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Antigout Potential of Selected Malaysian Traditional Vegetables/Ulam

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The discovery of pharmacologically active plant-derived natural products as an alternative approach is highly needed for the purpose of disease treatment, specifically in gout remedy. This study was conducted to investigate the antigout potential of five selected Malaysian traditional vegetables/ulam, namely *Gynura bicolor*, *Barringtonia racemosa*, *Centella asiatica*, *Manihot esculenta* and *Lasia spinosa*. *n*-Hexane, ethyl acetate and methanol extracts of the selected traditional vegetables/ulam were analyzed for their xanthine oxidase inhibitory activity and phytochemical contents. The highest total phenolic and total flavonoid contents were observed in methanol extracts of *L. spinosa* and *M. esculenta* with the values of 1.68 ± 0.05 mg GAE/g and 1.26 ± 0.05 mg RE/g, respectively whereas methanol extract of *G. bicolor* showed the highest total anthocyanin content. *G. bicolor* extracted in three sequential solvents were found to possess the strongest xanthine oxidase inhibition with the lowest IC_{50} values. Therefore, the presence of phytochemical compounds in *G. bicolor* extracts might contribute to its antigout activity, suggesting this plant as antigout agent.

Keywords: Traditional vegetables, Ulam, Gout, Total flavonoid content, Total anthocyanin content, Xanthine oxidase inhibition.

INTRODUCTION

Gout is classified as a type of inflammatory arthritis triggered by the accumulation of urate crystals at the synovial fluid of joints and tissues [1]. The deposition of excess uric acid and failure of excreting uric acid from the body are the two main factors of developing gout [2]. The imbalance in uric acid level is probably affected by genetic factor, increased purine-rich diet, obesity, excessive alcohol consumption, kidney damage as well as certain medications such as diuretics and aspirin [3]. Additionally, dehydration may increase the risk of gout flares [4]. The symptom of gout disease is associated with the swelling and pain at the patient's joints and it may lead to movement limitations [3]. However, gout is treatable using modern medication as the first line of the therapy.

Despite the effectiveness of synthetic drugs for gout treatment, its application has been detrimental to some patients due to its adverse effect, including the toxicity to kidneys and liver [5]. Therefore, it is encouraged to explore the complementary and alternative cure from the natural products, particularly the food such as ulam that is common for consumption by the locals. In general, ulam or commonly known as traditional vegetables or salad is an edible food that often consumed as a side dish and considered to be the daily diet of the elders and also it contains rich nutrient and phytochemical content which is beneficial to human health [6,7]. In addition, these traditional vegetables are easy to be found as they are widely cultivated by villagers and available in the markets at affordable price.

To date, there is lack of study regarding the phytochemical content and antigout properties of these five selected Malaysian traditional vegetables, namely *Gynura bicolor*, *Barringtonia racemosa*, *Centella asiatica*, *Manihot esculenta* and *Lasia spinosa*. A detailed study on the selected samples was carried out through the sequential solvent extraction for the evaluation of their phytochemical contents such as phenolic, flavonoid and anthocyanin and also xanthine oxidase inhibition ability as well as their correlation.

EXPERIMENTAL

Sample collection and identification: Malaysian traditional vegetables or ulam samples including *G. bicolor* (local name: Sambung Nyawa Ungu), *B. racemosa* (local name: Putat), *C. asiatica* (local name: Pegaga), *M. esculenta* (local name: Pucuk Ubi) and *L. spinosa* (local names: Geli-geli or Keladi Keris) were chosen based on the dietary habits of the locals. The fresh samples were collected from the villages and markets located at Pagoh, Johor and Wakaf Bharu, Kelantan, Malaysia. Plant identification was carried out by the botanists and also through the assistance of databases such as Malaysia Biodiversity Information System (MyBIS), Flora Fauna and Useful Tropical Plants website as references.

Sample extraction: The samples were freeze-dried for two nights to remove its moisture while still maintain its nutritional value [8]. Sequential solvent extraction was selected as the extraction method in this study and three solvents *viz. n*-hexane, ethyl acetate and methanol with varying polarities used in the extraction.

Quantitative analysis of phytochemicals: In the current study, Folin-Ciocalteau and aluminium colorimetric methods were used to analyze the contents of phenolic and flavonoid compounds [9,10] whereas the content of anthocyanin was determined using pH-differential method [11]. The absorbance was measured using ultraviolent-visible (UV-Vis) spectrophotometer and each analysis was done in triplicates.

