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Xanthine Oxidase Inhibitory and DPPH Radical Scavenging Activities of Some Primulaceae Species

(Aktiviti Perencatan Xantina Oksidase dan Hapus-sisa Radikal DPPH oleh Beberapa Spesies Primulaceae)

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ABSTRACT

Xanthine oxidase (XO) is an enzyme that catalyzes the metabolism of hypoxanthine and xanthine into uric acid. XO also serves as an important biological source of free radicals that contribute to oxidative damage involved in many pathological processes. Antioxidant effects of several Primulaceae species have been reported but their XO inhibitory activity has not been investigated. Thus, this study was conducted to determine the XO inhibitory and free radical scavenging activities of Primulaceae species and to correlate these activities with their total phenolic contents (TPC). A total of 129 extracts of different plant parts of twelve Primulaceae species were assayed for XO inhibition spectrophotometrically at 290 nm using allopurinol as a positive control. The antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and TPC of the extracts were determined by the Folin-Ciocalteau method. The Pearson correlation analysis indicated that the TPC of the extracts showed moderate positive correlations with XO inhibition (r=0.31, p<0.05) and DPPH antioxidant activity (r=0.31, p<0.05) for all of the dichloromethane extracts. Amongst the extracts tested, the dichloromethane extract of the roots of Labisia pumila *var.* alata *showed the strongest inhibitory effects for XO (IC_{50} 4.8 µg/mL) and DPPH free radical capacity (IC_{50} 1.7 µg/mL). The results suggested that Primulaceae species, particularly the dichloromethane extract of* L. pumila *var.* alata *roots, are the potential source of useful leads for the development of XO inhibitors.*

Keywords: Labisia pumila *var.* alata; *Primulaceae; total phenolic content; xanthine oxidase inhibition*

ABSTRAK

Xantina oksidase (XO) merupakan enzim yang memangkinkan metabolisme hipoxantina dan xantina kepada asid urik. XO juga merupakan sumber biologi radikal bebas yang menyebabkan kerosakan oksidatif yang terlibat dalam kebanyakan proses patologi. Kesan antioksidan beberapa spesies Primulaceae telah dilaporkan tetapi aktiviti perencatan XO masih belum dikaji. Oleh itu, tujuan kajian ini adalah untuk menentukan aktiviti perencatan XO dan hapus-sisa radikal bebas oleh spesies Primulaceae dan mengkorelasi aktiviti ini dengan kandungan fenolik total (TPC). Sejumlah 129 ekstrak daripada bahagian tumbuhan yang berbeza daripada dua belas spesies Primulaceae telah dinilai untuk aktiviti perencatan XO secara spektrofotometri pada 290 nm menggunakan alopurinol sebagai kawalan positif. Aktiviti antioksidan ditentukan dengan asai hapus-sisa radikal bebas 2,2-difenil-1-pikrilhidrazil (DPPH) dan TPC dalam ekstrak ditentukan oleh kaedah Follin-Ciocalteu. Analisis korelasi Pearson menunjukkan bahawa TPC dalam ekstrak mempunyai korelasi yang sederhana positif dengan aktiviti perencatan XO (r=0.31, p<0.05) dan antioksidan DPPH (r=0.31, p<0.05) bagi kesemua ekstrak diklorometana. Antara kesemua ekstrak yang diuji, ekstrak diklorometana akar Labisia pumila *var.* alata *menunjukkan kesan perencatan XO (IC_{50} 4.8 µg/mL) dan radikal bebas DPPH (IC_{50} 1.7 µg/mL) yang kuat. Hasil kajian ini menunjukkan bahawa spesies Primulaceae, terutamanya ekstrak diklorometana akar* L. pumila *var.* alata*, merupakan sumber berpotensi yang berguna untuk pembangunan agen perencat XO.*

Kata kunci: Kandungan fenolik total; Labisia pumila *var.* alata; *perencatan xantina oksidase; Primulaceae*

