Anti-gout potential of selected Malaysian local fruits

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Abstract. This study aimed to investigate the in vitro xanthine oxidase (XO) inhibitory activity and phytochemical content of guava, water rose apple, Malay gooseberry, pineapple and ambarella. The xanthine oxidase inhibitory activity was measured spectrophotometrically at 295 nm. The phytochemical analysis tested were total phenolic, total flavonoid and total anthocyanin contents of each methanolic extract of the fruits. The highest amount of phenolic was found in ambarella (0.245 mg GAE/g) while guava had the highest amount of flavonoid (0.472 mg RE/g). Meanwhile, water rose apple had the highest anthocyanin content (5.001 mg c-3-gE/g). For the XO inhibitory activity, water rose apple displayed the lowest IC50 value (26.86 µg/mL), showing better anti-gout activity as compared to that of other fruit samples. Positive correlation between total phenolic content and XO inhibitory activity was also observed in this study. Further study on the isolation of bioactive compounds from the fruit samples that act as XO inhibitor is greatly needed in the future.

1. Introduction

Gout is a common metabolic disorder caused by chronic elevation of serum uric acid (SUA) levels beyond the saturation point of monosodium urate (MSU) crystal formation that lead to deposition of MSU crystal in peripheral joints and tissues [1]. This disease can be treated using allopurinol, synthetic xanthine oxidase inhibitor commonly used in the clinical management of gout. However, it gives side effect to the patients. Due to this factor, research on medicinal plants has increased worldwide and treating disease using natural sources is gaining new interest of researchers [2]. As reviewed by Abu Bakar et al. [3], many previous studies have shown the effectiveness of phytochemical compounds as source of therapeutic agents in treating gout by lowering the uric acid level and act as xanthine oxidase inhibitors.

Phytochemical compounds such as flavonoid, anthocyanins, and phenolic which known to have antioxidant and anti-inflammatory properties can be found mainly in fruits and vegetables [4, 5]. Local fruits such as guava, Malay gooseberry, water rose apple, pineapple and ambarella can be easily found and being consumed by the people. These fruits are believed to have anti-gout properties. Hence, this study aimed to evaluate the xanthine oxidase inhibitory activity and phytochemical compounds of the methanol extract of guava, Malay gooseberry, water rose apple, pineapple and ambarella pulp.
2. Materials and Methods

2.1 Preparation of sample and extract
The sample and extraction was prepared with some modifications [6]. The selected fruits (guava, ambarella, water rose apple, Malay gooseberry and pineapple) were sorted out and washed thoroughly to remove dirt. After that, they were separated into seed, pulp and fruit peels using a table knife. The edible part of fruits were cut into small pieces and stored at -80 °C before lyophilized using a freeze dryer. The lyophilized fruits were grounded into powder form using blender and kept at -20 °C. For extraction, the methanolic extract was prepared by mixing 1 g of the lyophilized fruit powder with 80 % methanol (v/v) at a ratio 1:10. The mixture was placed in a conical flask that wrapped with an aluminium foil and agitated at 200 rpm with the aid an orbital shaker for 30 min. After 30 min, the mixture was filtered through filter paper (Whatman No.4) to obtain a clear solution. The supernatant was collected and subsequently been used for determination of total phenolic, flavonoid, anthocyanin as well as XOI activity.

2.2 Determination of total phenolic content (TPC)
The phenolic content was determined using Folin-Ciocalteu method with slight modification [7]. 1 mL of pulp extract (1 mg/mL) was mixed thoroughly with 5 mL of Folin-Ciocalteu reagent solution using vortex and allowed to stand at room temperature for 5 min. Then, 4 mL of (75 g/L) sodium carbonate was added into the mixture and allowed to stand for 30 min at room temperature. The absorbance was measured at 765 nm after 30 min using spectrophotometer. Gallic acid was used as a reference standard and the total phenolic content was determined from the calibration curve. The results were expressed as milligram gallic acid equivalents 100 g dry weight.