Antigout assay: Antigout potency was tested *in vitro* using xanthine oxidase inhibition assay [12]. The xanthine oxidase inhibition ability of selected samples was analyzed at the concentrations of 10, 25, 50, 75 and 100 µg/ml. Allopurinol was used as the positive control in the assay. IC_{50} was obtained from the logarithmic function curve of the plot of different concentrations (10-100 µg/mL) against the percentage of xanthine oxidase inhibition in order to evaluate the effectiveness of each selected sample in response to their xanthine oxidase inhibition.

RESULTS AND DISCUSSION

Total phenolic content: In present study, the range of total phenolic content among the selected Malaysian traditional vegetables in hexane, ethyl acetate and methanol extracts was from 0.01 ± 0.001 to 1.68 ± 0.05 mg GAE/g (Table-1). *n*-Hexane and methanol extracts of *L. spinosa* (0.10 ± 0.003 and 1.68 ± 0.05 mg GAE/g, respectively) as well as the ethyl acetate extracts of *G. bicolor* and *C. asiatica* (0.12 ± 0.02 and 0.12 ± 0.01 mg GAE/g, respectively) were found to have the highest phenolic content as compared to other samples in each extract. This study indicates that phenolic compounds were present in all the selected samples, but the maximum amount of phenolic was observed in the samples extracted in methanol. Naczk & Shahidi [13] stated that large amount of phenolic compounds could be efficiently extracted using a more polar solvent. In fact, phenolic compound is soluble in polar solvents due to the presence of hydroxyl group [14]. Therefore, methanol solvent could maximize the extraction of phenolic compounds from the selected Malaysian traditional vegetables.

TABLE-1
TOTAL PHENOLIC CONTENT OF SELECTED MALAYSIAN TRADITIONAL VEGETABLES/ULAM

Samples	Total phenolic content (mg GAE/g)		
	Solvent extracts		
	Hexane	Ethyl acetate	Methanol
Gynura bicolor	0.03±0.001^{d}	0.12±0.02^{a}	0.26±0.05^{a}
Barringtonia racemosa	0.01±0.001^{e}	0.04±0.01^{c}	0.75±0.33^{a}
Centella asiatica	0.03±0.005^{c}	0.12±0.01^{a}	0.59±0.06^{ab}
Manihot esculenta	0.08±0.01^{b}	0.07±0.002^{bc}	0.39±0.05^{bc}
Lasia spinosa	0.10±0.003^{a}	0.09±0.05^{ab}	0.68±0.05^{a}

Results are expressed in mean ± standard deviation (n = 3).
$^{a-e}$Values with different lowercase within the same column are significantly different at $p < 0.05$.

Total flavonoid content: In overall, total flavonoid content ranged from 0.05 ± 0.01 to 1.26 ± 0.05 mg RE/g (Table-2) and this result shows that the presence of flavonoid compounds were observed in all the samples. Among the samples extracted in hexane and ethyl acetate solvents, *G. bicolor* had the highest total flavonoid contents with the values of 0.15 ± 0.02 and 1.08 ± 0.14 mg RE/g, respectively. Meanwhile, methanol extract of *M. esculenta* significantly exhibited the highest flavonoid content (1.26 ± 0.05 mg RE/g) compared to other methanolic samples. Amount of flavonoid were varied among the samples regardless of the sequential solvents in the present study. Cultivars and climate could be the factors influencing the structure of bioactive compounds in plants [15,16]. Hence, this could be the reason, which leads to a variation in total flavonoid content of the selected Malaysian traditional vegetables.

TABLE-2
TOTAL FLAVONOID CONTENT OF SELECTED MALAYSIAN TRADITIONAL VEGETABLES/ULAM

Samples	Total flavonoid content (mg RE/g)		
	Solvent extracts		
	Hexane	Ethyl acetate	Methanol
Gynura bicolor	0.15±0.02^{a}	1.08±0.14^{a}	0.46±0.12^{c}
Barringtonia racemosa	0.10±0.01^{bc}	0.64±0.19^{abc}	0.51±0.01^{c}
Centella asiatica	0.12±0.02^{ab}	0.90±0.46^{ab}	0.49±0.08^{c}
Manihot esculenta	0.09±0.02^{c}	0.54±0.15^{bc}	1.26±0.05^{a}
Lasia spinosa	0.05±0.01^{d}	0.30±0.07^{c}	1.11±0.04^{b}

Results are expressed in mean ± standard deviation (n = 3).
$^{a-e}$Values with different lowercase within the same column are significantly different at $p < 0.05$.

