

# Antioxidant and Xanthine Oxidase Inhibitory Activities of *Swietenia Macrophylla* and *Punica Granatum*

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**Abstract**— Gout is an inflammatory arthritis characterised as hyperuricaemia associated with elevation of blood uric acid by xanthine oxidase (XO) in purine metabolism pathway. Hence, the inhibition of XO has gained the therapeutic interest for gout therapy. Although *Swietenia macrophylla* (SM) and *Punica granatum* (PG) uses as folk medicine to treat metabolic disorder, their XO inhibitory activity is yet to be elucidated. This study investigated the XO inhibitory and antioxidant activities of *Swietenia macrophylla* (SM) and *Punica granatum* (PG). This study suggested that phenolic and flavonoid compounds in extracts could have contributed towards the antioxidant activity of PG and SM. However, only methanol extract of PG seeds exhibited inhibition to XO whereas other PG extracts showed no inhibitory activity. The methanol extract of SM seed showed the highest xanthine oxidase inhibition. Both plants exhibited radical scavenging properties. SM extracts were suggested to possess both DPPH radical scavenging and xanthine oxidase inhibitory activity; whereas PG extracts possessed significant antioxidant activity but showed minimal or no xanthine oxidase inhibitory activity.

**Keywords**—Antioxidant, *Punica granatum*, *Swietenia macrophylla*, Xanthine oxidase

## I. INTRODUCTION

Gout is a chronic inflammatory arthritis presented with excruciating pain, inflammation, erythema and swelling during the acute attack [1]. this chronic metabolic disorder is due to hyperuricaemia, leading to deposition of monosodium urate monohydrate crystals in joints or kidneys causing gouty arthritis or uric acid nephrolithiasis [2][3]. Hypeuricaemia is characterised as elevation of serum urate level to more than 7.0 mg/dL for men and more than 5.7 mg/dL for women [4].

Xanthine oxidase (XO) associated in the development of gout disease where the uric acids are produced as end product in purine metabolism pathway. XO will catalyze the

hydroxylation of hypoxanthine into xanthine. Xanthine and hypoxanthine will undergo oxidation by XO into uric acid [5][6]. Uric acids produced from the oxidation of hypoxanthine and xanthine by XO in the purine metabolic pathway [5]-[7]. Hence, occurrence of the abnormalities in this pathway elevates the uric acids level.

Allopurinol, Food and Drug Administration (FDA) approved XO inhibitor that has been widely used as an anti-gout drug to reduce serum urate level [3][8]. However, patients has the risk of developing allopurinol hypersensitivity syndrome which characterised as fever, rashes, renal impairment, hepatic dysfunction, leucocytosis and eosinophilia on its administration [8][9]. Alternative therapeutic agents especially medicinal plants, which possess lesser side effects are widely used to treat gout. Several medicinal plants from India, Philippine, Vietnam and northeastern America with higher level of phenolic and flavonoids compounds have shown the xanthine oxidase inhibitory (XOI) activity [10]-[13].

*Punica granatum* (PG) from Punicaceae family, or commonly known as pomegranate is a native plant from middle east that has been cultivated in various regions [14][15]. Pomegranate fruit has been used as folk medicine to treat diseases such as diabetes, diarrhea and arthritis [15][16]. Previous studies have proven that pomegranate can effectively suppress the inflammation by inhibits the inflammatory enzymes, cyclooxygenase and lipoxygenase as well as reduce joint damage incidence in tested species with arthritis [17]. Thus, potential bioactive compounds from pomegranate may contribute to the development of new therapeutic agent for gout treatment. Meanwhile, *Swietenia macrophylla* (SM) from family Meliaceae is known as 'sky fruit', is native to tropical America, Mexico, and South America. SM was reported to possess anti-inflammatory, antimicrobial, antifungal, and antihyperglycaemic activities. The leaf of SM had shown to possess antioxidant activity. The limonoid compounds in the fruit of SM were found to inhibit the superoxide anion generation by human neutrophils in response to formyl-l-methionyl-l-leucyl-l-phenylalanine (fMLP) during onset of inflammation. In Malaysia, SM is used as a folk medicine to treat diabetes and hypertension [18]-[21].

