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ORIGINAL ARTICLE

Evaluation of Xanthine Oxidase Inhibitory Potential and *In vivo* Hypouricemic Activity of *Dimocarpus longan* Lour. Extracts

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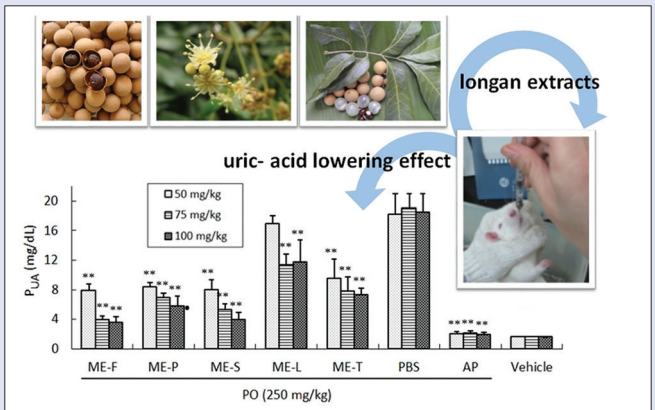
ABSTRACT

Background: Longan is a fruit tree known to contain many phenolic components, which are capable of protecting people from oxidative damage through an anti-inflammatory mechanism. It may be also worthwhile to study the effect on lowering uric acid activity. **Materials and Methods:** This study investigates the lowering of uric acid using longan extracts, including flowers, pericarps, seeds, leaves, and twigs, on potassium-oxonate-induced hyperuricemia mice and its inhibitory actions against xanthine oxidase (XO) activities. **Results:** The findings revealed that ethyl acetate fraction of longan extracts exhibited strong XO-inhibitory activity, and the flower extracts (IC_{50} = 115.8 μg/mL) revealed more potent XO-inhibitory activity to those of pericarps (118.9 μg/mL), twigs (125.3 μg/mL), seeds (262.5 μg/mL), and leaves (331.1 μg/mL) *in vitro*. In addition, different dosages of longan extract (50–100 mg/kg) were administered to hyperuricemic mice. The lowering effect of longan extracts on uric acid at 75 mg/kg markedly reduced plasma uric acid levels in decreasing order: Flowers (80%) > seeds (72%) > pericarps (64%) > twigs (59%) > leaves (41%), compared with allopurinol (89%). Finally, 10 isolated phytochemicals from longan flowers were then examined *in vitro*. The results indicated that proanthocyanidin A2 and acetonylgeraniin A significantly inhibited XO activity *in vitro*. This is the first report providing new insights into the urate-reducing effect of phenolic dimer and hydrolyzable tannin, which can be developed to potential hypouricemic agents.

Key words: *Dimocarpus longan* Lour., hypouricemic effect, xanthine oxidase inhibition

SUMMARY

- Longan flower extracts possess more potent XO-inhibitory activity than pericarps, twigs, seeds, and leaves *in vitro*
- The lowering effect of longan flowers and seeds extracts markedly reduced plasma uric acid levels as compared to allopurinol *in vivo*
- The extract proanthocyanidin A2 and acetonylgeraniin A were demonstrated potent XO inhibitory activity *in vitro*.



The uric acid-lowering effects of methanol extract from longan flower, pericarp, seed, leaf and twig tissues on mice with PO-induced hyperuricemia.

Abbreviations used: PO: Potassium-oxonate, XO: xanthine oxidase, HE: n-hexane, EA: ethyl acetate, i.p.: intraperitoneal, PBS: phosphate-buffered saline, AP: allopurinol, P_{UA}: plasma uric acid.

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INTRODUCTION

Longan (*Dimocarpus longan* Lour.) is an evergreen fruit tree belonging to the Sapindaceae family. It is native to temperate and tropical Asia and widely cultivated in Southern China and Southeast Asia. Many people love to eat longan due to its delicate flavor and sweet taste; the fruit is also used as a medicament by tonifying heart blood, spleen qi, strengthening postpartum weakness, and calming the spirit, especially when dried.

