CHEMISTRY AND TECHNOLOGY OF MEDICINAL COMPOUNDS AND BIOLOGICALLY ACTIVE SUBSTANCES

ХИМИЯ И ТЕХНОЛОГИЯ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ И БИОЛОГИЧЕСКИ АКТИВНЫХ СОЕДИНЕНИЙ

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RESEARCH ARTICLE

Screening medicinal plant extracts for xanthine oxidase inhibitory activity

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Abstract

Objectives. *The study aimed to test the ethanol extracts of ten medicinal plants for xanthine oxidase inhibitory activity.*
Methods. *The degree of xanthine oxidase inhibitory activity was determined by measuring the absorbance spectrophotometrically at 290 nm, which is associated with uric acid formation. The selected medicinal plants included Piper lolot C.DC. (Piperaceae), Pandanus amaryllifolius R.(Pandanaceae), Brassica juncea L. (Brassicaceae), Piper betle L. (Piperaceae), Perilla frutescens L. (Lamiaceae), Anacardium occidentale L. (Anacardiaceae), Polygonum barbatum L. (Polygonaceae), Artocarpus Altilis P. (Moraceae), Vitex negundo L. (Verbenaceae), Annona squamosal L. (Annonaceae), which were selected based on folk medicine.*
Results. *The results showed that the Piper betle L. has a strong ability to inhibit xanthine oxidase with an IC_{50} value of up to 1.18 μg/mL, compared to allopurinol 1.57 μg/mL. Different parts of Piper betle L. were compared and the leaves of Piper betle L. showed the best value for xanthine oxidase inhibitory and antioxidant activity.*
Conclusions. *Piper betle L. showed the best potential for inhibition of xanthine oxidase among ten medicinal plants. Piper betle L. leaf extract showed strong xanthine oxidase inhibitory and antioxidant activity, compared to the whole plant, and the stem extract, which promises to be applied in the treatment of gout.*
Keywords: *anti-gout, xanthine oxidase inhibitors, medicinal plants*

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НАУЧНАЯ СТАТЬЯ

Скрининг экстрактов лекарственных растений на ингибирующую активность ксантиноксидазы

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Аннотация

Цели. *Исследование было направлено на проверку этанольных экстрактов десяти лекарственных растений на ингибиторную активность ксантиноксидазы.*
Методы. *Степень ингибирующей активности ксантиноксидазы определяли путем спектрофотометрического измерения поглощения при 290 нм, вызываемого образованием мочевой кислоты. В состав отобранных лекарственных растений вошли перец-лолот (Piperaceae), пандан (Pandanaceae), горчица сарептская (Brassicaceae), бетель (Piperaceae), перилла обыкновенная (Lamiaceae), кешью (Anacardiaceae), конопля (Polygonaceae), хлебное дерево (Moraceae), прутняк китайский (Verbenaceae), сахарное яблоко (Annonaceae), отобранные на основе их применения в народной медицине.*
Результаты. *Результаты показали, что бетель обладает сильной способностью ингибировать ксантиноксидазу со значением IC_{50} до 1.18 мкг/мл по сравнению с аллопуринолом 1.57 мкг/мл. Были проведены сравнения различных частей бетеля, и листья бетеля показали наилучшие показатели ингибирования ксантиноксидазы и антиоксидантной активности.*
Выводы. *Бетель показал лучший потенциал ингибирования ксантиноксидазы среди десяти лекарственных растений. Экстракт листьев бетеля показал сильное подавление ксантиноксидазы и антиоксидантную активность по сравнению с целым растением и экстрактом стебля, которые применяются при лечении подагры.*

Ключевые слова: *антиподагра, ингибиторы ксантиноксидазы, лекарственные растения*

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INTRODUCTION

Gout is the most common inflammatory arthritis characterized by hyperuricemia, arthritis, tophaceous deposits, and renal calculi associated with a high serum uric acid level [1]. The prevalence and incidence of gout disease have increased annually due to changes in diet and lifestyle, such as fast food, lack of exercise, etc. [2]. Globally, the reported prevalence of gout ranges from 0.1% to approximately 10%, and the incidence from 0.3 to 6 cases per 1000 people per year [3]. Xanthine oxidase (XO) inhibitors are the mainstay of the therapy to reduce serum urate levels in patients with gout [4]. XO is an enzyme involved in the purine metabolism of purine which catalyzes the oxidation of hypoxanthine to xanthine and of xanthine to the uric acid [5]. Inhibition of XO helps to increase uric acid excretion and reduce uric acid production, which reduces the risk of gout [6]. Allopurinol, a clinically available drug is widely

used for the management of hyperuricemia in patients with gout, was proved to have some adverse effects consisting of hypersensitivity reactions, skin rash, and gastrointestinal distress [7, 8]. For these reasons, the search for alternative therapeutic strategies, particularly those that involve the use of natural products with low side effects, is gaining interest. There is an increase in research, which investigate medicinal plants that contain chemical constituents with potential biological activity for the treatment of diseases, including gout treatment [9].