Total anthocyanin content: Table-3 displays the total anthocyanin content of the selected samples extracted in three different sequential solvents. As shown in the result, the presence of anthocyanin was observed in methanol extract of all the samples and also in ethyl acetate extract of *G. bicolor*. Anthocyanin belongs to the class of phenolic compounds and its solubility could be seen in highly polar solvents such as water and methanol [17]. Therefore, the compound was not detected in hexane extract of the samples possibly because of its non-polar property and the presence of high level of wax, oil and Fchlorophyll in the solvent [18]. In contrast, high anthocyanin content was found in ethyl acetate and methanol extracts of

TABLE-3
TOTAL ANTHOCYANIN CONTENT OF SELECTED MALAYSIAN TRADITIONAL VEGETABLES/ULAM

Samples	Total anthocyanin content (c-3-gE/g)		
	Solvent extracts		
	Hexane	Ethyl acetate	Methanol
Gynura bicolour	ND	0.36 ± 0.30[a]	30.75±50.21[a]
Barringtonia racemosa	ND	ND	13.03±2.19[a]
Centella asiatica	ND	ND	2.78 ±1.85[a]
Manihot esculenta	ND	ND	0.58±0.30[a]
Lasia spinosa	ND	ND	0.63±0.34[a]

[ND]Not detected; Results are expressed in mean ± standard deviation (n=3); [a]Values with different lowercase within the same column are significantly different at $p < 0.05$.

G. bicolor. Main anthocyanin from *G. bicolor* leaves such as bicolnin, bicolmalonins and rubrocinerarin were identified by Shimizu *et al*. [19] reported that the natural pigment of reddish purple on its leaves was derived from the mixture of this compound. Thus, it is suggested that *G. bicolor* contains major components of anthocyanin compared to others.

Xanthine oxidase inhibition assay: Xanthine oxidase percentage inhibition among the samples from *n*-hexane, ethyl acetate and methanol extracts is shown in the Tables 4-6. Most of the samples extracted from these solvents exhibited xanthine oxidase inhibition potency at varying concentrations. The highest percentages of xanthine oxidase inhibition recorded at 100 µg/mL were observed in hexane extract of *L. spinosa* (98.70 ± 0.12%), ethyl acetate extract of *C. asiatica* (99.33 ± 0.25%) and methanol extract of *G. bicolor* (98.70 ± 0.00%) compared to other samples of each extract. Besides, the second and third highest percentages inhibition were found in *n*-hexane and ethyl acetate extracts of *G. bicolor*, ethyl acetate extract of *M. esculenta* and methanol extract of *B. racemosa* with the record of more than 90%.

The IC_{50} was used in the study to determine the effectiveness of the samples that inhibit 50% of xanthine oxidase enzyme where the lower the IC_{50} value, the higher the potential for the selected Malaysian traditional vegetables in reducing the formation of uric acid in the body, directly lowering the risk of gout development. As a result, *G. bicolor* in ethyl acetate and methanol extracts as well as *M. esculenta* in ethyl acetate extract had the lowest IC_{50} values of 0.002 µg/mL, 1.93 µg/mL and 2.25 µg/mL, respectively.

Sequential extraction using different solvents might contribute to different xanthine oxidase inhibition ability among the samples. The extraction solvents are varied in their polarities,

TABLE-4
PERCENTAGE OF XANTHINE OXIDASE INHIBITION OF SELECTED MALAYSIAN TRADITIONAL VEGETABLES/ULAM IN HEXANE EXTRACT

Concentrations of sample (µg/mL)	Percentage of xanthine oxidase inhibition (%)				
	Gynura bicolor	*Barringtonia racemosa*	*Centella asiatica*	*Manihot esculenta*	*Lasia spinosa*
10	54.37 ± 0.51	Not detected	3.85 ± 0.51	17.78 ± 2.35	29.04 ± 1.43
25	48.15 ± 2.89	14.52 ± 0.26	Not detected	19.70 ± 1.43	20.59 ± 1.36
50	84.15 ± 8.27	Not detected	22.96 ± 1.35	68.15 ± 14.36	13.48 ± 6.03
75	60.45 ± 0.09	42.55 ± 0.67	70.34 ± 3.50	78.47 ± 13.16	77.27 ± 0.18
100	94.88 ± 0.18	51.34 ± 1.90	44.37 ± 0.92	80.46 ± 7.05	98.70 ± 0.12

Results are expressed in mean ± standard deviation (n = 3).