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Table 1: Pharmacological activities of *Dimocarpus longan* Lour. in different experimental models reported by previous investigators

Part	Extract, fraction or isolate[a]	Model	Capacity methods[b]	Pharmacological activity	Ref.
Flower	Extract with boiled H_2O (100°C) for 5 min Oral administration of LFWE (125-500 mg/kg/day) is neuroprotective via antioxidative, anti-inflammatory, and anti-apoptotic mechanisms	*In Vitro* *In Vivo*	LPIA, Parkinsonian animal model	Antioxidant activity Neuroprotective effect	Lin *et al*. 2012
Flower	Extract with boiled H_2O (100°C) for 30 min A combined effect of decreased exogenous lipid absorption, normalization of hepatic PPAR-α gene expression, suppression of pancreatic activity and SREBP-1c and FAS gene expression, and higher fecal triglyceride output	*In Vivo*	SCC, TGB, EET	Antiobesity Hypolipidemic effect	Yang *et al*. 2010
Flower	Methanol extract was fractionated with HE, EA, BuOH, H_2O Proanthocyanidin A2 and (-)-epicatechin from EA sub-fractions with excellent activity of delaying LDL oxidation	*In Vitro*	DPPH, ORAC, LDL	Antioxidant activity	Hsieh *et al*. 2008
Flower	Ethanol extract - extract twice with 95% ethanol for 24 h at room temperature; water extract - reflux with H_2O for 30 min Ethanol extract possesses higher proanthocyanidin and flavonoid contents than water extract, therefore enhancing the anti-inflammatory activity and showing prominent inhibitory effects on nitric oxide and prostaglandin E2 production	*In Vitro*	ABTS, TEAC, NORSA, LPS-stimulation, iNOS expression	Antioxidant activity Anti-inflammatory activity	Ho *et al*. 2007
Pericarp	50% ethanol extract was fractionated with DEE, EA, BuOH EA fraction showed the highest scavenging activity and reducing power	*In Vitro*	DPPH, ABTS, HRSC, FRAP	Antioxidant activity	Yang *et al*. 2014
Pericarp	Extract twice with boiled H_2O (100°C) for 60 min H_2O extraction exhibited radical scavenging, reducing activity and liposome protection activity; and inhibited lipopolysaccharide (LPS)-induced nitric oxide production in macrophages	*In Vitro* *In Vivo*	DPPH, ABTS, FRAP, CPE, LPS-stimulation	Antioxidant activity Anti-inflammatory activity	Huang *et al*. 2012
Pericarp	High-pressure-assisted extract with 50% ethanol (500 mL) at 500 MPa for 2.5 min Gallic acid, corilagin, ellagic acid were identified by comparing with high-pressure extract and conventional extract of longan; which exhibited higher extraction effectiveness in terms of higher extraction yield, higher phenolic content, and higher antioxidant and anticancer activity with shorter extraction time	*In Vitro*	DPPH, SRSA, LPIA, MTT, HepG2, A-549, SGC-7901 testing	Antioxidant activity Anticancer activity	Prasad *et al*. 2009
Pericarp	95% ethanol extract employing MAE & SE method MAE processed advantages compared to SE method and exhibits excellent antioxidant activities	*In Vitro*	DPPH, HRSC, FRAP	Antioxidant activity	Pan *et al*. 2008
Pericarp	Extract twice with methanol-acetone-H_2O (4.5:4.5:1) for 20 min at 4°C; two representative polyphenols were separated and purified 4-*O*-methylgallic acid exhibited stronger antioxidant capability than (-)-epicatechin	*In Vitro*	DPPH, HRSC, FRAP, SRSA	Antioxidant activity	Sun *et al*. 2007
Pulp	Polysaccharides extracted with H_2O using cellulase enzymolysis and ultrasonic cell disintegration LPP (peroral administration of 100 mg/kg/day) can significantly stimulate lymphocyte/macrophage activation and cytokine/antibody secretion	*In Vivo*	Immuno-suppressed mice models	Immunomodulatory activity	Yi *et al*. 2011
Fruit	Extract with boiled H_2O (100°C) for 2 h Immunohistochemical studies revealed that the number of cells immunopositive for BDNF, pCREB, or pERK 1/2 was significantly increased in the hippocampal dentate gyrus and CA1 regions after longan extract treatment for 14 days	*In Vivo*	Passive avoidance task	Memory-enhancing effects	Park *et al*. 2010
Pulp	Polysaccharides extracted with H_2O for 4.5 min at 680W in an ultrasonic cell disintegrator 3-4 times LPP (doses ranging 100 to 200 mg/kg) had potent immune-modulatory effects in S180 tumor mice model and exhibited significant effect on DTH response, macrophage phagocytosis and ConA-stimulated splenocyte proliferation	*In Vitro* *In Vivo*	DPPH, HRSC, S180 tumor mice models	Antioxidant activity Immunomodulating Antitumor effect	Zhong *et al*. 2010
Seed	Reflux triplicate with 90% ethanol for1.5 h at 70°C Ellagic acid and *p*-coumaric acid-glycoside had important contribution to the potent antioxidant activity Gallic acid, corilagin, (*S*)-flavogallonic acid, methyl-ellagic acid glucopyranoside and ethyl gallate showed a very little contribution to the total antioxidant activity of longan seed extract	*In Vitro*	DPPH, FRAP, ORAC	Antioxidant activity	Chen *et al*. 2014
Seed	50% ethanol extracted triplicate for 24 h and was partitioned with EA, EA/MeOH, BuOH, H_2O The P01-SI01 fraction showed the strongest activity against *Staphylococcus aureus* and methicillin- resistant *S. aureus*, which were found to contain gallic acid, corilagin, ethyl gallate and ellagic acid	*In Vitro*	DDT, MIC	Antimicrobial activity	Tseng *et al*. 2014