Vietnam is home of about 12000 species of greatly appreciated plants and about 36% of which have medicinal properties [10]. Some plants and their phytochemicals are worth investigating as possible inhibitors, of XO inhibition as they have been used as food or food supplements for many years and found to be safe for human bodies [11]. Many medicinal plants have traditionally been used in folk medicine to treat a variety of complications such as gout. In fact, several plants have been reported in pharmacopeia as antigout products, and most of them have demonstrated this activity experimentally. However, these plants are underutilized and require additional research to confirm this effect. Therefore, this work aimed to identify medical plants with antigout potential of *Piper lolot* C.DC., *Pandanus amaryllifolius* R., *Brassica juncea* L., *Piper betle* L., *Perilla frutescens* L., *Anacardium occidentale* L., *Polygonum barbatum* L., *Artocarpus Altilis* P., *Vitex negundo* L., *Annona squamosal* L. by evaluation of the *in vitro* XO inhibitory activity of them. The total phenolic and flavonoid content of the tested extracts was also determined, identifying the importance of these compounds as XO inhibitors.

MATERIALS AND METHODS

Materials and Chemicals

The leaves or whole plants samples of ten plants (Table 1) were collected in An Giang province, Vietnam, in February 2020, during dry season which is appropriated for harvesting these plants. They were washed for removing residue of some plant and dust, then dried under natural air flow in shadow until the moisture content diminished to 12% and then grounded and stored in a sealed bag for further use. The plants were authenticated by the Department of Ecology and Evolutionary Biology, Faculty of Biology and Biotechnology, Ho Chi Minh City University of Science, Vietnam National University.

Absolute ethanol (C_2H_5OH), methanol (CH_3OH), sodium nitrite ($NaNO_2$), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), aluminum chloride ($AlCl_3$), dimethyl sulfoxide (DMSO), ferric chloride ($AlCl_3$), diclofenac sodium and other reagents of analytical grade were obtained from *Merck* (Darmstadt, FR, Germany). Folin-Ciocalteu's reagent, quercetin, xanthine oxidase (XO) (4.5 U/mL, from bovine milk), xanthine, ascorbic acid, allopurinol, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), and gallic acid (GA) were provided by *Sigma-Aldrich* (Singapore).

Preparation of plant extract

The dried plant powder (20 g) was extracted at 45°C in absolute ethanol for 45 min with the 10:1 mL/g solvent-to-sample ratio. Subsequently, the extract was filtered by vacuum filtration, and the filtrate was concentrated by a vacuum rotary evaporator (*BUCHI*, USA) to remove excess solvent.

Qualitative phytochemical screening

Phytochemical screening of the of medicinal plant extracts was used to determine the presence of bioactive compounds: polyphenols, flavonoids, alkaloids, and tannins [12–14].

Determination of total polyphenol content

The polyphenol concentration in the extracts was determined by the Folin–Ciocalteu's assay with slight modification [15]. In summary, 40 µL of the diluted extract at different concentrations was thoroughly mixed with 200 µL of Folin–Ciocalteu's reagent. The mixture was kept for reaction for 5 min at 25°C, followed by the addition of 600 µL of Na_2CO_3 20 w/v % and 3160 µL of distilled water. The mixture absorbance was measured at the 760 nm wavelength using a Genesys 10S UV-vis Spectrophotometer (*Thermo Fisher Scientific*, USA). Total polyphenol content was determined in milligram of gallic acid equivalent per gram of sample (mg GAE/g). The calibration curve for gallic acid was created to calculate the phenolic content as the following equation: $y = 0.0013x - 0.0262$ ($R^2 = 0.994$) where y is the absorbance and x is the concentration as gallic acid equivalents (mg GAE/mL).

Determination of total flavonoid content

The concentration of polyphenols in the extracts was quantified using the aluminium chloride colorimetric assay method [16]. Briefly, 0.5 mL of the extract dissolved in methanol was mixed with 2 mL of distilled water and then 0.15 mL of $NaNO_2$ 5%. The mixture was incubated for reaction in 5 min, followed by the addition of 0.15 mL of 10% $AlCl_3$. Then, 1.0 mL of NaOH 1M and 1.2 mL of distilled water were added. The absorbance of the mixture was measured at the 425 nm wavelength. The number of total flavonoids was shown as milligrams of Quercetin equivalents per gram of sample (mg QUE/g), using quercetin to perform the calibration curve: $y = 0.001x - 0.0048$ ($R^2 = 0.9986$) where y is the absorbance and x is concentration as quercetin equivalents (µg/mL).

