Comparison of Antioxidant Activity and Phytochemical Content of Borneo Wild Berry, *Rubus fraxinifolius* (Rogimot)

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ABSTRACT *Rubus fraxinifolius,* locally known as Rogimot, is an underutilized edible fruit and grown wildly around Mount Kinabalu, Sabah. Antioxidant activities and phytochemicals content in three different parts (i.e., fruit, stem and leaves) of this plant were analyzed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2-2'-Azinobis(3-ethylbenzothiazoline)-6-sulphonic acid (ABTS), as well as ferric reducing/antioxidant power assay (FRAP). Samples were freeze-dried and extracted using 5 different solvents namely dH2O, absolute ethanol, 80% (v/v) ethanol, absolute methanol and 80% (v/v) methanol. The result of antioxidant tests showed that 80% (v/v) methanol crude extract display higher antioxidant value compared to the other solvents extract. Phytochemical analysis from these extracts showed that the TPC and TFC were higher in the leaves at 56.32 ± 0.05 (mg GAE/g) and 31.36 ± 1.05 (mg CE/g), respectively. Meanwhile, TAC and TCC were found higher in the fruit flesh at $22.27 \pm 1.28 \times 10^{-14}$ (mg C-3-GE/g) and 10.02 ± 0.22 (mg BC/g), respectively. The same trend was found for antioxidant assay, where leaves show highest values as compare to the other plant parts. These finding suggested that the leaves of *R. fraxinifolius* has a potential to be used as a natural antioxidative for human health.

KEYWORDS: Rogimot, *Rubus fraxinifolius*, antioxidant activity, phytochemical, Kundasang.

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INTRODUCTION

Antioxidant from natural sources has shown a significant benefit in preventing numerous human diseases (Juliet & Sivakumar, 2017). Recently, research on natural antioxidant has increased in various fields including food biology, food chemistry, cosmetic and other medical healthcare industries (Duda Chodak & Tarko, 2007; Li *et al*., 2014). Various food advertisers have begun to take note and publicising this fact (Anbudhasan *et al.*, 2014). This triggered intense research not only on edible part of a plant such as fruit but also among the underutilised parts; such as stem, flower, root, bark and leaves.

Rubus plants can be found in all continents except Antarctica and it is distributed from low and tropic to subtropics region (Menzies, 2002; Yang & Pak, 2006). More than eight species of *Rubus* found above 1200 m at Mount Kinabalu (Corner & Beaman, 1996) including *Rubus fraxinifolius*. This species locally known as "Rogimot" among Dusun and Kadazandusun tribes of Sabah, Malaysia. Meanwhile, in Indonesia, *Rubus fraxinifolius* is known as "Arben" and this plant is planted in Cibodas Botanical Garden, which is an institute of *ex situ* conservation area (Surya, 2012). This plant used in relieving morning sickness, strengthening pregnant woman, aiding in childbirth, stimulating the uterus at the beginning of child birth and used in relieving menstrual cramp (Hummer, 2010). The utilization of this wild berries species in Sabah is never being scientifically. However, this species is utilized by local communities around Kundasang, Sabah as minor components of their diet. Due to a huge diversity of wild berries, this study aims to investigate the antioxidant and phytochemical content of wildly grown *Rubus fraxinifolius*.

MATERIALS AND METHODS

Sample Collection

Rubus fraxinifolius was collected from Desa Aman, Kundasang, Sabah. Samples were collected and identified by Mr. Johnny Gisil, botanist from Institute for Tropical Biology & Conservation, University Malaysia Sabah. Plant specimen was deposited in BORNEENSIS, Universiti Malaysia Sabah (identification number BORH 3391). The fruits, stem, and leaves were cleaned, cut into small pieces and leave in -80°C for 24 h before placed into the freeze-drying machine. The freeze-dried samples were then grounded using grinder into fine powders and sieved to get uniform size particles, then kept in an air-tight container in -20°C (Abu Bakar *et al.*, 2009).

Extraction

Powdered samples were extracted with 1:10 (sample:solvent) of distilled water (dH_2O), absolute ethanol (ABS ETOH), 80% (v/v) ethanol (80% ETOH), absolute methanol (ABS MEOH), and 80% (v/v) methanol (80% MEOH) at a room temperature on orbital shaker set at 200 rpm for 2 hours (Velioglu *et al*., 1998). The extracts are then centrifuged at 1400 x g for 20 minutes and the supernatant transferred into a beaker. The pellet then re-extracted (2x) under identical conditions. The supernatant then combined and evaporated under vacuum for drying using rotary evaporator except for distilled water (dried with freeze dryer). The temperature of rotary evaporator was set under the solvent boiling point (40°C) and the extracts are kept in 4°C until further analysis. Upon test, all extracts were dissolved back using 80% (v/v) methanol (Harborne, 1998).