INTRODUCTION

Xanthine oxidase (XO) enzyme is responsible for the oxidation of hypoxanthine to xanthine and subsequently of xanthine to uric acid, inducing production of oxygen-derived free radicals such as the hydroxyl ($OH^{\cdot}$) and superoxide anion ($O_2^{\cdot -}$) radicals (Ozyurek et al. 2009). Free radicals may cause oxidative damage to cells and living tissues, while overproduction of uric acid can lead to increased risk of hyperuricaemia. These phenomena are reportedly associated with gouty conditions and cardiovascular diseases. Thus, inhibition of XO helps to reduce production of uric acid and prevent from occurrence of other XO-related diseases (Higgins et al. 2012). Allopurinol is a common drug used clinically to treat hyperuricemia and gout. It reduces the plasma uric acid by inhibiting XO. XO inhibitors have also been found in a wide

variety of medicinal plants (Umamaheswari et al. 2007). Besides, naturally occurring antioxidants particularly from plants are intensively being studied (Krishnaiah et al. 2011) due to their capacity that may help to protect and minimise damage to the cells brought on by oxidative stress.

Several Myrsinaceae genera have been recently revised and reclassified into the Primulaceae family (APG III, 2009). *Ardisia, Rapanea* and *Maesa* are the largest ex-Myrsinaceae genera (Stone 1989). In Malaysia, some species of *Ardisia* such as *Ardisia colorata, Ardisia crassa* and *Ardisia odontophylla* have been traditionally used for rheumatism (Burkill 1966). To the best of our knowledge, biological or pharmacological activities of Primulaceae species related to XO inhibition activity and anti-hyperuricemia effect have not been reported. Primulaceae species yielded interesting phenolic- and triterpenoid-rich secondary metabolites. The presence of benzoquinones, flavonols and triterpenoid saponins seem to be common in the family and the distribution is quite abundant especially in the genera of *Ardisia, Embelia, Maesa* and *Myrsine* (Kobayashi & Mejía 2005; Manguro-Arot & Williams 1997; Manguro-Arot et al. 2003; Tuntiwachwuttikul et al. 1997).

In the present study, different plant parts of twelve Primulaceae species were investigated for XO inhibitory activity and free radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH). Total phenolic content (TPC) was also determined to correlate its relationship with XO and DPPH inhibitory activities.

MATERIALS AND METHODS

CHEMICALS

Chemicals used in this study included dichloromethane, ethanol and methanol of analytical grade that were purchased from BDH Laboratory Supplies (Poole, UK), whereas phosphate buffer, XO enzyme solution, xanthine solution, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Follin-Ciocalteau reagent, HCl, Na_2CO_3, allopurinol, ascorbic acid and gallic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

PLANT COLLECTION

Twelve species of Primulaceae were collected from various forests in the Peninsular Malaysia between October and December 2008 (Table 1). The plant specimens were identified by Mr. Kamaruddin Salleh from the Herbarium of Forest Research Institute Malaysia (FRIM). Voucher specimens of the plants were deposited at the Herbarium of Universiti Kebangsaan Malaysia, Bangi. Plant parts, such as leaf, stem, twig, bark and root were individually separated to afford 43 plant specimens. Each specimen was air-dried and then ground into powder.

PREPARATION OF EXTRACTS

Each specimen was successively extracted using Soxhlet apparatus with dichloromethane and then methanol. Aqueous extraction of the methanol residue

TABLE 1. List of Primulaceae species used in the study

Species	Locality	Voucher Specimen Number	Traditional Uses
1. *Ardisia colorata* Roxb.	Pasoh, Negeri Sembilan	29815	Cough, diarrhoea, lameness, lumbago, rheumatism (Burkill 1966)
2. *Ardisia crassa* C.B.Clarke	Pasoh, Negeri Sembilan	29816	Rheumatism (Burkill 1966)
3. *Ardisia elliptica* Thunb.	Kota Tinggi, Johor	29814	Pain associated with the heart (Burkill 1966), as well as ear pain, fever, diarrhoea (Mat-Salleh & Latiff, 2002)
4. *Ardisia hulletii* Mez	Pasoh, Negeri Sembilan	29818	NR
5. *Ardisia kunstleri* King & Gamble	Pasoh, Negeri Sembilan	29817	NR
6. *Ardisia pachysandra* (Wall.) Mez	Pasoh, Negeri Sembilan	29820	NR
7. *Ardisia porosa* C.B. Clarke	Pasoh, Negeri Sembilan	29819	NR
8. *Embelia coriacea* Wall. ex A. DC.	Kuala Kubu, Selangor	29812	NR
9. *Embelia myrtillus* (Hook.) Kurtz	Fraser Hill, Pahang	29811	NR
10. *Labisia pumila* (Blume) Fern.-Vill var. *alata* (Scheff.) Mez	Pasoh, Negeri Sembilan	29809	Childbirth, dysentery, dysmenorrhea, flatulence, gonorrhea, 'sickness in the bones' (Burkill 1966)
11. *Maesa ramentacea* (Roxb.) A. DC.	Gunung Berembun, Pahang	29813	Liver problem, dermatosis (Burkill 1966)
12. *Rapanea porteriana* (Wall. & A. DC. Mez	Port Dickson, Negeri Sembilan	29810	NR