2.3 Determination of total flavonoid content (TFC)
The flavonoid content was measured using aluminium chloride colorimetric assay with some modifications where rutin was used as a standard [8, 9, 10]. 1 mL of extracts of an aliquot or rutin standard solution were added to a 10 mL volumetric flask that containing 4 mL of distilled deionized water. Then, 0.3 mL of 5 % sodium nitrite solution (NaNO₂) were added into each volumetric flask. After 5 min, 0.6 mL of 10 % aluminium chloride (AlCl₃) were added. Then, 2 mL of 1M sodium hydroxide (NaOH) and 2.1 mL of distilled water were added after 6 min and mixed using vortex. The absorbance was measured against prepared reagent blank at 510 nm. The results were expressed as mg of rutin equivalents/ 100 g of dry mass.

2.4 Determination of total anthocyanin content (TAC)
Total anthocyanin content was measured using spectrophotometric pH differential method [11, 12, 13]. 0.5 mL of the extract was mixed thoroughly with 3.5 mL of 0.025 M potassium chloride buffer pH 1. Then, the mixture was mixed with vortex and allowed to stand for 15 min. After that, the absorbance was measured at 515 and 700 nm against a distilled water blank using spectrophotometer. Then, same extract was combined with 3.5 mL of 0.025 M sodium acetate buffer pH 4.5 and the absorbance was measured at the same wavelength after being allowed to stand for 15 min. The results were expressed as mg of cyanidin-3-glucoside equivalents in 100 g of dried sample (mg c-3-gE/100 g dried sample).

2.5 In vitro xanthine oxidase inhibitory (XOI) activity
The XOI activity was measured spectrophotometrically at 295 nm under an aerobic condition with some modifications [2, 14, 15, 16]. In this inhibition test, 100 µg/mL of allopurinol was used as a positive control. The mixture was prepared by mixing 300 µL of 50 mM sodium phosphate buffer (pH 7.5), 100 µL of the sample solution, 100 µL of freshly prepared enzyme solution and 100 µL of distilled water. After that, the mixture was pre-incubated at 37 °C for 15 min. Then, 200 µL of a substrate solution (0.15 mM of xanthine) was added to the mixture and incubated at 37 °C for 30 min. The reaction was stopped with the addition of 200 µL of 0.5 M HCl. Next, the absorbance was measured using UV/VIS
spectrophotometer against blank prepared in the same way but the enzyme solution was replaced with phosphate buffer. Meanwhile, another reaction mixture (control) was prepared using 100 µL of dimethylsulfoxide (DSMO) instead of test compounds in order to have maximum uric acid formation. The equation was used to evaluate the degree of XO inhibitory activity. Thus, XOI activity was calculated, in which α is the activity of XO without test extract and β is the activity of XO with test extract.

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\text{% XO inhibition} = (1 - \beta/\alpha) \times 100
\]

2.6 Statistical analysis

All the results were expressed as mean ± standard deviation. The data for correlation analysis between phytochemical content and xanthine oxidase inhibitory activity was analyzed using Pearson’s correlation using Statistical Package for the Social Sciences (SPSS) software version 22.0.

3. Results and Discussion

Table 3.1. Phytochemical contents and xanthine oxidase inhibitory activity of guava, water rose apple, pineapple, Malay gooseberry and ambarella.

| Sample           | Total phenolic content (mg GAE/g) | Total flavonoid content (mg RE/g) | Total anthocyanin (mg c-3-gE/g) | Xanthine oxidase - IC\textsubscript{50} (µg/mL) |
|------------------|-----------------------------------|-----------------------------------|---------------------------------|---------------------------------------------|
| Guava            | 0.101 ± 0.002                     | 0.472 ± 0.013                     | 4.609±0.1681                    | 102.7                                       |
| Ambarella        | 0.245 ± 0.062                     | 0.046 ± 0.006                     | 3.986±0.4081                    | 36.1                                        |
| Water rose apple | 0.095 ± 0.005                     | 0.149 ± 0.015                     | 5.001±0.4171                    | 26.86                                       |
| Malay gooseberry | 0.106 ± 0.003                     | 0.321 ± 0.001                     | 4.832±1.1023                    | 44.52                                       |
| Pineapple        | 0.244 ± 0.004                     | 0.033 ± 0.002                     | 1.804±0.4380                    | 39.62                                       |