To date, XO activity of seeds and peels extract of both medicinal plants from local species have not been evaluated. This recent study was aimed to evaluate the methanolic and aqueous extracts of peel and seeds both medicinal plants for their antioxidant and xanthine oxidase inhibitory activities.

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## II. MATERIALS AND METHODS

### A. Preparation of Extractions

Fruits of *S. macrophylla* and *P. granatum* were collected from months of June 2012 to July 2012 locally in Kuala Lumpur, Malaysia. The fruits were sent for authentication at The Forest Research Institute Malaysia (FRIM), Malaysia. Prior for the extractions, peels and seeds of fruits were oven-dried separately for 3 to 4 days. For aqueous extraction, grinded-powder was soaked in 1000 mL of sterile distilled water and kept under 60 °C for 3 hours. Subsequently, the mixture was filtered and freeze-dried by Freeze drier (Freezone Freeze Dry System Labconco). For methanol extraction, grinded powder was suspended in 1000 mL methanol and stored in dark for 3 days at room temperature. The methanolic extract was filtered and concentrated by rotary evaporator (R-215, Buchi) to yield the crude extract. The working stock concentration of crude methanolic and aqueous extracts was prepared by dissolving crude extract in dimethyl sulfoxide (Sigma-Aldrich, USA) and deionised water respectively.

### B. Total Flavonoids Content Determination (TFC)

Total flavonoids content of extract was examined by aluminium chloride colorimetric assay with modification [22][23]. In this assay, 10 $\mu$ L of extract was mixed well with methanol, 10% aluminium chloride (Fisher Scientific, USA), 1 M potassium acetate (Ajax Finechem Pty Ltd, Australia) and deionised water. The mixture was incubated for 30 minutes at room temperature and absorbance was measured at 450 nm by spectrophotometer (opsysMR, Dynex). Quercetin (Fisher Scientific, USA) was used as the standard reference. The experiments were done in triplicate. The total flavonoid content was expressed in terms of  $\mu$ g quercetin equivalent/ g dry weight ( $\mu$ g QE/g DW).

### C. Total Phenolic Content Determination (TPC)

Total phenolics content of extracts were determined by Folin-ciocalteu's reagents with modification [24]. Folin-ciocalteu's reagent (Sigma-Aldrich, USA) was added into 10  $\mu$ L extracts and incubated for 5 minutes in room temperature. Then, 80  $\mu$ L of 7.5% sodium carbonate (Fisher Scientific, USA) was added and incubated for 30 minutes at room temperature. The absorbance was determined at 630 nm using microplate reader (opsysMR, Dynex). The experiment was done in triplicate. Gallic acid (Fisher Scientific, USA) was used as standards. The total phenolic contents were expressed as  $\mu$ g gallic acid equivalent/g dry weight ( $\mu$ g GAE/g DW).

### D. Antioxidant Activity Determination

The antioxidant activity of the extract was determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay with modification [25]. Extracts were mixed with 300  $\mu$ M methanolic DPPH and incubated in dark at room temperature for 30 minutes. The absorbance was measured by microplate reader (opsysMR, Dynex) at 490 nm. The experiment was done in triplicate. Ascorbic acid was used as the standard antioxidant. DPPH radical scavenging activity was calculated by using the equation: % DPPH radical

scavenging activity =  $(A_0 - A_s)/A_0 \times 100$  %, whereby  $A_0$  is the absorbance without extract and  $A_s$  is the absorbance of sample extract. The percentage of inhibition of extracts that showed greater than 50% inhibition at 100  $\mu$ g/mL was tested further to determine the corresponding IC<sub>50</sub> values.

### E. In vitro Xanthine Oxidase Inhibitory Activity

*In vitro* xanthine oxidase inhibitory (XOI) activity of extract was measured spectrophotometrically [26]. The extracts in different concentrations were pre-incubated with 800  $\mu$ L of 150  $\mu$ M xanthine for 10 minutes at 25 °C. After pre-incubation, the reaction was initiated by adding 100  $\mu$ L of XO and incubated for another 5 minutes. The enzymatic reaction was terminated by adding 100  $\mu$ L of 1 M hydrochloric acid to the mixture and absorbance measured at 295 nm by using Shimadzu UV-Vis Spectrophotometer. Allopurinol was used as reference control. The XOI activity of extracts was calculated as % xanthine oxidase inhibition activity =  $1 - (XOI_s / XOI_o) \times 100$  %, whereby  $XOI_s$  is enzyme activity with test sample and  $XOI_o$  is enzyme activity without sample.

