Evaluation of phytochemical content and antioxidant activity in *Rubus fraxinifolius* fruit extracts using different solvents

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ABSTRACT *Rubus fraxinifolius* is one of the wild berries (Rogimot) species native to the highland regions of Southeast Asia, including Sabah, Malaysia. Despite their ethnobotanical significance, studies on its phytochemistry and antioxidant potential remain limited. This study compares the antioxidant activity and phytochemical content of fruit extracts from *Rubus fraxinifolius* using, 80% methanol, 70% acetone and distilled water. Antioxidant properties were evaluated using DPPH and FRAP assays, while total phenolic content (TPC), total flavonoid content (TFC) and total anthocyanin content (TAC) were also quantified. The results showed that the methanolic extract of *R. fraxinifolius* had the highest TPC (36.70±0.56 ± 0.44 mg GAE/g), TFC (12.15 ± 0.13 mg QE/g), and TAC (76.41± 71 mg C3G/ 100g) values. Antioxidant analysis showed 80% methanol exhibited the highest DPPH scavenging activity, IC_{50} = 64.47±5.55 µg/mL, and highest FRAP activity, 71.69±2.88 (µg TE/mL) compared to 70% acetone and distilled water. These findings showed significant variation in phytochemical content and antioxidant activity based on the solvent used, highlighting the importance of solvent selection in optimising bioactive compound extraction. Further research to evaluate its detailed phytocompound components and bioactivity is strongly warranted.

KEYWORDS: *Rubus Fraxinifolius*; Rogimot; Antioxidant; Flavonoids; Anthocyanins
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INTRODUCTION

Plants, as a natural source of food, are rich in bioactive phytochemicals that provide valuable health benefits. Among these are the genus *Rubus*, under the Rosaceae family, comprising various berry-producing shrubs like raspberries and blackberries (Joshi *et al*., 2024). This includes 12 subgenera and more than 700 species and can grow at elevations up to 4,500 meters above sea level (Bhuyan & Dutta, 2021; Foster *et al*., 2019). Berries are valued for their nutritional, medicinal, and economic importance (Joshi *et al*., 2024). Furthermore, they contain high secondary metabolites content as anthocyanins, phenolics, flavonoids, terpenoids, ellagitannins and saponins (Foster *et al*., 2019). Several *Rubus* species has been reported in the highlands of Sabah, Malaysia including *Rubus rosifolius*, *Rubus moluccanus* L., *Rubus fraxinifolius* Poir., and *Rubus alpestris* Blume (Abu Bakar *et al*., 2016). Locally known as "Rogimot" among native Kadazandusun tribe, the fruit has been traditionally and medically used in the diet by the local communities (John *et al*., 2025; Shamsudin *et al*., 2019). In Borneo, *Rubus* plants are also found in Sarawak and in Kalimantan (Chai, 2000; Setiyadi *et al*., 2018).

Phenolic acids are dietary polyphenols that act as natural antioxidants, aiding plant growth and protect against environmental stress (Lin *et al*., 2016). Similarly, flavonoids are essential plant polyphenolic compounds which contribute to human health through their antimicrobial, antioxidant, anti-inflammatory, and anticancer properties (Jomova *et al*., 2025). Previously, Desmiaty *et al*. reported that methanolic extracts of *R. fraxinifolius* leaves had higher total phenolic content and antioxidant activity than n-hexane and ethyl acetate extracts, indicating that solvent choice can

significantly affect phytochemical extraction (Desmiaty *et al*., 2018). Previous reports showed that the fruits of *R. fraxinifolius* from Sabah contain phenolic compounds, flavonoids, carotenoids and anthocyanins (Abu Bakar *et al*., 2016; Shamsudin *et al*., 2019) with the methanolic fruit extract showed better antioxidant activities (Abu Bakar *et al*., 2016; Shamsudin *et al*., 2019). However, research on the effect of extraction solvents on the phytochemical recovery of *Rubus fraxinifolius* fruits from Sabah, Malaysia remains limited. Therefore, this study aims to expand current knowledge by investigating the effects of different extraction solvents (methanol, acetone, and water) on the phytochemical composition and antioxidant properties of *R. fraxinifolius* fruit extracts.

