



Research article

Investigation of xanthine oxidase inhibitors in bioactive components of *Jatropha podagrica* stem bark

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ABSTRACT

This study was conducted to examine the activity of antioxidants against xanthine oxidase in bioactive extracts from *Jatropha podagrica* stem bark. Among the extracts, the EtOAc extract showed the strongest antioxidant activity and xanthine oxidase inhibition. Column chromatography isolation using different ratios of 'ethyl acetate-hexane' (2:8, 3:7, and 4:6) yielded five fractions (M1-M5), which were identified and confirmed in the previous study. Specifically, the fractions included methyl gallate and gallic acid (M1); methyl gallate, gallic acid, and fraxetin (M2); methyl gallate, fraxetin and tomentin (M3); methyl gallate and fraxetin (M4); and fraxetin (M5). Employing allopurinol as the positive control (IC_{50} = 20.2 μ g/mL), M3, was found to have the highest level of xanthine oxidase inhibitors (IC_{50} = 69.7 μ g/mL), followed by M5 (fraxetin, IC_{50} = 106.9 μ g/mL). The results of this study proposed *Jatropha podagrica* as an alternative and potential source for the treatment of gout symptoms.

Keywords: Xanthine Oxidase, *Jatropha podagrica*, Inhibitor, Stem, Bark.

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INTRODUCTION

Xanthine oxidase (XOD) catalyzes the breakdown of purine nucleotides to produce uric acid, which motivates oxidation reaction to generate free radicals. During this enzymatic reaction, reactive oxygen species (ROS) are also released. ROS are linked to aging and a number of conditions such as diabetes and Alzheimer's disease [1]. The increase of uric acid levels in the blood (7 mg/mL in males and 6 mg/mL in females) leads to precipitation of urate crystals, mainly concentrating on the joints of hands and feet, leading to acute arthritis. Inhibiting the activity and preventing the oxidation of xanthine oxidase minimizes the formation of uric acid in the blood thereby preventing arthritis. Flavonoids are a group consisting of different hydroxylated polyphenolic compounds in natural products. These compounds were studied and showed different properties such as enzyme inhibition, antiviral, antimicrobial. Xanthine oxidase was reported blocked by flavonoids inhibition ability which is a promising possibility to use flavonoids as a remedy to reduce both uric acid and superoxide concentration in tissues of gout and ischemia patients [2]. *Plumeria rubra* (PR) was considered an alternative for allopurinol due to its high level of flavonoids, better therapeutic activity and fewer side effects. The XOD inhibitory activity, *in-vitro*, exhibited the highest inhibition activity of IC_{50} = 23.91 μ g/mL. Serum uric acid level in the rats was significantly reduced by 43.77% when a dosage

of 400 mg/kg of methanol flower extract (PR-ME) was used [3].

Isolates (Baicalein, baicalin and wogonin) from *Scutellaria rivularis* have been recorded to contain a high level of XO inhibitors. The amount of Cytochrome C reduced and modified xanthine oxidase inhibition were used to evaluate the antioxidant activity [4]. In another study, baicalein and baicalin were confirmed with electron spin resonance (ESR) technique. Both demonstrated strong activity in eliminating the superoxide radical [5].

In a study on *Acacia confusa* heartwood extract, Lin, Chang and Chang reported that okanin displayed the strongest XO inhibitory activity, *in vitro*, with an IC_{50} value of 0.076 μ M, followed by melanoxetin (0.274 μ M), allopurinol (4.784 μ M), and 7,8,3',4'-tetrahydroxyflavone (10.488 μ M). Further studies demonstrated that melanoxetin has a better XOD inhibition effect than the commercial drug, allopurinol. At the same dosage, XOD and xanthine exothermic reactions released less heat with melanoxetin than allopurinol. Furthermore, 34.6 and 24.5 μ M were the obtained value of the Michaelis constants (K_m) of XOD and xanthine reaction for melanoxetin and allopurinol, respectively. The molecular docking studies indicated that melanoxetin and allopurinol both occupied the same binding site. Also, the carbonyl and multihydroxyl in melanoxetin may be the reason that melanoxetin has a higher binding

affinity to XOD than allopurinol [6].

The development of nature-based medicines is a global trend, which aims to enhance the efficacy of the treatments of various diseases and to minimize, if not eradicate, side effects. A number of medicinal plants are currently being used extensively for anti-gout remedies. Therefore, conducting research to discover innovative xanthine oxidase inhibitors from natural resources is among the oriented approaches to contribute to anti-hyperuricemic treatments.

Taking advantage of our previous work, this study was aimed to verify the strong antioxidants found in the ethyl acetate (EtOAc) extract of the stem bark of *Jatropha podagrica* [7]. The XOD inhibition of these antioxidants was tested and results are reported in this paper.