Antioxidant Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical assay: Free radical scavenging activity of the sample was determined by DPPH as a model of free radical assay adapted from Yang *et al*. (2011). The percentage of antioxidant activity (AA) in samples was calculated as follow.

$$AA\ (\%) = [1- (A_1 – A_2)/ A_0] \times 100\% \quad (1)$$

A_0 is the absorbance of the control, A_1 is the absorbance in the presence of sample and A_2 is the absorbance of a sample without DPPH radical. The AA (%) of all samples is plotted. The result of DPPH expressed as IC_{50} value (extract concentration which able to inhibit 50% of the used DPPH amount).

ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) assay: ABTS free radical decolorization assay of extract was determined from method by Chun *et al.* (2005). All percentage of antioxidant activity (AA) in samples was calculated following formula (1). ABTS expressed as IC_{50} value (extract concentration which able to inhibit 50% of the used ABTS amount).

FRAP (Ferric reducing/antioxidant power) assay: FRAP procedure was conducted with slight modification (Benzie & Strain, 1996). Standard calibration curve of a fresh working solution of Fe (II) was run in different concentration ranged from 0 to 100 µg/ml and later plotted against its concentration. The results expressed as the concentration of antioxidant having a ferric reducing ability in 1 gram of sample (mM Fe^{2+}/g).

Phytochemical Content

Determination of total phenolic content (TPC): TPC of 80% MEOH extract was determined using Folin-Ciocalteu reagent (Velioglu *et al.*, 1998). For standard, gallic acid calibration curve ranged from 0 to 1000 µg/ml was run. The result of TPC expressed as mg gallic acid equivalent per g of dried weight sample (mg GAE/g).

Determination of total flavonoid content (TFC): TFC were determined by a colorimetric method which adapted from Dewanto *et al.* (2002) using catechin as the reference standard. The result of TFC was expressed as mg catechin equivalent per g of dried weight sample (mg CE/g).

Determination of total anthocyanin content (TAC): TAC was determined by spectrophotometric pH differential protocol based on Giusti & Wrolstad (2011). The TAC value of sample was calculated using the following equation.

$$TAC = A \times Mw \times DF \times 1000/ (\varepsilon \times C) \quad (2)$$

where A is absorbance = $(A_{515} - A_{700})$ pH 1.0 - $(A_{515} - A_{700})$ pH 4.5; Mw is molecular weight for Cyanidin-3-glukosida=449.2; DF is dilution factor for sample, ε is the molar absorptivity of cyanidin-3-glukosida = 26,900; C is the concentration of the buffer mg/ml. The result of TAC expressed as mg cyanidin-3-glucoside (C-3-GE) equivalent to g dried weight sample.

Determination of total carotenoid content (TCC): TCC were determined according to Hess *et al*. (1991). The result of TCC expressed as mg β-carotene (BC) equivalent to g dried weight sample.

Statistical Analysis

All experiments were carried out in five replicates using three independent experiments. All results presented as mean ± standard deviation (S.D). Data were statistically analyzed using one-way ANOVA and Duncan post-hoc test comparison method with a significance level of $p \leq 0.05$.

RESULT AND DISCUSSION

Results in Table 1 shows that leaves of *R. fraxinifolius* had highest antioxidant capacity compared to fruit and stem part. Leaves extract of 80% MEOH showed higher antioxidant activities using DPPH, ABTS and FRAP assays, with value of 48.97 ± 0.33 µg/ml, 51.49 ± 2.52 µg/ml and 214.09 ± 2.42 mM Fe^{2+}/g, respectively. In contrast, fruits extract of dH_2O showed weakest antioxidant potential of DPPH, ABTS and FRAP assay; 808.34 ± 16.96 µg/ml, 351.36 ± 18.91 µg/ml and 48.57 ± 2.8 mM Fe^{2+}/g, respectively. The choice of solvent play an essential roles in term of ability of extracting the secondary metabolites from plant (Said *et al.,* 2018), and can affect the antioxidant mechanism (Abdul Latiff *et al.,* 2017). The results is found in line with Abu Bakar *et al*. (2016), however in this study the leaves extract identified as the predominant parts that contributed to this biological activity. Ethnomedical knowledge from *Rubus* family had been gradually investigated and documented. Instead of being good antioxidant, *R. fraxinifolius* also showed weak antibacterial activity against *Bacillus cereus* (Galvec, 2016).

Leaf extracts of 80% MEOH were selected for phytochemical tests. Result in Table 2 shows the value of total phenolic, flavonoid, anthocyanin and carotenoid for fruit, stem, and leaves with significant differences (p<0.05). It was found that leaves of *R. fraxinifolius* showed the highest phenolic content (56.32 ± 0.05 mg GAE/g) among all tested plant parts, while fruit parts show the lowest total phenolic (8.7±0.10 mg GAE/g). Meanwhile, the highest flavonoid content (31.36 ± 1.05 mg CE/g) was observed on leaves extract, while fruits part showed lowest flavonoid (7.91 ± 0.19 mg CE/g). Both of total phenolic and flavonoid content obtained in the current studies are relatively higher than those reported by Abu Bakar *et al*. (2016). According to Galvec (2016), *R. fraxinifolius* was proven to contain flavonoids and phenolic compounds, as well as other phytochemicals such as alkaloid, phytosterols, tannins and terpenoids. These compounds have been used in management and prevention of diseases that associated with free radicals (Abdul Majid, 2017).