Table 3.1 shows the results for total phenolic content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC) and xanthine oxidase (XO) inhibitory activity of guava, water rose apple, pineapple, Malay gooseberry and ambarella pulp methanol extract. The range of TPC of fruit pulp extracts was from 0.095 to 0.245 mg GAE/g. The highest amount of TPC was found in ambarella, followed by pineapple, Malay gooseberry, guava and water rose apple. This might be due to the diverse phytochemical contents in the fruits. Previous study showed that the unripe fruit contained higher TPC as compared to that of ripe fruit [17]. This supported the current study where the lowest amount of TPC in water rose apple due to its maturity stage (ripe) and it also had some blemish parts on the surface. Thus, it affects the phenolic component of the fruit.

Meanwhile, TFC ranged from 0.033 to 0.472 mg RE/g. The highest TFC was found in guava, followed by Malay gooseberry, water rose apple, ambarella and pineapple. The lowest amount of TFC in pineapple may be due to the antioxidant capacity of flavonoids which suffer from the influence of oxygen in the atmosphere, because their easy auto-oxidation [18]. Anthocyanin content can be refer to dark red colour of fruits [19]. Water rose apple pulp extract had the highest anthocyanin content while pineapple pulp had the lowest anthocyanin content compared to other fruits. The range of total anthocyanin content of fruits in this study was from 1.804 to 5.001 mg c-3-gE/g. Few factors have been identified to affect the phytochemical contents in fruits such as radiation from sun, temperature variation and climatic conditions at a geographical location [20- 22].

In addition, water rose apple had the lowest IC\textsubscript{50} value (concentration needed to inhibit xanthine oxidase activity by 50%) with 26.86 µg/mL as compared to that of other fruit samples where the smaller
IC₅₀ value, the better inhibition of xanthine oxidase activity. Meanwhile, the highest IC₅₀ value was found in guava with 102.7 µg/mL. Based on the results obtained, it can be said that all fruits in this study had the ability to inhibit XO activity which consequently preventing the gout disease.

**Table 3.2. Correlation coefficients of each analysis**

|          | TPC   | TFC   | TAC   | XOI activity |
|----------|-------|-------|-------|--------------|
| Pearson correlation | 1     | -.736** | -.685** | .180         |
| Sig. (2-tailed)      | .002  | .005  | .521  |              |
| Pearson correlation  | -.736** | 1     | .571* | -.757**      |
| Sig. (2-tailed)      | .002  | .026  | .001  |              |
| Pearson correlation  | -.685** | .571* | 1     | -.059        |
| Sig. (2-tailed)      | .005  | .026  | .833  |              |
| Pearson correlation  | .180  | -.757** | -.059 | 1            |
| Sig. (2-tailed)      | .521  | .001  | .833  |              |

*Note: *. Correlation is significant at the 0.05 level (2-tailed)
**. Correlation is significant at the 0.01 level (2-tailed).

As shown in Table 3.2, negative correlation was found between TFC and XO inhibitory activity (r² = -0.757). The same result was shown between TAC and XO inhibitory activity (r² = -0.059). Meanwhile, positive correlation was found between TPC and xanthine oxidase inhibitory activity (r² = 0.189). This was in line with the previous study [23].

4. Conclusion

Ambarella had the highest amount of TPC while guava had the highest amount of TFC. Moreover, water rose apple displayed the highest amount of TAC and strongest inhibition of XO activity. Hence, further study on the isolation of bioactive compounds present in these fruits that act as XO inhibitor is greatly needed in future.

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