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Table 1: Contd...

Part	Extract, fraction or isolate[a]	Model	Capacity methods[b]	Pharmacological activity	Ref.
Seed	Extract triplicate with hot H_2O (70-75°C) for 60 min Longan seed exhibited antifungal activity against the opportunistic yeasts. Ellagic acid showed the most potent antifungal activity followed by corilagin and gallic acid	*In Vitro*	DAD	Antifungal activity	Rangkadilok *et al.* 2012
Seed	Longan seed extract was purchased from Joben Bio-Medical Co (Taiwan) Gallic acid, corilagin and ellagic acid was carried using an HPLC technique	*In Vitro* *In Vivo*	XO	Urate reducing effect	Hou *et al.*, 2012
Seed	Extract with methanol after delipidation with hexane High ellagitannin content in longan seeds exhibited strong antioxidant capacity	*In Vitro*	DPPH, ORAC, FRAP	Antioxidant activity	Sudjaroen *et al.* 2012
Seed	Polysaccharides extracted with H_2O for 3 h at 80°C were used in mice in a swimming test LSP (doses ranging 50-100 mg/kg) can increase swimming time and HG whilst reducing SUN and BLA levels, indicating an ameliorating effect on physical fatigue in mice	*In Vivo*	FST, HG, SUN, BLA	Antifatigue effect	Zheng *et al.* 2010
Seed	95% ethanol extracted triplicate for 24 h, and was fractionated with PE, $CHCl_3$, EA, BuOH Gallic acid (1), ethyl gallate (2), 1-β-*O*-galloyl-d-glucopyranose (3), methyl brevifolin carboxylate (4), brevifolin (5), corilagin (6), ellagic acid (7), 4-*O*-α-l-rhamnopyranosyl-ellagic acid (8) were isolated. DPPH: antioxidant order 1>2>4>6>7>5>3>8; SRSA: antioxidant order 1>2>6>7>4>3>5>8	*In Vitro*	DPPH, SRSA	Antioxidant activity	Zheng *et al.* 2009
Seed Pulp Whole fruit	Dried seed powders and pulps of longan were extracted three times with hot water (70-75°C) for 1 h; whole fruits (peel, pulp, seed) were boiled in water for 3 h High levels of gallic acid, ellagic acid and corilagin in dried longan seeds exhibited strong scavenging activities when comparing fresh seed and dried pulp extracts Both fresh and dried longan seed had higher activity than dried pulp and whole fruit in the ORAC assay Longan seed extract also showed significantly inhibited tyrosinase activity	*In Vitro*	DPPH, ORAC, SRSA	Antioxidant activity Anti-tyrosinase activity	Rangkadilok *et al.* 2007
Seed Pulp	Reflux triplicate with 50% ethanol for1 h at 70°C The seeds showed a much higher antioxidant activity and phenolic content than the pulp	*In Vitro*	ABTS, FRAP, FCR	Antioxidant activity	Soong *et al.* 2004