NR=Not reported

was subsequently carried out using reflux method. Dichloromethane and methanol solutions were vacuum evaporated to give the dichloromethane and methanol extracts, while aqueous solutions were freeze-dried to afford the aqueous extracts. A total of 129 extracts were obtained and the percentage yield of each extract was calculated based on its dry weight. Every extract was kept in an airtight container at 4°C until use.

XANTHINE OXIDASE INHIBITION ASSAY

The assay was carried out based on a procedure reported by Umamaheswari et al. (2007) with slight modification. Briefly, the assay mixture consisting of the test extract solution (100 µg/mL), phosphate buffer (0.05 M, pH7.5) and XO enzyme solution (0.2 units/mL) was pre-incubated at room temperature (25°C) for 15 min, then mixed with xanthine solution (0.15 mM) and incubated for another 30 min at 25°C, after which HCl (1N) was added. A blank solution was similarly prepared but the XO enzyme was added after HCl addition. Each mixture was measured at 290 nm using a UV-Vis spectrophotometer. The percentage of XOI activity was calculated as follows (Umamaheswari et al. 2007):

$$\text{Percentage Inhibition (\%)} = \left(\frac{(A-B)-(C-D)}{(A-B)} \right) \times 100,$$

where A is the enzyme activity without test extract; B is the control of A without test solution and enzyme and C and D are the activities of the test extract with and without enzyme, respectively. Allopurinol (10, 25, 50 and 100 µg/mL) was used as a positive control. Extracts showing ≥ 60% XO inhibition were further assayed at 10, 25 and 50 µg/mL to determine the half maximal inhibitory concentration (IC_{50}) values.

2, 2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH) FREE RADICAL SCAVENGING ACTIVITY

The assay was carried using a method described by Choi et al. (2002) with slight modification. Briefly, a mixture of test extract (250 µg/mL in ethanol) and ethanolic DPPH solution (0.3 mM) was shaken and left to stand at room temperature for 30 min. Then, the absorbance was measured at 518 nm using a UV-Vis spectrophotometer. A blank solution comprised a mixture of ethanol without DPPH solution and test extract, while a negative control had DPPH solution and ethanol. Ascorbic acid solution was used as a positive control. Percentage of DPPH free radical scavenging activity was calculated as follows (Choi et al. 2002):

$$\text{Percentage of DPPH free radical scavenging activity (\%)} = 1 - \left(\frac{A_1 - A_2}{A_0} \right) \times 100,$$

where A_0 is the absorbance of negative control; A_1 is the absorbance of DPPH solution with test extract and A_2 is the absorbance of blank. Extracts having ≥ 60% inhibition were further assayed at 10, 50 and 100 µg/mL to determine the IC_{50} values.

TOTAL PHENOLIC CONTENT

TPC was estimated using Follin-Ciocalteau method (Slinkard & Singleton 1977). Test extract (50 µL) was mixed with 1.58 mL of distilled water and 0.1 mL of 2N Follin-Ciocalteau reagent. After 8 min, 0.3 mL of Na_2CO_3 (0.2 g/mL) was added. The mixture was incubated at room temperature for 2 h in the dark. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer. Gallic acid solutions (100-500 µg/mL) were used to construct a calibration curve. TPC was expressed as gallic acid equivalents (GAE) in milligrams per gram of dried material.

STATISTICAL ANALYSIS

All data were analysed using SPSS version 16.0. Every test extract was assayed in triplicate (n=3) and the values were represented as mean ± SEM. IC_{50} values of the active extracts were determined using Probit analysis. Degree of correlation between TPC in the extracts and activities of XO and DPPH inhibition were determined using bivariate Pearson's product-movement correlation coefficient (r). A value of r=1.0 was interpreted as perfect correlation, whereas a value between 0 and 1 indicated positive correlation. Significance level was denoted by a value of p<0.05.

