Ethnobotanical note, total phenolic content, total flavonoid content, and antioxidative activities of wild edible vegetable, *Crassocephalum crepidioides* from Kota Belud, Sabah.

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Abstract. *Crassocephalum crepidioides* or locally known as “tanduk manggarang” (Bajau) or “gipun” (Dusun) is an underutilized weed that has been consumed as wild edible vegetable by Sama Bajau people in Kota Belud, Sabah. The villagers obtained the plant from local traders at fresh market or foraging the plant from forest. Correlation between TPC and TFC with IC\(_{50}\) of DPPH and ABTS scavenging activities were analysed by using Pearson’s correlation. The ethanolic extract exhibited the highest TPC (175.06±0.574 µg/ml) and TFC (139.72±0.923 µg/ml), followed by hot water extract with TPC of 54.45±0.818 µg/ml and TFC of 25.07±0.156 µg/ml. The distilled water extract showed the lowest TPC (29.98±0.918 µg/ml) and TFC (19.96±0.538 µg/ml). Antioxidant activities also demonstrated the same trend, ethanolic extract displayed the highest percentage of antioxidant activity of DPPH (85.4±1.64 %) and ABTS (85.2±0.57 %), followed by hot water extract with 65.4±3.87 % for DPPH and 79.4±3.2 % for ABTS. Distilled water extract had the lowest antioxidant activities for both DPPH (55.0±0.7 2%) and ABTS (71.35±2.61 %). The IC\(_{50}\) of DPPH assay were decreasing in the subsequent order; distilled water>hot water>ethanolic. Meanwhile, the IC\(_{50}\) of ABTS were decreasing in the following order; hot water>distilled water>ethanolic. There was negative high correlation between TPC in *C. crepidioides* leaves extracts with their IC\(_{50}\) of DPPH and ABTS assays. Following the same trend, there was also negative high correlation between TFC in *C. crepidioides* leaves extracts with their IC\(_{50}\) of DPPH and ABTS assays. As a conclusion, this readily available wild edible vegetable could be a poten resource of natural antioxidant for rural populace in Sabah, Malaysian Borneo.

1. Introduction

There are number of diseases occurrence that can be related with oxidative stress, where oxidative damage of cell components were triggered by reactive oxygen species (ROS) and free radicals accumulation in human cells. These diseases include cardiovascular diseases, diabetes, cancer, vital
and organ failure [1]. Wild leafy vegetable are often neglected by agriculture industry, indigenous people in Sabah were still utilized the forest produce as their source of food. The traditional knowledge is not just important to select source of food from natural surroundings, but also serves as basis for community centred effective conservation and management strategy [2].

*C. crepidioides* is known among Sama Bajau people in Kota Belud as “tanduk manggarang”, it is also known by other vernacular names, such as “gipun” (Dusun), and “sawi enggang” (Malay). This species is from Asteraceae family, herbaceous plant with succulent stem, and can grow up to 20 cm above the ground. Meanwhile, in Africa, it is known as “ebolo” and planted in commercial farm, the height of plant can reach 100 to 180 cm [3]. This plant is also eaten as traditional vegetable in Taiwan and Okinawa, Japan [4].

In Kota Belud, Sabah, Malaysian Borneo, this species grow wildly in forest, rubber plantation area, and as a secondary successor in abandoned crop land [5]. Local Sama Bajau people have been consumed it as traditional vegetables, they obtained their supply from forest foraging or purchasing it from local market [6]. Previously record had shown that, other than consumed as food plant, *C. crepidioides* also have been used as traditional medicine to treat hepatitis related ailment, fever, edema, and malaria [4] *C. crepidioides* can also provide consumer with numerous beneficial nutrition such as calcium, kalium, natrium, magnesium, iron, fibre, and amino acids [7].

### 2. Methodology

#### 2.1. Plant sample

Plant samples were obtained from Kota Belud, Sabah in November 2017. The identification process of plant was assisted by Mr. Mohd Aminur Faiz and Mr. Jemson Jumian from Sandakan Herbarium (SAN). Specimen (FAK406) was also prepared and housed in Borneensis Herbarium, Universiti Malaysia Sabah (BORH). The morphological and ecological data of plant was recorded.

#### 2.2. Extractions

Prior to extraction process, fresh leaves was separated from the plant, and washed thoroughly using tap water to remove dirt and debris. The sample later air dried and grinded using electrical blender. Powdered form of leaves was stored in air tight container in refrigerator.

