing technique on holistic quality of bamboo shoot (Bambusa vulgaris). [Dissertation] Indian Institute of Technology Delhi; India
[22] Zhang D and Hamauzu Y 2004 Phenolics compounds and their antioxidant properties in different tissues of carrots (Daucus carota L.). Food, Agriculture, & Environment 2(1) pp. 95-100.
[23] Maisuthisakul P, Pasuk S, and Ritthiruangdej P 2008 Relationship between antioxidant properties and chemical composition of some Thai plants Journal of Food Composition and Analysis 21 pp. 229-40
[24] Iwansyah A C, Damanik R M, Kustiyah L, and Hanafi M 2016 Relationship between antioxidant properties and nutritional composition of some galactopoietics herbs used in indonesia: a comparative study Int. J Pharm Pharm Sci, 8(12) pp. 236-243
[25] Yusoff M M, and Iwansyah A C 2011 Comparative evaluation of total phenolics and free radical scavenging activity of aqueous extracts of Labisia pumila var. alata from Malaysia and Indonesia. International Conference on Biotechnology and Food Science, IEEE, April 1-3, 2011. Bali, Indonesia pp.4-8
[26] Stewart A D and Moorthy F N 1933 Lethal properties of aqueous extract of young bamboo shoots. The Indian Medial Gazette pp.320-324