MATERIALS AND METHODS

Materials

J. podagrica Hook stem bark was preliminarily sterilized with 1% NaOCl and rinsed thoroughly under tap water. Sample JS-M2020 was then dehydrated in thermal drying chamber and stored at the Laboratory of Research and Applied Biochemistry Laboratory (CRETECH, Vietnam Academy of Science and Technology, Vietnam) [7].

Jatropha Podagrica Stem Bark Bioactive Component isolation

As reported in the previous study [7], bioactive compounds in *J. podagrica* Hook stem bark were isolated and purified by column chromatography technique to obtain five (5) bioactive fractions (M1-M5). They were identified and confirmed as methyl gallate (present in the first four fractions), gallic acid (present in the first two fractions), fraxetin (present in the last four fractions), and tomentin (present in the third fraction).

Bioactive Activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay, and β -carotene bleaching test were employed to measure the antioxidant activity in the plant extracts [8-10]. Adapting the method described by Minh et al. (2019) [11], xanthine oxidase inhibition capability of isolated fractions (M1-M5) was measured by spectrophotometry.

Statistical Analysis

For statistical measurement, MiniTab® Version 19 with one-way variance analysis (ANOVA) ($p < 0.05$) was employed to process the data. Three replicates were done for each trial using a complete randomized scheme.

RESULTS AND DISCUSSION

Antioxidant and Xanthine Oxidase (XOD) Inhibition of *Jatropha podagrica* stem bark extracts obtained by different solvents

Table 1 summarizes the antioxidant activities of extracts from *Jatropha podagrica* stem bark obtained by different solvents via four techniques: DPPH, ABTS free radical scavenging, β -carotene bleaching and XOD assays. Standards used for the assays were BHT

and Allopurinol.

Table 1. Bioactive activities of *Jatropha podagrica* stem bark extracts obtained by different solvents

Extract	Antioxidant activity		LPI (%) β -carotene	Enzyme activity IC ₅₀ (μ g/mL) XOD
	DPPH	ABTS		
MeOH	626.8 \pm 1.3 ^b	1783.0 \pm 1.9 ^b	50.2%	725.0 \pm 8.4 ^c
Hexane	991.8 \pm 2.7 ^a	2102.1 \pm 2.8 ^a	35.9%	1022.7 \pm 7.5 ^a
EtOAc	46.4 \pm 0.3 ^d	119.7 \pm 1.4 ^d	80.7%	571.0 \pm 5.1 ^d
Aqueous	462.9 \pm 1.5 ^c	1263.3 \pm 1.2 ^c	53.8%	999.3 \pm 6.4 ^b
BHT*	10.1 \pm 0.1 ^e	49.7 \pm 0.6 ^e	93.1%	-
Allopurinol*	-	-	-	20.2 \pm 0.8 ^e

* Positive control. Data reported are the means \pm SD (standard deviation); Small alphabetical symbols illustrate significant differences at $p < 0.05$; BHT- butylated hydroxytoluene; LPI-lipid peroxidation inhibition

The antioxidant activities of stem bark extracts from *J. podagrica* were expressed in the value of IC₅₀ and ranges from 46.4 - 991.8 μ g/mL for DPPH assay and 119.7 - 2102.1 μ g/mL for ABTS assay. In terms of lipid peroxidation inhibition (LPI) value by β -carotene (β -c) bleaching method, the result ranges from 35.9% - 80.7% (Table 1). The EtOAc extract from *J. podagrica* stem bark showed the highest antioxidant activity in all three methods employed.

The results (Table 1) also revealed that all extracts of stem bark of *J. podagrica* were highly active against XO (IC₅₀ = 571.0 - 1022.7 μ g/mL) with the EtOAc extract exhibiting the highest XOD inhibition effect (IC₅₀ = 571.0 μ g/mL) followed by the MeOH extract (IC₅₀ = 725.0 μ g/mL) while positive control, allopurinol, exhibited an IC₅₀ = 20.2 μ g/mL.