[a]LFWE: Longan flower water extract; PPAR-α: Peroxisome proliferator-activated receptor-α; SREBP-1c: Sterol regulatory element binding protein-1c; FAS: Fatty acid synthase; HE: *n*-hexane; EA: Ethyl acetate; BuOH: *n*-butanol; LDL: Low-density lipoprotein peroxidation inhibition assay; DEE: Diethyl ether; MAE: Microwave-assisted extraction; SE: Soxlet extraction; LPP: Longan pulp polysaccharide; BDNF: Brain-derived neurotrophic factor; pCREB: Phosphorylated cAMP response element binding protein; pERK: Phosphorylated extracellular signal-regulated kinase; DTH: Delayed-type hypersensitivity; MeOH: Methanol; LSP: Longan seed polysaccharide; HG: Hepatic glycogen; SUN: Serum urea nitrogen; BLA: Blood lactic acid; PE: Petroleum ether. [b]LPIA: Lipid peroxidation inhibitory activity; SCC: Serum cholesterol clearance; TGB: Triglyceride biosynthesis; EET: Energy expenditure tests; DPPH: 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging assay; ORAC: Oxygen radical absorbance capacity assay; ABTS: 2,2'-amino-di(2-ethyl-benzothiazoline sulphonic acid-6)ammonium salt (ABTS) radical-scavenging assay; TEAC: Trolox equivalent antioxidant capacity; NORSA: Nitric oxide radical scavenging assay; iNOS: Inducible nitric oxide synthase; HRSC: Hydroxyl radical scavenging capacity; FRAP: Ferric ion reducing antioxidant power; CPE: Carrageenan paw edema; SRSA: Superoxide radical scavenging activity; MTT: [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide reduction assay; HepG2: Human hepatocellular liver carcinoma; A-549: Human lung adenocarcinoma; SGC-7901: Human gastric carcinoma; DDT: Disc diffusion test; MIC: Minimum inhibitory concentration test; DAD: Disc agar diffusion; XO: Xanthine oxidase inhibition; FST: Forced swimming test; FCR: Folin-Ciocalteu reagent assay

Previous reports depicted that longan flowers, fruits, pericarps, pulps, and seeds are known to contain many phenolic components, which are capable of protecting people from oxidative damage through an anti-inflammatory mechanism.[1,2] Several scientific studies validated that longan extract with different extraction technologies display a wide range of therapeutic activities as reviewed herein [Table 1] (i.e., antifungal,[3] antimicrobial,[4] anti-inflammatory,[1,2] antioxidant,[1,2,5-16] antiobesity,[17] hypolipidemic,[17] antifatigue,[18] anticancer,[9] antitumor,[16] neuroprotective,[7] immunomodulatory,[16,19] anti-tyrosinase,[10] memory-enhancing,[20] and urate-reducing effect)[21] [Table 1].

To our knowledge, longan extracts, rich in phenolics, have been well characterized chemically; in this regard, most of the therapeutic properties of natural phenolics and flavonoids have been ascribed to their enzyme inhibitory and antioxidant activity.[22-24] Taking into account the high levels of phenolics and flavonoids in longans,[10,25] this *in vivo* investigation performed the uric acid-lowering effect of longan extracts (including flowers, pericarps, seeds, leaves, and twigs) in potassium-oxonate (PO) treated mice. According to our preliminary screening, longan flower extracts have been shown to contribute beneficially to lowering the levels of uric acid. In an attempt to pursue this anti-gout effect, we isolated 10 compounds from longan flower [Figure 1], such as acetonylgeraniin A, chebulagic acid, chebulinic acid, corilagin, (-)-epicatechin, gallic acid, geraniin, proanthocyanidin A2, procyanidin B2, and protocatechuic acid. Therefore, this study also intended the evaluation of longan flower extracts for their possible inhibitory activity against xanthine oxidase (XO) *in vitro* [Figure 1].

MATERIALS AND METHODS

Chemicals

XO purified from bovine milk, allopurinol, PO, and xanthine were purchased from Sigma-Aldrich (St. Louis, MO, US). The solvents used for extraction and column chromatography, including methanol, n-hexane, ethyl acetate (EA), n-butanol, and acetone, were of analytical grade and supplied by J.T. Baker (Phillipsburg, NJ, US).