Table 1. Antioxidant activities of different part of *R. fraxinifolius*

Samples	DPPH assay (IC_{50}) (μg/ml)	ABTS assay (IC_{50}) (μg/ml)	FRAP assay (mM Fe^{2+}/g)
Fruit			
dH_2O	808.34±16.96^{i}	351.36±18.91^{g}	48.57±2.8^{k}
ABS ETOH	756.30±48.43^{i}	208.58±5.23^{f}	50.00±1.76^{k}
80% ETOH	707.53±83.38^{i}	204.49±5.60^{f}	55.33±3.57^{j}
ABS MEOH	322.03±6.69^{h}	185.39±2.02^{e}	58.24±2.83^{j}
80% MEOH	233.11±8.14^{f}	164.53±1.57^{d}	64.09±2.23^{i}
Stem			
dH_2O	261.80±12.97^{g}	351.36±18.9^{g}	86.02±1.44^{h}
ABS ETOH	228.62±6.24^{f}	213.05±12.70^{f}	88.46±3.08^{h}
80% ETOH	169.24±3.07^{e}	195.09±10.45^{f}	91.74±1.96^{g}
ABS MEOH	126.24±1.38^{d}	89.84±2.41^{b}	99.61±2.17^{f}
80% MEOH	103.22±1.07^{c}	77.55±2.35^{b}	126.64±2.77^{d}
Leaf			
dH_2O	184.62±44.86^{e}	157.50±6.44^{cd}	96.08±1.90^{f}
ABS ETOH	95.99±2.03^{b}	146.41±9.62^{c}	117.46±2.36^{e}
80% ETOH	106.58±2.88^{c}	145.19±3.99^{c}	154.06±2.12^{c}
ABS MEOH	98.38±0.47^{b}	59.24±0.29^{a}	184.15±2.46^{b}
80% MEOH	48.97±0.33^{a}	51.49±2.52^{a}	214.09±2.42^{a}

Value are expressed as means ± SD (n=3). Mean followed by the different letters (within column) are significantly different at $p< 0.05$ by Duncan test.

Table 2. Phytochemicals content of 80% (v/v) methanol extract in 3 different parts of *R. fraxinifolius*

	Total Phenolic (mg GAE/g)	Total Flavonoid (mg CE/g)	Total Carotenoid (mg BC/g)	Total Anthocyanin (mg C-3-GE/g)
Fruit	8.7±0.10^{c}	7.91±0.19^{c}	10.02±0.22^{a}	(22.27±1.28)^{a}
Stem	26.66±0.08^{b}	25.02±0.84^{b}	8.09± 0.27^{b}	ND
Leaf	56.32±0.05^{a}	31.36±1.05^{a}	7.71±0.21^{b}	11.87±3.40^{b}

Value are expressed as means ± SD (n=3). Means followed by the different letters (within row) are significantly different at $p<0.05$ by Duncan test. ND=Not Detected

Meanwhile, carotenoid content was higher in fruit part compared to stem and leaves. Even though carotenoid is responsible for many of the orange, yellow and red hues of plant fruit, leaves, and flower, but they are also found to be the in a green plant tissue (Dutta *et al.*, 2005; Eldahshan & Singab, 2013). Leaves and stem of *R. fraxnifolius* also contain carotenoid but the colors are hidden by other dominant pigments such as chlorophyll, and these can be seen during decomposition of chlorophyll as it is partially responsible for fall coloration after chlorophyll in leaves has been destroyed (Handelman *et al.*, 1991). This might explain the total carotenoids that are found in the leaves and stem of *R. fraxinifolius.*

The intensity and type of colour produced by plant depend on the present of anthocyanin, where if the hydroxyl group are predominant then the colour becomes more to bluish shade, and if methoxyl group prevail, the colour becomes more into red (Herediaa *et al.*, 1998; Horbowicz *et al.*, 2008). In this study, the results showed that the fruit part of *R. fraxinifolius* had higher anthocyanin content (22.27±1.28 mg C-3-GE/g dried sample) compare to the other plant parts, followed leaves (11.87±3.40 mg C-3-GE/g dried sample). On the other hand, there are no anthocyanins detected in

stem part. Total anthocyanin content in this current study was slightly lower than total anthocyanin as reported by Abu Bakar *et al.* (2016). This is maybe due different location of sample collection.

CONCLUSION

In this paper, extracts from different part of *Rubus fraxinifolius* have been analyzed for their antioxidant activities and phytochemicals content. For extraction method, it was found that 80% (v/v) methanol exhibits a good recovery of potent antioxidant component from *R. fraxinifolius*. In terms of its contents, group of astounding phytochemicals such as phenolic, flavonoid, carotenoid and anthocyanin were detected in plant extracts. In addition, leaves extract had significant potential as an antioxidative when tested with DPPH, ABTS and FRAP antioxidant assay compared to fruits and stem. Finding in this study might help to ascertain the potency of *R. fraxinifolius* for their nutraceutical and medical health applications. Consumption of this fruit are recommended as new alternative sources of edible wild fruit which could offer a protection against free radical damage to human body.

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