METHODOLOGY

Sample Collection and Extraction

Rubus fraxinifolius fruit samples were obtained from Kg. Tudan, Tambunan, Sabah, Malaysia. The fruits were identified by Mr. Johnny Gisil, a botanist from Universiti Malaysia Sabah. Fresh fruits were frozen and freeze-dried using a Labconco FreeZone Freeze Dryer system (Kansas City, MO, USA). Dried fruits were blended into powder and kept in -20°C for further analysis. Fruit powder samples were extracted using several solvents (80% methanol (v/v), 70% acetone (v/v) and distilled water respectively, by agitation at 250rpm for 2 hours using mechanical shaker, to make a final crude extract concentration of 20 mg/mL. For anthocyanin analysis, 50mg/mL of extract was used. After agitation, the solution was centrifuged for 10 minutes at 3000 rpm, the supernatant was filtered using Whatman paper No. 1 and kept at -20°C until further use.

Total Phenolic Content (TPC)

The total phenolic content was quantified as described by Velioglu *et al*. (Velioglu *et al*., 1998). Briefly, 100 µL of sample extract was mixed with 750 µL of Folin-Ciocalteu reagent (diluted 1/10 with distilled water), vortexed and incubated in the dark for 5 minutes. 750 µL of sodium carbonate solution (60 g/L) was added, and the reaction mixture was left for 90 minutes. Absorbance was measured at 725nm using a microplate reader. The standard was Gallic acid (0 to 100 µg/mL), results were expressed as milligrams of gallic acid equivalent per gram of dried sample (mg GAE/g).

Total Flavonoid Content (TFC)

Total flavonoid content assay was based on the method by Chang *et al.* with modifications (Chang *et al*., 2002). 120µL of the extract/standard was mixed with 360 µL of methanol, followed by the addition of 24 µL of 10% (w/v) aluminium chloride solution. The mixture was left to stand for 6 minutes. Then, 24 µL of 3.0 M potassium acetate was added, and the reaction was left to stand for 5 minutes. Finally, 680 µL of distilled water was added. From this mixture, 300 µL was transferred into a microplate well and the absorbance was measured at 415nm. A control blank was prepared by mixing 120 µL of methanol with 180 µL of distilled water. A control sample was prepared by mixing 30 µL of plant extract with 270 of distilled water in each well. Quercetin (0 - 100µg/mL) was used as the standard and results were expressed as milligrams of quercetin equivalent per gram of dried sample (mg QE/g).

Total Anthocyanin Content (TAC)

Total anthocyanin content was measured following the method by Giusti and Wrolstad with slight modifications (Giusti & Wrolstad, 2001) as given in Equation (1). In briefly, 25 µL of extract (50 mg/mL) was added in a microplate and mixed with 175 µL of potassium chloride buffer (0.025M, pH 1.0). The mixture was left for 15 minutes. Absorbance was measured at 515 and 700 nm using distilled water as the blank. In another microplate, 25 µL of the extract was mixed with 175 µL of

sodium acetate buffer (0.025M, pH 4.5), followed by a 15-minute incubation. Absorbance was measured at 515nm and 700nm.

$$\text{Total anthocyanin content} = A \times Mw \times DF \times 1000 / (\varepsilon \times l) \quad (1)$$

A, absorbance = (A515-A700) pH 1.0 – (A515 – A700) pH 4.5, Mw for cyanidin-3-glucoside = 449.2, DF is a dilution factor of the samples = 8, ε = is the molar absorptivity of cyanidin-3-glucoside = 26900, and l = pathlength in cm. The result was expressed as mg cyanidin-3-glucoside (C3G) equivalent per 100g samples.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

DPPH free radical scavenging assay was adapted using the methods described by Clarke *et al*. (2013) and Baliyan *et al*. (2022) and is shown in Equation (2). A 0.3 mM DPPH solution was prepared by dissolving 0.0118 g of DPPH powder in 100 mL of absolute methanol. 100 µL of the extract at varying concentrations (4–5000 µg/mL) was mixed with 100 µL of the DPPH solution in a microplate well. The mixture was incubated in the dark for 30 minutes. Absorbance was measured at 540 nm.

$$\text{Antioxidant activity percentage, AA (\%)} = [(Ac - As)/Ac] \times 100\% \quad (2)$$

AA = Antioxidant activity percentage, Ac = Absorbance of methanol only with DPPH reagent
As = Absorbance of sample with DPPH reagent, The AA (%) of all samples is plotted and the result of DPPH is expressed as IC_{50} value (half maximal inhibitory concentration).