Plant material and extractions

The male flowers, pericarps, seeds, leaves, and twigs of *Dimocarpus longan* Lour. were collected from private farms in Hongjia (Xuejia District, Tainan City, Taiwan). Dried materials (flowers, pericarps, seeds, and twigs) were ground into powder and extracted with 95% methanol at room temperature for 24 h, and then passed through a Whatman no. 1 filter paper. This procedure was repeated 3 times for the residues, and the filtrates were combined. All solvents were removed under reduced pressure for isolation of the extract. Longan leaf powders were extracted with 70% acetone triplicate. The total acetone extracts were filtered and evaporated in a vacuum. The percentage yield so obtained was 10.2%, 11.9%, 10.2%, 26.7%, and 4.5% toward flower, pericarp, seed, leaf, and twig samples, respectively. To these extracts, H_2O was added individually and the resulting extracts were successively partitioned with HE, EA and distilled water to yield soluble fractions. All the fractions were further concentrated under vacuum at 50°C; the crude dried extracts obtained were used directly for XO assay. In context, the chemical constituents identified in longan flower have been characterized with those reported.[6,21] Briefly, the freeze-dried methanol extract was re-dissolved in methanol and then sequentially partitioned with HE, EA, *n*-butanol, and H_2O. The EA fraction that showed prominent antioxidant activity was further chromatographed on Sephadex LH-20 (Pharmacia) with H_2O-MeOH (2:3) and MCI-CHP-20P (Mitsubishi) with H_2O-MeOH (1:0–1:1). The structures of some of the compounds isolated are given in Figure 1.

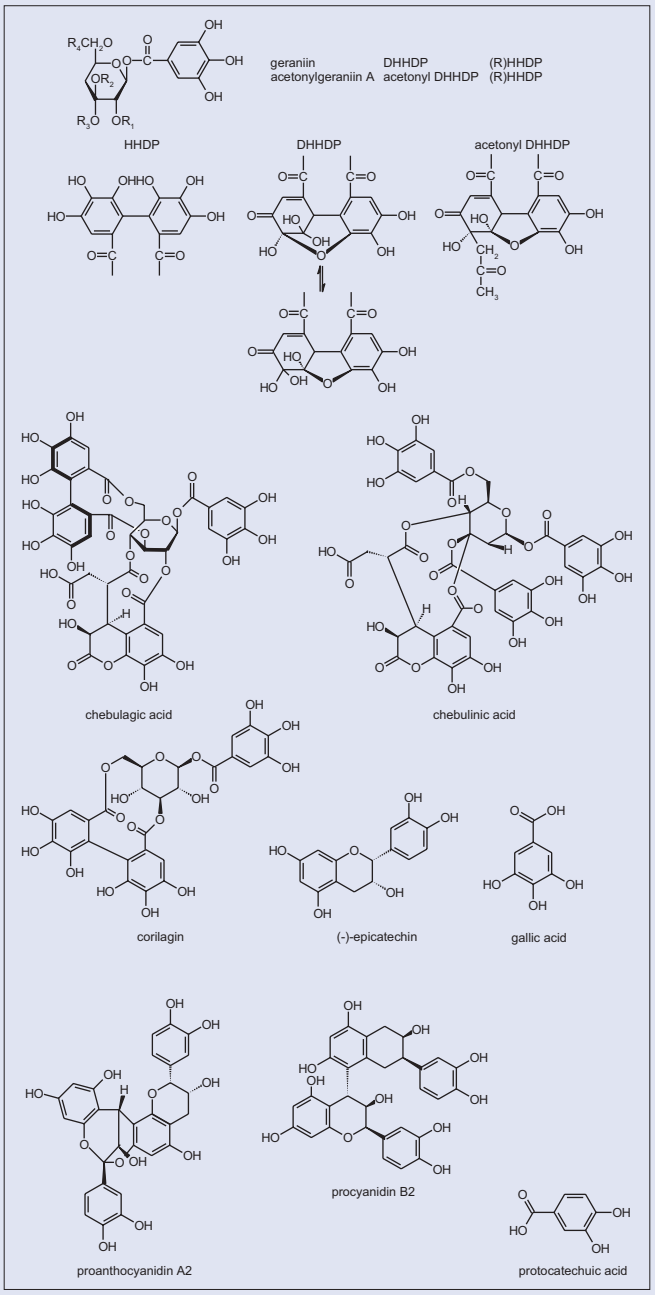

Figure 1: Chemical structures of typical constituents isolated from *Dimocarpus longan* Lour.