### F. Statistical Analysis

Data were presented as mean  $\pm$  standard deviation (mean  $\pm$  SD) in triplicate by SPSS 18.0 software. Student T-test was used to determine the significance of results yield by assays whereby  $p < 0.05$ .

## III. RESULTS

### A. Total Flavonoids Content Determination

The total flavonoids content of SM and PG was determined through aluminum chloride colorimetric method. Table 1 shows, the total flavonoid content of SM and PG extracts expressed in terms of  $\mu$ g quercetin equivalent/ g dry weight ( $\mu$ g QE/g DW). Aqueous extracts for both PG seed and peels showed higher flavonoids content compared to that of PG methanol extracts. The peel methanol extract of SM was shown to possess highest total flavonoid content followed by peel aqueous extract, seed methanol extract and seed aqueous extract.

### B. Total Phenolics Content Determination (TPC)

The total phenolics content of SM and PG was determined through aluminum chloride colorimetric method. Table 2 shows, total phenolic contents were expressed as  $\mu$ g gallic acid equivalent/g dry weight ( $\mu$ g GAE/g DW). The working concentration of TPC determination was 1000  $\mu$ g/mL. PG peel extracts for both aqueous and methanol extraction had higher phenolics content compared to seed extracts in pomegranate. At the concentration of 1000  $\mu$ g/mL, the total phenolic content of SM was shown to decrease in the following order: peel methanol extract, peel aqueous extract, seed methanol extract, and seed aqueous extract. The peels of SM were shown to possess higher phenolic content than the seeds.

TABLE I  
TOTAL PHENOLIC AND FLAVONOIDS COMPOUND CONTENT OF *SWIETENIA MACROPHYLLA* AND *PUNICA GRANATUM* EXTRACTS

Plant Extracts		TPC ( $\mu\text{g GAE/g DW}$ )	TFC ( $\mu\text{g QE/g DW}$ )
<i>Swietenia macrophylla</i>	Seed Aqueous	$0.05 \pm 0.0004^*$	$0.04 \pm 0.0010^*$
	Methanol	$0.08 \pm 0.0006^*$	$0.07 \pm 0.0015^*$
	Peel Aqueous	$0.15 \pm 0.0035^*$	$0.12 \pm 0.0025^*$
	Methanol	$0.28 \pm 0.0057$	$0.30 \pm 0.0027^*$
<i>Punica granatum</i>	Seed Aqueous	$0.01 \pm 0.0001^*$	$0.01 \pm 0.0003^*$
	Methanol	$0.01 \pm 0.0001^*$	$0.01 \pm 0.0001^*$
	Peel Aqueous	$0.06 \pm 0.0007^*$	$0.02 \pm 0.0002^*$
	Methanol	$0.02 \pm 0.0003^*$	$0.01 \pm 0.0001^*$

Data were expressed as means  $\pm$  SD (n=3).

\*  $p < 0.05$  statistically significant

PG peel extracts in these studies showed higher phenolics and flavonoids content compared to PG seed extracts. The peel of PG was previously reported to possess abundant phenolic acids, such as gallic acid and ellagic acid as well as flavonoids such as flavones, catechin and anthocyanidines [27][28]. In contrast, phenolics and flavonoids compounds acts as minor components in seed extracts as it comprises less than 5% of total phytochemical components of PG. Furthermore, PG aqueous extract possess higher flavonoids content compared to PG methanol extract in these studies. Previous study has suggested that phenolics and flavonoids in pomegranate extract suggested were more hydrophilic in nature rather than lipophilic [22]. Studies revealed that deionized water was the best extraction medium for PG seed extract as aqueous extract exhibited highest scavenging activity and highest phenolics content among extracts with different solvents [29]. These studies shown SM contained phenolic and flavonoid compounds in its seeds and peels, with the peels possess higher content of both compounds than the seeds. In both seeds and peels, the methanol extracts were shown to contain higher concentrations of total phenolic and flavonoid compounds compared to the aqueous extracts. Due to methanol solvent has stronger extraction capacity than deionized water thus greater number of bioactive components such as alkaloid, terpenoid, phenolic, flavonoid, tannin and saponin present in the methanol extracts in SM [30].