Xanthine Oxidase (XOD) Inhibition of *J. podagrica* stem bark fractions

Table 2. Xanthine Oxidase inhibition of *Jatropha podagrica* stem bark fractions

Fractions	Compounds	Chemical Formula	Reference	Enzyme activity IC ₅₀
				(μ g/mL) XOD
M1	Gallic acid	C ₇ H ₆ O ₅	[7]	221.3 \pm 6.4 a
	Methyl gallate	C ₈ H ₈ O ₅		
M2	Fraxetin	C ₁₀ H ₈ O ₅	[7]	139.5 \pm 5.6 c
	Gallic acid	C ₇ H ₆ O ₅		
	Methyl gallate	C ₈ H ₈ O ₅		
M3	Tomentin	C ₁₀ H ₁₀ O ₅	[7]	69.7 \pm 2.3 e
	Fraxetin	C ₁₀ H ₈ O ₅		
	Methyl gallate	C ₈ H ₈ O ₅		
M4	Fraxetin	C ₁₀ H ₈ O ₅	[7]	166.5 \pm 4.8 b
	Methyl gallate	C ₈ H ₈ O ₅		
M5	Fraxetin	C ₁₀ H ₈ O ₅	[7]	106.9 \pm 4.2 d
Allopurinol*	-	-	Standard	20.2 \pm 0.8 f

* Positive control. Data reported are means \pm SD (standard deviation); small alphabetical symbols illustrate significant differences at $p < 0.05$; XOD: xanthine oxidase inhibition

The inhibitory effects (XOD) of the isolated fractions (from the previous study [7]) is summarized and presented in Table 2.

The IC₅₀ value of isolated fraction **M3** (tomentin, fraxetin, methyl gallate) showed the highest XOD inhibition at 69.7 μ g/mL followed by the pure compound **M5** (fraxetin) (IC₅₀ = 106.9 μ g/mL). The obtained results were weaker than the standard allopurinol, which showed the IC₅₀ level at 20.2 μ g/mL (Table 2).

The concern of researchers regarding the use of drug, healthy foods, and natural herbicides are its safety and environmental

impact [10, 11]. Studies on *J. podagrica* revealed various compounds in the isolation procedures such as japodic acid, erytrinasinate, fraxidin [12], steroids, flavonoids [13, 29], japodagrins, diterpenoids, japodagrone [14], 3-acetylaleuritic acid, japodagrol [15], γ -sitosterol, and n-heptyl ferulate [16]. Researches on various biological properties (antioxidant, antitumor, antiproliferative, and antimicrobial) of *J. podagrica* extract were done using different parts of the plant. However, this study is the first to investigate the antigout (XOD inhibition) activity of the isolated antioxidant compounds (**M1–M5**) from the stem bark of *J. podagrica* [17].

Phenolic compounds, which possess hydrogen donors to neutralize free radicals, are considered the driving factor for antioxidant property. In the antioxidant assay, *J. podagrica* stem bark extract of EtOAc showed higher anti-radical scavenging activity (DPPH, ABTS and β -carotene oxidation) as compared to other extracts but lower antioxidant capability as compared to the positive control (BHT). On the other hand, the isolated compounds **M1–M5** showed stronger antioxidant activity than BHT [18].

Terpenoids and phenolics are believed to play major roles in XOD inhibition [19]. A number of investigations have found several compounds possessing both antioxidant and anti-gout properties [19–21]. In this study, isolated antioxidants M1 (methyl gallate, gallic acid), M2 (methyl gallate, gallic acid, fraxetin), M3 (methyl gallate, fraxetin, tomentin), M4 (methyl gallate, fraxetin), M5 (fraxetin) inhibited XOD at varying degrees, with **M3** exhibiting the highest inhibition ($IC_{50} = 69.7 \pm 2.3 \mu\text{g/mL}$).

Tamus communis L., a traditional medicine in Algeria, were used to search for XOD inhibitors. The inhibition ability of root extracts (methanol, chloroform and ethyl acetate) and aqueous protein extract against bovine, sheep and human milk XOD were dependent on the concentration of each solvents, with a strongest scavenging capacity found in ethyl acetate extract ($IC_{50} = 0.15, 0.09$ and 0.04 g/L, respectively). The antioxidant potential was verified by a total radical-trapping antioxidant parameter (TRAP) assay, which showed that the extracts have the same potential antioxidant active level as the cytochrome C reduction method. The antioxidant strength of examined extracts was arranged as follows: ethyl acetate extract > chloroform extract > protein [20–21].

CONCLUSIONS

This study documented the xanthine oxidase inhibitory activities, *in vitro*, of the antioxidants isolated from the stem bark of *Jatropha podagrica*. The findings showed that **M3** (tomentin, fraxetin, methyl gallate) is a potential source of natural antioxidants as well as an anti-gout agent.

In vivo tests are recommended to affirm the bioavailability of **M3** for the development of medicines for gout prevention and/or

treatment. This will also ascertain the value of this plant for sustainable cultivation by the agricultural sector.

DECLARATION

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Author Contributions

TNM conceived of the idea and implemented the experiments. TNM, CDG, YA, BQM and NQT analyzed data and wrote the manuscript. TNM and CDG revised the manuscript. All authors agreed to the final version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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