Table 2: XO-inhibitory activitiy of derived soluble fractions of *Dimocarpus longan Lour*

Part	Solvent-partitioned fractions[a]	Test concentration (μg/mL)	Inhibition (%)[b]
Flowers	HE	33	25.0±4.0
	EA	100	78.6±2.4
	H_2O	100	46.4±1.5
Pericarps	HE	100	68.4±2.5
	EA	50	79.2±6.3
	H_2O	100	15.8±3.0
Seeds	HE	50	8.3±1.8
	EA	50	78.9±3.6
	H_2O	100	20.8±1.6
Leaves	HE	50	25.0±3.3
	EA	100	42.1±1.8
	H_2O	100	31.6±5.5
Twigs	HE	100	68.4±4.2
	EA	50	79.2±3.0
	H_2O	100	15.8±2.2
Allopurinol[c]	-	50	98.2±0.4

[a]HE: *n*-hexane; EA: Ethyl acetate; [b]Values present mean±SD of triplicate experiments; [c]Positive control

Table 3: Inhibitory effects of isolated 10 phytochemicals from longan flower extracts against XO

Phytochemicals	Test concentration (μg/mL)	Inhibition (%)[a]
Acetonylgeraniin A	40	64.7±4.0
Chebulagic acid	40	16.0±4.0
Chebulinic acid	40	34.0±1.0
Corilagin	40	43.1±3.1
(-)-Epicatechin	40	NI[b]
Gallic acid	40	ND[c]
Geraniin	40	46.8±8.3
Proanthocyanidin A2	20	70.7±7.5
Procyanidin B2	40	24.3±8.8
Protocatechuic acid	40	22.3±1.5
Allopurinol[d]	50	98.7±0.4

[a]Values present mean±SD of triplicate experiments; [b]No inhibition; [c]Not determined; [d]Positive control

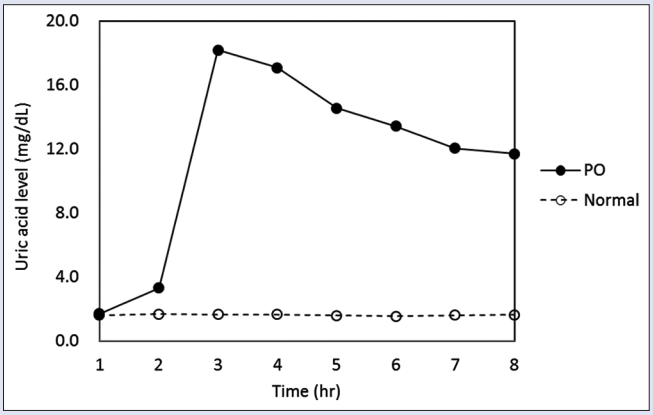

Figure 2: Time-course effect of potassium oxonate on hyperuricemic mice uric acid levels

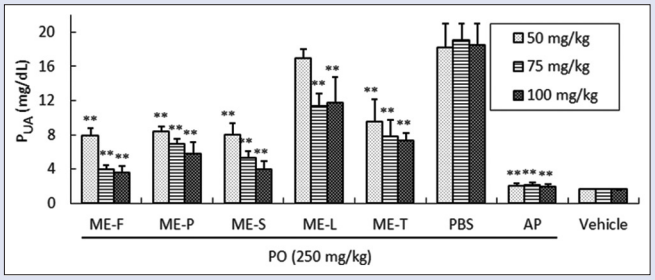

Figure 3: The uric acid-lowering effects of methanol extract from longan flower, pericarp, seed, leaf and twig tissues (50, 75, 100 mg/kg) on mice with PO-induced hyperuricemia. The results are presented as the mean ± SD ($n = 6$). $^{**}P < 0.01$ compared to the PO-treated group

Determination of xanthine oxidase-inhibitory activity

XO-inhibitory activity was measured spectrophotometrically based on the procedure reported by Nguyen *et al.*[26] The reaction mixture consisted of 50 µL of test samples or compounds, 35 µL of 50 mM phosphate buffer (pH 7.5), and 30 µL of XO solution (0.1 U/mL in 50 mM phosphate buffer, pH 7.5) and was prepared immediately before use. After preincubation at RT (25°C) for 15 min, 60 µL of substrate solution (150 µM xanthine in the same buffer) was added to the mixture to initiate the reaction. The assay mixture was then incubated at RT for 30 min. Afterward, 25 µL of stop solution (1 N HCl) was added, and the absorbance values were measured at 290 nm with a microplate reader (µQuant™, BIO-TEK Instruments Inc., USA). Allopurinol was used as a positive control. Three replicates were performed for each test sample, and the increased ultraviolet absorption at 290 nm indicated the formation of uric acid. The percentage inhibition ratio was calculated according to the following equation: % inhibition = (1 − B/A) × 100, where A is the change in absorbance per min without the test sample and B is the change in absorbance per min with the test material. The concentration of samples required to inhibit 50% of XO activity (IC_{50}) was estimated from the % inhibition versus concentration plot using a linear regression algorithm.[27]

Hyperuricemia model in mice

About 6–8-week-old male ICR mice weighing 25–30 g were purchased from BioLASCO Taiwan Co., Ltd. (Yilan, Taiwan), maintained in 12 h light/dark cycles, and housed at 23 ± 2°C for at least 1 week prior to the experiment. Animals were provided with a rodent diet and clean water *ad libitum*, except 1 h prior to drug administration when access to food was restricted. Animal tests used in this study were conducted under the guidelines of the International Association for the Study of Pain.[28] The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of National Taiwan University (IACUC Approval No: NTU-101-EL-14).