### C. Antioxidant Activity Determination

Antioxidant activity of SM and PG extracts were determined by using DPPH method at difference concentration of standard and extracts ranging from 20  $\mu\text{g/mL}$  to 100  $\mu\text{g/mL}$ . A standard curve of DPPH was constructed for  $\text{IC}_{50}$  determination.  $\text{IC}_{50}$  represents the concentration of test material that caused 50 % of scavenging activity, whereby lower  $\text{IC}_{50}$  value indicates greater scavenging activity. The  $\text{IC}_{50}$  values were calculated through linear regression against percentage of DPPH scavenging activity. The  $\text{IC}_{50}$  of PG peel aqueous extract ( $\text{IC}_{50} = 34.79 \mu\text{g/mL}$ ) showed highest DPPH scavenging activity followed by PG peel methanol extract ( $\text{IC}_{50}$  of 37.02  $\mu\text{g/mL}$ ) in reference to ascorbic acid that was

82.64  $\mu\text{g/mL}$ . All PG samples extract showed inhibition that was concentration-dependent that range from 20  $\mu\text{g/mL}$  to 100  $\mu\text{g/mL}$ . At the concentration of 100  $\mu\text{g/mL}$ , DPPH radical scavenging activities of SM and PG were summarized (Table 2).

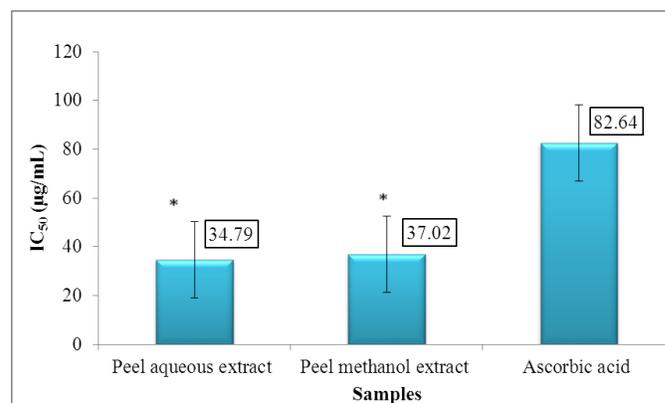


Fig. 1

$\text{IC}_{50}$  Values Of Radical Scavenging Activity Of The Ascorbic Acid, Aqueous And Methanol Extracts For Peel Of *Punica Granatum*.

Data were expressed as means  $\pm$  SD (n=3).

\*  $p < 0.05$  statistically significant

These studies showed that the presence of phenolic and flavonoids compounds in PG and SM may contribute to antioxidant potential by DPPH radical scavenging activities. The effect of antioxidants on DPPH radical scavenging is due to their hydrogen donating ability to reduce DPPH radical into stable diamagnetic molecule such as diphenyl picrylhydrazine [31]-[33]. Phenolic and flavonoid compounds are known as antioxidants for their redox properties, which are important in neutralizing free radicals, quenching singlet and triplet oxygen, decomposing peroxides and chelating metal ions [34][35]. The presence of hydroxyl groups in aromatic rings enables phenolics to possess redox properties and acts as reducing agent. Studies have shown strong correlations between total phenolics content and DPPH assay whereby antioxidant activities increased proportionally to the phenolic contents [29][36][37]. Flavonoids were suggested to contribute for the radical scavenging activity due to the presence of glycosides and free hydroxyls in structure whereby mechanism of action are via chelating process [29][38]-[40].

Some studies suggested that plant with good antioxidant activity does not necessarily show good scavenging activity with one particular assay [41]-[43]. For instance, methanolic extract of *Medicago sativa L.* had shown to possess higher  $\text{IC}_{50}$  value than the standard used for the DPPH radical scavenging, iron chelating and lipid peroxidation assays but showed lower  $\text{IC}_{50}$  value than the standard, ascorbic acid for 2, 2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), nitric oxide and superoxide scavenging assays [43]. Therefore, more antioxidant assays such as ferric reducing ability of plasma (FRAP), oxygen radical antioxidant capacity (ORAC) and thiobarbituric acid (TBA) assays should be carried out to more conclusively determine the antioxidant activity of the SM and PG extracts.