RESULTS

XANTHINE OXIDASE INHIBITORY ACTIVITY

Ninety-eight extracts (76%) demonstrated XO inhibitory activity with 14 extracts (10.9%) had inhibition greater than 50% (Table 2). These were mostly dichloromethane extracts, including *A. colorata* stem bark (with the highest inhibition of 73.5%), *A. crassa* stem bark, *A. elliptica* stem, *A. kunstleri* leaves, *A. pachysandra* roots, *E. coriacea* leaves and twig, *E. myrtillus* stem, *L. pumila* var. *alata* leaves and roots, *M. ramentacea* twig and *R. porteriana* leaves. Only methanol extract of *A. hulletii* leaves and aqueous extract of *R. porteriana* twigs were active. On the other hand, 31 extracts (24%) had no XO inhibitory activity. The results of this study demonstrated that the active extracts inhibited XO in a dose-dependent manner. IC_{50} values of active extracts are shown in Table 2. Dichloromethane extract of *L. pumila* var. *alata* roots showed the strongest activity with IC_{50} 4.8 µg/mL as compared to allopurinol (IC_{50} 0.2 µg/mL).

DPPH FREE RADICAL SCAVENGING ACTIVITY

In this study, all dichloromethane extracts exhibited DPPH free radical scavenging activity with 39 extracts (90.7%) showing ≥ 60% inhibition (Table 3). *L. pumila* var. *alata* root extract gave the most potent antioxidant activity with

TABLE 2. Inhibitory effect of various extracts of different plant parts of some Primulaceae species on xanthine oxidase (XO)

Species/standard	Plant parts	Percentage of XO inhibition of extracts at 100 mg/mL (%)[a]		
		Dichloromethane	Methanol	Aqueous
Ardisia colorata	Leaf	0.0	20.0±4.0	32.0±4.0
	Stem	0.0	0.0	0.0
	Stem bark	73.5±4.5 (5.6±0.5)[b]	0.0	31.0±4.0
	Twig	24.5±0.5	0.0	0.0
Ardisia crassa	Leaf	29.3±0.7	9.5±5.5	39.0±3.0
	Stem bark	54.0±6.0	0.0	0.0
	Twig	0.0	34.0±1.0	24.0±11.0
	Root	26.3±1.9	17.0±2.5	22.3±1.5
Ardisia elliptica	Leaf	29.0±20.1	26.0±5.0	0.0
	Stem	71.7±0.9 (5.4±0.3)[b]	36.5±2.5	0.0
	Twig	0.0	40.0±4.0	0.0
Ardisia hulletii	Leaf	34.7±1.3	61.7±0.9 (10.3±0.5)[b]	22.7±0.9
	Stem	0.0	31.3±7.3	17.7 ± 0.7
	Stem bark	5.0±2.0	28.0±0.6	27.3±0.7
	Twig	19.3±0.7	16.7±0.9	23.0±0.0
	Root	10.5±4.5	17.7±1.2	17.7±1.2
Ardisia kunstleri	Leaf	51.5±0.5	36.0±8.5	35.0±6.0
	Stem	0.0	34±4.0	27.5±2.5
	Stem bark	0.0	0.0	25.3±2.9
	Twig	0.0	0.0	0.0
	Root	15.0±4.0	0.0	26.5±5.5
Ardisia pachysandra	Leaf	22.0±3.0	21.5±1.5	16.3±1.8
	Stem	16.0±0.0	29.0±1.7	19.3±0.3
	Stem bark	13.3±1.7	25.0±0.6	24.3±1.3
	Twig	34.0±4.5	41.7±2.9	22.3±3.9
	Root	50.7±0.9	25.6±2.5	8.0±0.0
Ardisia porosa	Stem	21.7±1.7	27.0±4.0	10.3±0.3
	Stem bark	18.7±1.2	36.7±1.8	16.7±3.3
	Twig	10.7±1.2	25.7±1.9	10.7±1.2
	Root	21.0±3.0	33.5±0.5	17.3±0.9
Embelia coriacea	Leaf	67.5±6.5 (9.0±1.0)[b]	0.0	17.5±2.5
	Stem	27.0±3.0	0.0	45.0±4.0
	Stem bark	0.0	0.0	39.0±1.0
	Twig	69.0±3.0 (8.6±0.6)[b]	41.5±10.5	39.0±7.0
Embelia myrtillus	Leaf	47.8±5.3	48.3±8.7	24.8±6.2
	Stem	51.5±2.4	29.2±5.5	0.0
Labisia pumila var. *alata*	Leaf	69.6±1.3 (8.6±1.1)[b]	29.3±4.3	0.0
	Root	67.7±11.2 (4.8±0.8)[b]	24.7±2.3	13.0±2.0
Measa ramentacea	Leaf	4.5±0.5	0.0	34.5±11.5
	Twig	60.0±2.0 (14.6±0.2)[b]	27.0±3.0	23.0±1.0
Rapanea porteriana	Leaf	54.8±5.9	23.5±7.2	0.0
	Stem	0.0	8.9±2.9	26.2±0.8
	Twig	22.8±4.9	36.7±5.4	60.6±1.9 (13.9±0.4)[b]
Allopurinol (positive control)		90.7±2.2 (0.2±0.1)[b]		