Experimental hyperuricemia was induced in mice by intraperitoneal (i.p.) injections of the uricase inhibitor PO as described previously.[29] The mice were i.p. injected with phosphate-buffered saline (PBS) containing PO (250 mg/kg) 1 h after administration of test samples to increase blood urate levels. The names of the samples are as follows: Methanol extracts of the flower (F), pericarp (P), seed (S), leaf (L), and twig (T) of longan were shortened for ME-F, ME-P, ME-S, ME-L, and ME-T, respectively. Mice were randomly assigned into the following seven groups for different treatments ($n = 6$): (1) Vehicle group (normal control); (2) PO + allopurinol (AP, 10 mg/kg) group (positive control); (3) PO + PBS group (negative); (3) PO + ME-F group; (4) PO + ME-P group; (5) PO + ME-S group; (6) PO + ME-L group; (7) PO + ME-T group. For the comparative study, three dosages (100, 75, and 50 mg/kg) were delivered to ensure the utilization of this extract.

Measurement of plasma uric acid level

Uric acid in tail vein blood was measured 2 h after PO injection using commercial Ektachem clinical chemistry slides from Johnson & Johnson clinical diagnostics (US).

Statistical analysis

All data were expressed as the mean ± standard deviation ($n = 3$). The significance of difference was performed with Duncan's new multiple range test, and $^{*}P < 0.05$ and $^{**}P < 0.01$ were considered statistically significant.

RESULTS

Time course potassium-oxonate effects on plasma uric acid levels in mice

The effects of PO on mice plasma uric acid (P_{UA}) levels are shown in Figure 2. Uric acid levels in nonhyperuricemic, vehicle-treated mice were 1.6 ± 0.05 mg/dL. However, treatment with uricase inhibitor PO resulted in a significant elevation of P_{UA} levels reaching to 18 ± 2.8 mg/dL after 2 h, followed by slow decrease in urate levels 8 h postinjection [Figure 2].

Xanthine oxidase-inhibitory activity of longan crude extracts and derived soluble fractions

The tested longan crude extracts inhibited XO in a concentration-dependent manner. Longan flowers showed the best XO-inhibitory activity, with an IC_{50} value of 115.8 µg/mL, followed by pericarps (118.9 µg/mL), twigs (125.3 µg/mL), seeds (262.5 µg/mL), and leaves (331.1 µg/mL), respectively. These results are in accordance with Hou *et al.*,[21] who reported that longan seed extract had dose-dependent XO-inhibitory activity with an IC_{50} value of 277.8 µg/mL. Comparisons of XO-inhibitory activity results [Table 2] in various derived soluble fractions from longan extracts indicated that there are abundant XO-inhibitory phytochemicals present in longan extracts, especially in the EA fraction [Table 2].

Xanthine oxidase-inhibitory activity of phytochemicals from longan flower extracts

The inhibitory effects of isolated 10 phytochemicals [Figure 1] from longan flower extracts against XO are given in Table 3. The experimental evidence indicates that proanthocyanidin A2 and acetonylgeraniin A showed an excellent activities' profile for inhibition to XO compared to that of the standard, allopurinol, as indicated by inhibition (%). In addition, proanthocyanidin A2, a phenolic dimer belonging to the class of condensed tannins, has been shown to display superior antioxidant activity to that of ascorbic acid in previous research works;[6,30] while some studies revealed that acetonylgeraniin A, a hydrolyzable tannin, has an antihypertensive effect.[31,32] In this study, proanthocyanidin A2 and acetonylgeraniin A were found to inhibit XO *in vitro*, which may be potentially useful for the treatment of gout [Table 3].