Ferric Reducing/Antioxidant Power (FRAP) Assay

The FRAP assay applied the method of Russo *et al*. (2013) with modifications. In brief, 20 µL of the extract (1mg/mL)/ standard solution was mixed to 180 µL of freshly prepared FRAP reagent. The FRAP reagent content was 100 mL of acetate buffer (300 mM, pH 3.6), 10 mL of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution, and 10 mL of 20 mM ferric chloride solution. The reaction mixture was incubated at 37°C in the dark for 40 minutes. Absorbance was then measured at 593 nm. Trolox (0 – 100 µg/mL) was used as standard and results were expressed as Trolox equivalent (TE) µg/mL.

Statistical analysis

Statistical analysis was conducted using one-way ANOVA, followed by Tukey's post hoc test for multiple comparisons. Differences were considered statistically significant at $p \leq 0.05$. Experiments were done in triplicates. Statistical tests were conducted by using GraphPad Prism (version 8.0, San Diego, CA, USA) and results are presented as mean ± standard deviation (SD).

RESULT AND DISCUSSION

Table 1 shows the total phenolic, total flavonoid and total anthocyanin content and antioxidant activities of *R. fraxinifolius* fruits methanolic, acetonic and aqueous extracts. The total phenolic content (TPC) for 80% methanol was 36.70±0.56 mg GAE /g, significantly higher than distilled water and 70% acetone, 35.27±0.35 and 31.01±0.46 mg GAE /g, respectively. The total flavonoid content (TFC) for 80% methanol also exhibited higher value, 12.15±0.13 mg QE/g, compared to 9.97±0.19 mg QE/g in distilled water and 8.39±0.08 mg QE/g in 70% acetone extracts. Overall, we found that 80% methanol is the most effective solvent for phenolic compounds extraction from *R. fraxinifolius*. In our study, the values obtained for the 80% methanolic extract in TPC and TFC tests were slightly higher than reported previously by Shamsudin *et al.* (TPC: 8.7±0.10 mg GAE/g, TFC: 7.91±0.19 mg QE/g) and Abu Bakar *et al.* (TPC: 11.09±0.10 mg GAE/g), which could be due to variation in methodology or sampling locations (Abu Bakar *et al*., 2016; Shamsudin *et al*., 2019). Further comparison with other studies showed that the total phenolic content was similar to Serbian wild grown raspberry

(36.23±0.43 mg GAE/g) (Veljković *et al*., 2019) and slightly higher than the Romanian blackberry (*Rubus fruticosus* L.) extracted with methanol (27.89±2.11 mg GAE/g) (Albert *et al*., 2022). Among the Japanese wild berries tested, *Rubus trifidus* Thunb showed similar TPC content (36.77±0.43 mg GAE/g) compared to other species (Kumazawa *et al*., 2024).

The total anthocyanin analysis showed that 80% methanol recovered more anthocyanin content (76.41±0.71 mg C3G equivalent/100g) compared to 70% acetone (73.56±0.86 mg C3G equivalent/100g) and distilled water (52.72±1.20 mg C3G equivalent/100g). A previous study showed that methanolic extract is more efficient for recovering anthocyanins and flavonols in blackberries (*Rubus spp*.), whereas acetonic extract was more effective in extracting flavan-3-ols and ellagitannins (Liao *et al*., 2021), showing that polar compounds are more suitable in extracting anthocyanins from plants. Despite this, when compared to the total anthocyanin content from dried Romanian Blackberry (773.30±68.34 mg C3G equivalent/100g), the anthocyanin content found in our study is significantly low (Albert *et al*., 2022), this pattern is also observed in Japanase wild berries (5.97 ± 0.18 μmol/100 g) and Japanese blackberries (71.12 ± 2.59 μmol/100 g) (Kumazawa *et al*., 2024), suggesting the influence of climate or species variation in anthocyanin distribution.