[a]Value represents mean ± SEM (n=3); [b]Mean half maximal inhibitory concentration (IC_{50}) of extract with XO inhibitory activity ≥ 60%, determined from 10, 25, 50 and 100 µg/mL

TABLE 3. DPPH radical scavenging activity and total phenolic content of dichloromethane extracts of different plant parts of some Primulaceae species

Species/standard	Plant parts	DPPH inhibition at 250 µg/mL (%)[a]	IC_{50} of DPPH inhibition (µg/mL)[a,b]	TPC (mg GAE/ g)[a,c]
Ardisia colorata	Leaf	89.5±1.5	22.6±0.5	102±6.0
	Stem	78.1±3.4	58.4±2.4	98±3.0
	Stem bark	94.3±2.3	14.8±0.1	173±1.0
	Twig	91.1±3.0	11.6±0.6	120±2.0
Ardisia crassa	Leaf	40.0±0.5	-[d]	98±1.8
	Stem bark	47.2±6.1	-[d]	132±7.9
	Twig	90.5±2.0	12.5±1.2	168±6.0
	Root	42.8±8.8	-[d]	100±2.4
Ardisia elliptica	Leaf	94.1±2.0	19.9±0.1	101±1.3
	Stem	94.5±1.5	9.0±1.3	141±5.7
	Twig	79.9±7.9	10.5±0.9	90±1.0
Ardisia hulletii	Leaf	88.6±2.0	26.3±0.3	182±1.3
	Stem	87.9±2.1	5.3±0.2	103±2.0
	Stem bark	89.3±0.3	13.3±1.1	128±4.7
	Twig	83.2±4.7	9.9±0.7	168±6.0
	Root	87.3±0.8	9.5±0.5	166±4.0
Ardisia kunstleri	Leaf	89.0±3.2	13.9±0.4	280±2.0
	Stem	92.5±1.5	7.3±0.3	134±4.0
	Stem bark	91.3±3.5	17.1±0.0	146±2.0
	Twig	89.3±2.7	11.9±0.6	126±0.0
	Root	87.8±1.3	4.2±0.4	163±5.0
Ardisia pachysandra	Leaf	91.5±3.9	12.4±0.7	189±9.0
	Stem	90.9±1.6	15.4±1.1	343±1.0
	Stem bark	89.8±2.3	9.6±0.7	106±2.3
	Twig	88.7±3.7	7.5±0.3	176±2.0
	Root	93.9±2.6	8.8±0.2	459±1.0
Ardisia porosa	Stem	88.4±0.4	6.7±0.2	152±5.8
	Stem bark	86.0±2.0	4.5±0.8	192±6.0
	Twig	94.9±1.2	15.1±0.5	87±2.4
	Root	88.9±4.9	13.6±3.4	187±3.1
Embelia coriacea	Leaf	89.5±2.8	26.7±1.7	95±0.7
	Stem	90.7±1.3	11.3±0.1	104±2.0
	Stem bark	92.0±0.0	18.5±0.3	111±3.0
	Twig	66.8±2.4	45.0±3.0	103±5.0
Embelia myrtillus	Leaf	91.4±1.6	20.7±0.1	154±4.0
	Stem	81.3±2.9	16.8±0.2	214±0.0
Labisia pumila var. *alata*	Leaf	92.8±1.1	11.6±0.9	241±5.8
	Root	92.9±1.7	1.7±0.3	332±4.7
Measa ramentacea	Leaf	76.5±5.2	30.5±1.0	97±5.3
	Twig	67.7±1.7	36.7±0.8	104±0.0
Rapanea porteriana	Leaf	56.4±2.1	-[d]	130±1.2
	Stem	87.4±6.9	8.6±0.6	196±5.0
	Twig	87.5±1.3	15.0±0.3	196±10.0
Ascorbic acid (positive control)		92.9±0.6	5.4±0.3	-[d]