TABLE-5
PERCENTAGE OF XANTHINE OXIDASE INHIBITION OF SELECTED MALAYSIAN TRADITIONAL VEGETABLES/ULAM IN ETHYL ACETATE EXTRACT

Concentrations of sample (µg/mL)	Percentage of xanthine oxidase inhibition (%)				
	Gynura bicolor	*Barringtonia racemosa*	*Centella asiatica*	*Manihot esculenta*	*Lasia spinosa*
10	64.74 ± 0.68	Not detected	89.33 ± 1.60	77.92 ± 10.16	80.30 ± 34.13
25	85.33 ± 1.18	Not detected	9.33 ± 2.92	68.30 ± 23.96	54.37 ± 29.52
50	20.74 ± 0.93	Not detected	82.07 ± 2.23	72.00 ± 31.87	62.22 ± 32.72
75	52.17 ± 0.16	28.94 ± 0.64	99.12 ± 0.18	93.69 ± 1.43	81.68 ± 4.05
100	98.54 ± 0.18	56.97 ± 1.66	99.33 ± 0.25	93.07 ± 0.06	58.98 ± 6.27

Results are expressed in mean ± standard deviation (n=3).

TABLE-6
PERCENTAGE OF XANTHINE OXIDASE INHIBITION OF SELECTED MALAYSIAN TRADITIONAL VEGETABLES/ULAM IN METHANOL EXTRACT

Concentrations of sample (µg/mL)	Percentage of xanthine oxidase inhibition (%)				
	Gynura bicolor	*Barringtonia racemosa*	*Centella asiatica*	*Manihot esculenta*	*Lasia spinosa*
10	79.41 ± 24.13	84.74 ± 0.51	Not detected	7.26 ± 0.26	11.56 ± 4.24
25	85.63 ± 1.03	Not detected	55.85 ± 0.93	19.85 ± 3.22	54.37 ± 39.52
50	87.56 ± 0.00	Not detected	Not detected	24.89 ± 0.89	37.48 ± 0.26
75	96.94 ± 2.41	79.97 ± 0.27	58.75 ± 0.59	85.66 ± 0.62	45.86 ± 11.71
100	98.7 ± 0.00	97.68 ± 0.06	87.15 ± 1.36	70.02 ± 1.10	76.75 ± 21.08

Results are expressed in mean ± standard deviation (n=3).

thus affecting the variety and amount of the bioactive compounds being extracted from the plant samples [20]. Therefore, the potency of inhibiting the xanthine oxidase enzyme among *L. spinosa*, *C. asiatica* and *G. bicolor* is likely related to their greater amount of phytochemical constituents extracted from different solvents. Moreover, ethyl acetate and methanol extracts of *G. bicolor* as well as ethyl acetate extract of *M. esculenta* possessed potent xanthine oxidase inhibition abilities with their lower record of IC_{50} values so these samples could also be recommended to be a good source of antigout agent.

Correlation between phytochemical contents and xanthine oxidation inhibitory activity: In this study, total phenolic content, total flavonoid content and xanthine oxidase inhibitory activity were positively correlated among the samples. This revealed that the presence of phenolic and flavonoid compounds in Malaysian traditional vegetables is therapeutically important for their antigout potential and may contribute as a major xanthine oxidase inhibitor. Few studies have been proved the role of phytochemical constituents attributed to antigout properties of traditional vegetables. *Vitex negundo* is traditionally consumed as an ulam in Malay community and its secondary metabolites such as flavonoids, steroids, tannins and terpenoids have shown potent activity in xanthine oxidase superoxide (XOD) scavenging assays [21]. Additionally, *Brassica oleracea* leaves is a kind of traditional food as an ulam used by Sama-Bajau people of Kampung Taun Gusi in Kota Belud, Sabah, Malaysia and the plant was found to have xanthine oxidase inhibition ability due to the presence of phenolic acids and anthocyanin [22,23].

Conclusion

In conclusion, *Gynura bicolor* was found to be the most effective in antigout activity because its *n*-hexane, ethyl acetate and methanol extracts possessed higher xanthine oxidase percentage inhibition in addition to their greater amount of phytochemicals where the content of phenolic and flavonoid as well as xanthine oxidase inhibition were correlated. The findings in this study also revealed that the hexane extract of *Lasia spinosa*, methanol extract of *Barringtonia racemosa* and ethyl acetate extract of *Centella asiatica* and *Manihot esculenta* showed greater antigout potential. Further study is recommended to identify the compounds, which are responsible to the xanthine oxidase inhibition and more *in vitro* assays are required to warrant the ability of Malaysian traditional vegetables or ulam as an alternative agent in gout treatment.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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