- **Ethanol extract** Five grams of dried and powdered *C. crepidioides* sample was mixed and macerated with 50 ml 95% ethanol at room temperature 25°C ± 2°C. The extract was filtered by using Whatman paper No.1, and was further evaporated to dryness with rotary evaporator at 40°C under reduced pressure. The crude extract was kept in vial at -20°C [8].

- **Boiling water extract** Five grams of dried and powdered *C. crepidioides* was added to 50 mL of distilled water that was boiled to 100°C. The mixture is stirred, and was filtered by using Whatman paper No. 1. The sample further freeze dried to remove water. The crude extract was kept in vial at -20°C [8].

- **Distilled water extract** Five grams of dried and powdered *C. crepidioides* was added to 50 mL of distilled water at room temperature 25°C ± 2°C. The mixture is stirred, and was filtered by using Whatman paper No. 1. The sample further freeze dried to remove water. The crude extract was kept in vial at -20°C [8].

#### 2.3. Determination of total phenolic content

Total phenolic content assay was determined as described in [9] with modification. 0.2 ml of diluted plant extract in 95% ethanol added to 1 ml of Folin-Ciocalteu reagent (10%). The mixture was mixed, and after 10 minutes, 0.8 ml sodium bicarbonate (7.5%) was added. The mixture was incubated at room temperature (25 °C ± 2 °C) for 90 minutes, and the absorbance was measured at 750 nm. The content of phenolic was expressed as mg gallic acid equivalent per gram of sample.
2.4. Determination of total flavonoid content
Total flavonoid content was determined using aluminium chloride complex assay. 0.4 ml distilled water was added to 0.2 ml of 1 mg/ml plant extract. Following that, 30 µl of 5% (w/v) sodium nitrite was added. The mixture rested for 5 minutes before 30 µl of 10% (w/v) aluminium chloride was added. 0.2ml of 1 M sodium hydroxide was added after 6 minutes, and afterward distilled water was added up to 2ml total volume. The absorbance was measured at 510nm. Rutin was used as standard, and the total flavonoid content was expressed as rutin equivalent (RE) [8].

2.5. 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay
The stock solution of DPPH was prepared in methanol (0.03 mM) and 1 ml of the sample extract with difference concentrations (0-1.0 mg/ml) added to 3 ml of DPPH stock solution. The mixtures later incubated in the dark at room temperature. After 30 minutes the absorbance of the preparations was taken at 517 nm by a UV DPPH is one of the free radicals widely used for spectrophotometer. IC$_{50}$ of DPPH scavenging activity of each extract was determined by using its calibration curve [8].

2.6. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) cation radical decolorization assay
The procedure for ABTS decolorization assay was carried out according to Re et al., [10] with suitable modification. ABTS solution (7.0 mM) was reacted with potassium persulfate (2.45 mM) to generate radical monocation of ABTS. This reaction exhibited blue-green color of ABTS$^{+}$. In order to prevent incomplete oxidation process the mixture was kept in dark room for 16 hours. The absorbance of 0.70 ± 0.02 units at 534 nm was obtained by diluting the mixture by using distilled water. 0.2 ml of plant extract added to 2.0 ml ABTS free radical solution, and the mixture was allowed to stand for 5 minutes. The absorbance value was measured at 734 nm by using spectrophotometer. Ascorbic acid was used as standard and the final result was expressed as µg ascorbic acid equivalent antioxidant capacity in 1 L of sample extract. IC$_{50}$ of ABTS antioxidant activity of each extract was determined by using its calibration curve.

2.7. Statistical analysis
The statistical analyses were performed by a one-way ANOVA and Pearson’s correlation test using Microsoft Excel 2016. The results were expressed as means ± SD to show variations in the various experiments. Differences are considered significant when $p<0.05$.

3. Results and Discussion
3.1. Taxonomy and description of Crassocephalum crepidioides from Kota Belud, Sabah
C. crepidioides is an angiosperm, species that was found in Northern Thailand was recorded flowering between September and March every year. It is a succulent herbaceous plant which a common successor in abandoned crops land, secondary forest, and rubber plantation area. Family: Asteraceae; Genus: Crassocephalum; Species: Crassocephalum crepidioides; Vernacular name: Tanduk manggarang (Bajau), Gipun (Dusun), Sawi enggang (Malay), red flower ragleaf (English), ebolo (Nigerian), benibanaborogiku (Japanese); Locality: Hilly area, rubber plantation, Kg. Rosok, Kg. Pandasan, Kota Belud, Sabah.