Table 1. Phytochemical contents and antioxidant activities of 80% methanol, 70% acetone and extracts of *R. fraxinifolius* fruit

Assays	80% Methanol	70% Acetone	Distilled water
Total Phenolic Content	36.70±0.56^{a}	31.01±0.46^{c}	35.27±0.35^{b}
Total Flavonoid Content	12.15±0.13^{a}	8.39±0.08^{c}	9.97±0.19^{b}
Total Anthocyanin Content	76.41±0.71^{a}	73.56±0.86^{b}	52.72±1.20^{c}
DPPH (IC50)	64.47±5.55^{b}	77.82±6.94^{a}	83.22±0.63^{a}
FRAP	71.69±2.88^{a}	57.53±5.33^{b}	48.19±4.08^{b}

Values are presented as mean ± standard deviation (SD). Values in the same row with different letters are significantly different ($p < 0.05$). Total Phenolic Content (TPC) expressed as expressed as milligrams of gallic acid equivalent per gram of dried sample (mg GAE/g). . Total Flavonoid Content (TFC) expressed as milligrams of quercetin equivalent per gram of dried sample (mg QE/g). Total Anthocyanin Content (TAC) expressed as mg of Cyanidin-3-glucoside (C3G) equivalent/100g, DPPH assay is expressed as IC50 μg/mL, Ferric Acid Reducing Potential (FRAP) assay expressed as Trolox equivalent (TE) μg/mL.

Based on Table 1, the DPPH activity in *R. fraxinifolius* 80% methanolic extract exhibited the lowest IC_{50} value (64.47 ± 5.55 μg/mL), indicating significantly higher antioxidant activity compared to 70% acetone and water extracts. This result showed better activity compared to Serbian wild raspberry fresh fruit samples (IC_{50} = 294.79 μg/mL) (Veljković *et al*., 2019). The FRAP assay also indicated that 80% methanol has the highest antioxidant activity, 71.69±2.88 TE μg/mL, compared to 70% acetone 57.53±5.33 TE μg/mL and water extract, 48.19±4.08 TE μg/mL. The DPPH and FRAP findings from this study also reflect the previous findings showing highest activity in 80% methanol extract of *Rubus fraxinifolius* from Kundasang, Sabah (Shamsudin *et al*., 2019). However, a previous study by Albert *et al*. reported that acetone extracts of *Rubus froticosus* exhibited higher antioxidant activity than methanolic and ethanolic extract (Albert *et al*., 2022). As a comparison, the Japanase wild *Rubus* species showed comparable FRAP activity to raspberry (*Rubus idaeus*) but lower than blackberry (*Rubus fruticosus*) (Kumazawa *et al*., 2024). The antioxidant capacity of *Rubus* fruits is closely associated with their content of phenolics, flavonoids, tannins, and anthocyanins (Muniyandi *et al*., 2019). Thus, further phytochemical analysis such as measuring tannin and other phycompound contents can be done to further evaluate the phytochemical constituents of the fruit.

The use of other green solvents such as ethanol is also suggested to compare their extracting capacity to methanol. The choice of solvent is a key factor in extraction, as it affects selectivity, solubility, and safety as solvents with a polarity similar to the target compounds usually give better results (Lee *et al*., 2024). Depending on the type of compounds being extracted, the mixtures of solvents can also be used to improve efficiency (Lee *et al*., 2024). As this is a preliminary study on the fruit of *R. fraxinifolius*, additional analytical techniques such as liquid chromatography can be employed to identify and characterise the specific compounds. Bioactivity assays are strongly warranted to further characterise the fruit's biological effects and potential toxicity. Additionally, further study could assess the difference in phytochemical content and antioxidant activity of native Bornean berries against commercial *Rubus* species such as blackberry or raspberry.

CONCLUSION

This preliminary study evaluated the effects of various extraction solvents from fruit of *R. fraxinifolius* for its phytochemical composition and antioxidant properties. In this study, 80% methanol was identified as the most effective solvent for extracting key phytochemicals, namely phenolics, flavonoids, and anthocyanins, followed by 70% acetone and distilled water. Extract obtained using 80% methanol also exhibited superior antioxidant activity, as determined by DPPH and FRAP assays. These findings highlight the potential health benefits of consuming native wild berry such as *R. fraxinifolius*, due to their rich phytochemical content and strong antioxidant properties.

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