In vivo hypouricemic effect determined in mice with potassium-oxonate-induced hyperuricemia

To further confirm the capabilities of methanol extract of longan flower, pericarp, seed, leaf, and twig tissues to reduce the uric acid level *in vivo*, a PO-induced hyperuricemia mice model was investigated. In vehicle control mice, the P_{UA} level was 1.6 ± 0.03 mg/dL. After 2 h of PO treatment, the level of P_{UA} had increased to 18.5 ± 0.4 mg/dL. As shown in Figure 3, oral administration of ME-F, ME-P, ME-S, ME-L, and ME-T (50, 75 and 100 mg/kg) significantly reduced plasma urate levels in hyperuricemic mice in a dose-dependent manner, as well as the reference (AP) group. The administration of allopurinol (PO + AP group) significantly reduced the level of P_{UA} by (2.0 ± 0.1 mg/dL) 89% as compared with the PO + PBS group ($P < 0.01$). At doses of 75 mg/kg of ME-F, ME-P, ME-S, ME-L, ME-T, or above, plasma urate levels of the PO-treated mice were significantly reduced by 80%, 64%, 72%, 41%, and 59%, respectively, relative to the PO + PBS group ($P < 0.01$). No significant difference existed between the dosages of longan extracts at 75 and 100 mg/kg. Conversely, the lowering effect on uric acid by longan extracts at 50 mg/kg on PO-induced hyperuricemic mice was found to be weaker than that observed for 75 and 100 mg/kg dosages. It is noteworthy that comparisons of these results indicate that ME-F and ME-S exhibit excellent hypouricemic effects. Remarkably, the methanol extract of the flowers was more potent than seeds in uric acid-lowering effects *in vivo* [Figure 3].

DISCUSSION

In recent years, there is an increasing interest in finding herbal plants and phytochemicals which possess the capacity to inhibit XO activity and reduce urate levels. Longan is a fruit used in herbal preparations in China, and though unpollinated longan flowers and nonedible fruit seeds are generally regarded as disposable byproducts, studies show that longan flowers, pericarps, and seeds contain high levels of phenolics and flavonoids, which exhibit high antioxidant activity and may be rendered suitable as protective agents [Table 1]. The activities of extracts from its flowers, pericarps, leafs, and twigs against XO are reported here for the first time. A study was demonstrated that longan seed extract and its active components (corilagin, gallic acid, and ellagic acid) inhibited XO dose dependently *in vitro*, but were less potent than allopurinol.[21] Our findings indicate that extracts from longan flowers have a great potential for preventing diseases caused by the XO-inhibitory activity *in vitro*, with an IC_{50} value of 115.8 μg/mL, followed by pericarps (118.9 μg/mL), twigs (125.3 μg/mL), seeds (262.5 μg/mL), and leaves (331.1 μg/mL). For the *in vivo* study, longan extract (75 mg/kg) was able to reduce P_{UA} levels and XO activities in hyperuricemic mice in a decreasing order: ME-F (80%) > ME-S (72%) > ME-P (64%) > ME-T (59%) > ME-L (41%), compared with allopurinol (89%). Meanwhile, 10 phytochemicals were identified from longan flower, and a superior XO-inhibitory activity in the type of phenolics was observed. The *in vitro* inhibitions of XO by proanthocyanidin A2 and acetonylgeraniin A are high when compared to allopurinol, which possess the hypouricemic activities for the first time. Others yield weak inhibitory activity against XO.

A toxicological study revealed no toxic effects of oral administration of longan seed extract during acute and repeated doses (4 and 13 weeks).[33] Besides, longan seed extract inhibited uric acid production and XO activity in normal liver cells (clone-9 cells) and was not cytotoxic under the concentration of 200 μg/ml.[21] The results suggested that its urate-reducing effect might be due to modulation of urate transporters (GLUT1 and GLUT9) and inhibition of circulating XO. It is of great interest that the XO-inhibitory effect of longan flowers and seeds, the byproduct materials, may provide some choices for prevention and/or treatment of hyperuricemia as valuable functional ingredients.

CONCLUSION

It can be concluded from the present finding that longan extracts possess potent *in vivo* hypouricemic effects in hyperuricemic rats pretreated with oxonate. The use of longan flowers and seeds in the treatment of gout could be attributed to its inhibitory effect on XO. These results also suggest that proanthocyanidin A2 and acetonylgeraniin A extractions from *Dimocarpus longan* Lour. flowers could be developed as potent XO inhibitors for clinical application.

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Conflicts of interest

All authors declare that they have no conflicts of interest.

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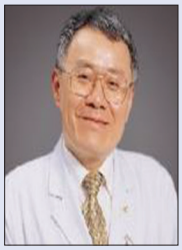

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