C. crepidioides from Kota Belud,Sabah  is herbaceous plant with fibrous roots (figure 1), flowering and grow throughout the year. Plant: 17-19 cm tall, herbaceous, with mucilaginous stem; leaves: alternate, serrated to denticate margin, acuminate to acute apex, mucilaginous blade, 7-13 cm length; flower: green (immature) scarlet red, sparsely; anthers: minute size <1.5mm. Geographical distribution: Africa; Asia: India, Pakistan, Sri Lanka, Myanmar, Laos, Cambodia, Thailand, Malaysia, Indonesia, Borneo, Philippines, Papua New Guinea, Taiwan, Japan (Okinawa) [11].

Sama Bajau people in Kota Belud, Sabah, consumed this plant as traditional vegetable. The preparation method as food including boiling, stir fried, or eaten fresh. Even in other African countries
this species is cultivated in farm or home garden. In Sabah, this plant is foraged from hilly forested area, and rubber plantation area for domestic consumption [3]. Other than Sama Bajau, Dusun is another indigenous tribe that is known consuming this species as traditional vegetable. This information was obtained from oral communication with Dusun from Kampung Serusop, Tuaran. Previous study reported that there are two species of *Crassocephalum* i.e. *C. crepidioides* and *C. rubens* in Asia, however the molecular analysis had shown that the occurrence of hybrid of two species in North of Thailand [11].

![Figure 1. A. Plant of *C. crepidioides*. B. Leaf of *C. crepidioides*](image)

3.2. **Total phenolic content (TPC) and total flavonoid content (TFC)**

TPC and TFC of *C. crepidioides* leaves extracts decreased in subsequent order ethanolic>hot water>distilled water. TPC of *C. crepidioides* for ethanolic extract was recorded at 175.06±0.574 µg/ml, and for hot water extract and distilled water extract were recorded at 54.45±0.818 µg/ml and 29.98±0.918µg/ml respectively (table 1). Meanwhile TFC for ethanolic extract of plant sample was recorded at 139.72±0.923 µg/ml. TFC for water extract and distilled water extract were recorded at 25.07±0.156 µg/ml and 19.96±0.538 µg/ml, respectively. These results are in agreement with previous studies on local vegetables where sample extracted with ethanol exhibiting higher TPC and TFC compare with sample that extracted with water, and more TPC and TFC in hot water extract compare to in lower temperature water extract [8, 12].

3.3. **DPPH free radical scavenging activity**

The DPPH scavenging activity of leaves extracts were ranging from 55.0 to 85.4 % (table 1). The highest percentage was recorded for ethanolic extract, followed by hot water extract, and distilled water extract. Meanwhile for IC<sub>50</sub> results had shown inverted trend, percentage of the IC<sub>50</sub> of DPPH assay were decreasing in the subsequent order; distilled water>hot water>ethanolic (figure 2). The lowest of IC<sub>50</sub> of plant extract indicates the highest antioxidant activity. The current results are in agreement with previous works, where the ethanolic extracts of local herbs and vegetables, e.g. *Cosmos caudatus*, *Eryngium foetidum*, *Ipomoea batatas*, *Manihot esculenta*, had shown highest
antioxidant compared to water extracts [8, 13]. Ethanol is known as a non-toxic organic solvent, which can act effectively to extract bioactive compounds from plant species [8].

**Table 1.** Average of TPC, TFC, ABTS, DPPH, IC$_{50}$ of DPPH, IC$_{50}$ of ABTS in different C. crepidioides extracts

| Sample     | TPC µg/ml  | TFC µg/ml  | DPPH % | ABTS % | IC$_{50}$ of DPPH µg/ml | IC$_{50}$ of ABTS µg/ml |
|------------|------------|------------|--------|--------|------------------------|------------------------|
| Ethanol    | 175.06 ± 0.574 a | 139.72 ± 0.923 a | 85.4 ± 1.64 a | 85.2 ± 0.57 a | 180.39 ± 1.94 a | 108.20 ± 5.76 a |
| Hot water  | 54.45 ± 0.818 b | 25.07 ± 0.156 b | 65.4 ± 3.87 ab | 79.4 ± 3.25 b | 386.84 ± 0.96 a | 458.66 ± 0.707 a |
| Distilled water | 29.98 ± 0.918 c | 19.96 ± 0.538 c | 55.0 ± 0.72 ab | 71.35 ± 2.61 b | 659.61 ± 0.87 a | 451.164 ± 2.28 a |

Mean ± S.D. (n = 3), Same superscripted number or letter within vertical columns are not significantly different ($p < 0.05$).