[a]Value represents mean ± SEM (n=3); [b]Mean half maximal inhibitory concentration (IC_{50}) of fraction with ≥ 60% DPPH radical scavenging activity, determined from 10, 50, 100 and 250 µg/mL; [c]Total phenolic content (TPC) is expressed as gallic acid equivalents (GAE) in milligrams per gram of dried material; [d]Value was not determined

IC_{50} value of 1.7 μg/mL compared to ascorbic acid (IC_{50} 5.4 μg/mL).

TOTAL PHENOLIC CONTENT

Calibration curve of gallic acid gave a linear regression of y = 0.0016x - 0.0372 with a value of r^2=0.993. All dichloromethane extracts showed high TPC, ranging from 87 to 459 mg GAE/g (Table 3). *A. pachysandra* leaf extract showed the highest TPC of 459 mg GAE/g dry weight followed by that of *A. pachysandra* stem (343 mg GAE/g) and *L. pumila* var. *alata* roots (332 mg GAE/g).

Significant positive correlations were found between the TPC of dichloromethane extracts with XO inhibitory activity (r=0.31, p<0.05) and with DPPH radical scavenging activity (r=0.31, p<0.05).

DISCUSSION

The inhibitory effect of *L. pumila* var. *alata* root extract on XO might be attributed to its traditional indication for bone-related problems (Burkill 1966). The methanolic leaf and root extracts of *L. pumila* var. *alata* were reported to contain flavonoids, including apigenin, kaempferol, myricetin, naringin and rutin, as well as phenolics (gallic acid, pyrogallol and caffeic acid) (Karimi & Jaafar 2011). Apigenin, kaempferol, myricetin and caffeic acid were known to be highly active against XO (Cos et al. 1998; Flemmig et al. 2011).

Antioxidant activity of the test extracts could be associated with the capability of their phytochemical components to donate hydrogen and to cause reduction of the DPPH free radicals (Brand-Williams et al. 1995). Findings from this study was consistent with the previous report whereby the methanolic extract of *L. pumila* var. *alata* leaves had high antioxidant activity (Karimi & Jaafar 2011). It also suggested the potential ability of the dichloromethane extracts to scavenge DPPH radicals. Information on the antioxidant activity of lipophilic plant extracts or extracts is scarce. However, there was an evidence that chloroform extract of *Calycopteris floribunda*, rich in phytosterols, triterpenoids, alkaloids, saponins, flavonoids, glycosides and tannins, had higher DPPH radical scavenging activity than that of its methanol extract (Satyanarayana & Eswaraiah 2010).

Crude methanol whole plant extract of *L. pumila* var. *alata* was previously reported to have high TPC with 193.5 mg GAE/g (Saputri & Jantan 2011). Such variation in phenolic content from this study could be influenced by the use of different solvent (Falleh et al. 2012). Nonetheless, in this study the dichloromethane root extract of *L. pumila* var. *alata* exhibited higher TPC than that of the whole plant, further revealing variation based on the different plant parts.

The moderate relationships might suggest that phenolics were not the only components responsible for the XO inhibition and DPPH radical scavenging activities. Several evidences were also available to substantiate that some terpenoids (triterpenoid and triterpenoid saponin) had XO inhibitory activity (Lin et al. 2011) and antioxidant effect against DPPH free radicals (Ponou et al. 2010).

CONCLUSION

This finding provided a basis for further study on isolation of active compounds through bioassay-guided for discovery of potential XO inhibitors that might be useful for the treatment of hyperuricaemia or inflammatory-related diseases. From this study, dichloromethane extract of *L. pumila* var. *alata* root was found to have strong activities for XO and DPPH inhibition that could be possibly due to presence of phenolic and non-phenolic compounds.

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