#### D. Xanthine Oxidase Inhibition

XOI activity of SM and PG extracts were determined by using xanthine as substrate with difference concentration of standard and extracts ranging from 20 µg/mL to 100 µg/mL. The XOI activity of SM and PG were summarized in Table 2.

TABLE II  
PERCENTAGE OF DPPH RADICAL SCAVENGING ACTIVITY AND XANTHINE OXIDASE INHIBITORY ACTIVITY

Samples (100 µg/mL)		DPPH Scavenging (%) <sup>a</sup>	XOI Activity (%) <sup>b</sup>
PG Peel	Aqueous extract	79.04 ± 0.721*	-
	Methanol extract	69.12 ± 1.442*	-
PG Seed	Aqueous extract	16.05 ± 2.171	-
	Methanol extract	22.19 ± 0.431*	15.53 ± 0.0010
SM Peel	Aqueous extract	36.40 ± 0.0127*	21.82 ± 0.0010*
	Methanol extract	11.66 ± 0.0035*	13.56 ± 0.0021
SM Seed	Aqueous extract	19.02 ± 0.0110*	20 ± 0.0010*
	Methanol extract	10.43 ± 0.0057	24.07 ± 0.0018*
Reference Standard	Ascorbic acid	61.86 ± 0.721	-
	Allopurinol	-	62.39 ± 0.0017

Data were expressed as means ± SD (n=3).

\*  $p < 0.05$  statistically significant

Phenolic and flavonoid compounds are reported to possess XOI activities. XOI activities of some plants were shown to correlated with their total phenolic contents [30][44]-[48]. Previous studies showed that structure-activity relationship of flavonoids will determine the XOI activity and radical scavenging activity [46]. The structure-activity of flavonoids influences the XO inhibition via interaction with the molecular target of flavonoids. Flavonoids that selected as potential XO inhibitor should consists either hydroxyl group at C-5 and C-7, double bond between C-2 and C-3 or planar structure in flavones are essential [29][45][46]. According to Nagao *et al*, lower inhibitory activities among some flavonoids are due to substitution of hydroxyl groups at C-3 and C-7 by glycoside or methyl group [45]. For instance, the flavonoids with potent XOI activity such as quercetin and kempeferol exhibit higher inhibition rate compared to the glycosides of these flavonoids [46][49]. Mo *et al* suggested that glycosylation of flavonoids on certain positions might interfere with the enzyme-binding process that eventually leads to lower inhibitory activity [50]. Other types of flavonoids that showed minimal or no XOI are such as catechin, non-planar flavones and isoflavones [45]. In these studies, all PG extracts do not possess XOI activity despite PG seed methanol extract was the only extract that exhibited XOI activity. Although the methanol extracts of SM seeds were shown to possess the highest XO inhibition, it contained lesser phenolic and flavonoid compounds compared to the SM peel extracts. These findings showed insight of the minimal XOI activity among PG and SM extracts and further validation of phenolics and flavonoids compounds can be done

by performing high performance liquid chromatography (HPLC) or further purification of extracts are required in future studies.

#### IV. CONCLUSION

XO is an enzyme involves in purine metabolic pathway and it is associated with the production of both free radicals and uric acid. Abnormalities of the purine metabolic pathway caused hyperuricemia and subsequently lead to development of gout disease. XO can generate reactive oxygen species (ROS) such as superoxide radicals and hydrogen peroxide leads to elevation in oxidative stress among gout patients. Inhibition of XO enzyme activity serves as important approach for gout treatment. In order to reduce the uric acid production, drug that can inhibit the oxidative activity of XO to form uric acid is very crucial in the treatment of gout. Natural products have gain interest to be potential source of new therapeutic agents for gout treatment as they shown to possess XOI activity and antioxidant activity, especially flavonoids and phenolics compounds were claimed to contribute for these activities. These studies revealed the antioxidant activities and xanthine oxidase inhibition of SM and PG. The isolation of the bioactive constituents from both extract warrants further study on the XOI activity. It is suggested that further purification such as silica gel vacuum chromatography and HPLC of extracts shall be done to validate the phenolic and flavonoids compounds which are present in the extracts. Furthermore, *in vivo* XOI activity assay can be done to further prove the anti-gout property of both medicinal plants.

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