3.4. **ABTS free radical scavenging activity**

The percentage of ABTS radical scavenging activity assay of leaves extracts were increased in the subsequent trend; ethanolic>hot water>distilled water (Table 1). The highest scavenging activity was recorded for ethanolic extract at 85.2±0.57%, followed by hot water extract (79.4±3.2 %) and distilled water extract (71.35±2.61 %). Meanwhile the IC$_{50}$ of ABTS assay had shown slightly different trend compared to IC$_{50}$ of DPPH assay, percentage of the IC$_{50}$ of ABTS assay were decreasing in the subsequent order; hot water>distilled water>ethanolic (Figure 2). Water is commonly used as solvent in food plants sample extract as it mimic the method of cooking, which often involve water i.e. boiling, blanching, beside water is also capable to extract polypeptides, anthocyanin, and tannins [8]. ABTS radicals react with both hydrophilic and lipophilic antioxidants [14].

![Figure 2. IC$_{50}$ of DPPH and ABTS scavenging activities in three different extracts of C. crepidioides; ethanol (EtOH), hot water (HW), and distilled water (DH2O).](image_url)

3.5. **Relationship between phytochemicals with antioxidant activities.**
Correlation matrix was created to explore the relationships between studied parameters. There were positive correlations between total phenolic content (TPC), total flavonoid content (TFC), DPPH and ABTS scavenging activities. The positive correlation between TPC with DPPH scavenging activity ($r = 0.983$, $p<0.05$) is stronger than positive correlation between TPC with ABTS scavenging activity ($r = 0.897$, $p<0.05$). Meanwhile the positive correlation between TFC with DPPH and ABTS, valued at $r = 0.954$, $p<0.05$ and $r = 0.837$, $p<0.05$ respectively (table 2). Low molecular weight of phenolic compounds in the extracts could contribute to higher antioxidant activity [13].

Table 2. Pearson’s correlation coefficient of total phenolic, total flavonoid of $C. crepidioides$ leaves extract with their IC$_{50}$ of DPPH and IC$_{50}$ of ABTS scavenging activities

| Pearson’s correlation | TPC       | TFC       |
|----------------------|-----------|-----------|
| DPPH %               | 0.983     | 0.954     |
| ABTS %               | 0.897     | 0.837     |
| IC$_{50}$ of DPPH    | -0.903    | -0.844    |
| IC$_{50}$ of ABTS    | -0.984    | -0.998    |

The highly negative correlations were shown between TPC or TFC, with IC$_{50}$ of DPPH and IC$_{50}$ of ABTS. TPC of $C. crepidioides$ leaves extracts showed significantly negative correlation with IC$_{50}$ of DPPH ($r = -0.903$, $p<0.05$) and IC$_{50}$ of ABTS ($r = -0.984$, $p<0.05$). Meanwhile correlation between TFC of $C. crepidioides$ leaves extracts with IC$_{50}$ of DPPH was $r = -0.844$, $p<0.05$ and with IC$_{50}$ of ABTS was $r = -0.998$, $p<0.05$. Highly negative correlation between TPC and TFC with IC$_{50}$ of DPPH and IC$_{50}$ of ABTS is indicating that significant content of TPC and TFC would further increase the antioxidant activities, and higher antioxidant activity would be expressed in lower IC$_{50}$ value. Same pattern of results were previously observe in test with different wild edible food plants i.e. $Ardisia$ elliptica, $Ardisia$ cymosa, $Ardisia$ fuliginosa, $Musa$ spp. [15-16]. Direct consumption of $Crassocephalum$ crepidioides as traditional vegetable was considered safe for human as it may help halting the reaction of free radicals and reactive oxygen species.

4. Conclusion
In conclusion, ethanolic extract of $C. crepidioides$ leaf has the highest overall performance in term TPC, TFC, DPPH scavenging activity, and ABTS scavenging activity as compared to hot and distilled water. IC$_{50}$ of DPPH and IC$_{50}$ of ABTS for ethanolic extract also shown the lowest value, this signify that higher antioxidants presence in the extract and trigger better scavenging activity. Underutilized $C. crepidioides$ could be a good choice of nutrients and antioxidant source for indigenous tribes in Kota Belud, Sabah, Malaysian Borneo. As this species is available in natural forest, and survive well in disturbed forest, and rubber plantation area. However, more study is needed to verify the species viability as effective herbal medicine.

5. References
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