Table 1. Phytochemical screening results from leaf extract of ten plants

Medicinal plants	Plant part used	Bioactive compounds			
		Polyphenols	Flavonoids	Alkaloids	Tannins
Piper lolot C.DC.	Whole plant	+	+	−	+
Pandanus amaryllifolius R.	Leaves	+	+	++	+
Brassica juncea L.	Whole plant	+	+	+	+
Piper betle L.	Whole plant	++	+	−	++
Perilla frutescens L.	Leaves	++	+	+	++
Anacardium occidentale L.	Leaves	++	++	−	++
Polygonum barbatum L.	Leaves	++	++	−	++
Artocarpus altilis P.	Leaves	++	++	+	++
Vitex negundo L.	Leaves	+	+	+	+
Annona squamosal L.	Leaves	++	++	++	++

− Not detected, + Slightly positive reaction, and ++ Strong positive reaction

In vitro XO inhibitory activity assay

XO enzyme catalyzes the conversion of hypoxanthine and xanthine into uric acid, a direct cause of gout [5]. XO inhibitory activity assay is widely used to determine the anti-gout activity of the plant extracts [17]. In this study, XO inhibitory activity of the extract was determined using an *in vitro* assay with slight modification to the Abd El-Rahman and Abd-ELHak method [18].

The assay was carried out on a 48-well plate. The reaction mixture included 250 µL extract in DMSO, 5% 175 µL sodium phosphate buffer (pH 7.5) and 150 µL enzyme (0.2 units/mL of XO in phosphate buffer). The mixture was incubated for 15 min at 37°C. Afterward, 300 µL of xanthine (mM) and incubated for 30 min at 37°C. The reaction was stopped with the addition of 125 µL HCl 1M. The absorbance was measured at 290 nm by Genesys 10S UV-Vis Spectrophotometer (*Thermo Fisher Scientific*, USA). Allopurinol was used as a positive control. The assay mixture without a sample was used as a negative control. XO inhibitory activity was expressed as the percentage inhibition of XO and calculated by Eq. 1:

$$\text{XO inhibition} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100\% \quad (1)$$

with A_{blank} is the absorbance at 290 nm of blank, A_{sample} is the absorbance at 290 nm of the sample.

DPPH radical scavenging activity

The free radical scavenging activity of the extracts was determined using the DPPH radical in methanol. The assay was carried out with a slight modification to the Sharma and Bhat method [19]. In the methanol solution, DPPH had a purple color that gradually changed to a yellow color in reaction with antioxidants. Briefly, 180 µL of DPPH in methanol was mixed with 120 µL of sample in the methanol at different concentrations. The reaction mixture was homogenized thoroughly by a vortex machine and kept in the dark at 25°C for 30 min. Ascorbic acid (Vitamin C) and methanol were used as the positive control and negative control, respectively. The absorbance of the mixture was measured at 517 nm using a Genesys 10S UV-Vis Spectrophotometer (*Thermo Fisher Scientific*, USA) to calculate the percentage of inhibition as follows:

$$\text{DPPH radical scavenging activity} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\% \quad (2)$$

where A_{control} is the absorbance of the negative control, and A_{sample} is the absorbance of the test solution.

Statistical analysis

All experiments were carried out in triplicate and the data were expressed as mean±standard deviation (SD).

RESULTS AND DISCUSSION

Phytochemical screening of medicinal plant extracts

Many natural compounds have shown the anti-XO enzyme ability such as polyphenols, flavonoids, alkaloids, tannins, etc [20]. The phytochemical screening of ten plants was carried out to identify the bioactive compounds, and the result is shown in Table 1. All extracts have the presence of flavonoids, polyphenols, and tannins with different quantities. All plants have the presence of alkaloids except *Piper lolot* C.DC., *Piper betle* L., *Ancardium occidentale* L., and *Polygonum barbatum* L. The presence of these diverse bioactive compounds indicated the potential for various biological activities. Polyphenols, which are secondary metabolites produced by higher plants, have a variety of biological effects, including antioxidant, anti-inflammatory, anti-carcinogenic, and anti-gout [21]. Flavonoids have the inhibitory activity of various enzymes, such as XO, peroxidase, and nitric oxide synthase, which are involved in the production of free radicals, resulting in less oxidative damage to macromolecules [22]. Tannins, water-soluble polyphenols, have a variety of *in vitro* bioactivities, the most well studied of which are antimicrobial and antioxidant properties [23]. Alkaloids show strong biological effects on human organisms, especially anti-inflammatory while inflammatory is the most popular gout symptom [24]. Preliminary phytochemical screening shows the potential of medicinal plants for gout treatment.

Total polyphenol content, total flavonoid content, and XO inhibitory activity of plant extracts

Polyphenols and flavonoids are considered the main bioactive chemical constituents and are found ubiquitously in plants [25]. Therefore, the total polyphenol content (TPC) and total flavonoid content (TFC) of the extracts were determined and the results are described in Table 2. The TPC of the three sample extracts ranged from 32.13 to 427.89 mg GAE/g, while the TFC ranged from 50.34 to 605.81 mg QUE/g. The *Piper betle* L. had the highest value of both TPC (427.89 mg GAE/g) and TFC (605.81 mg QUE/g). The significant value of TPC, and TFC which higher around 13–19 times than the lowest values. Therefore, *Piper betle* L. was predicted to show high potential in inhibiting XO inhibition because XO inhibition of plant extract may be related to TPC, TFC, and the chemical structures of individual phenolic.

The XO inhibitory activity of ten plants was also presented by their IC_{50} values, shown in Table 2. Most medicinal plants have XO inhibitory activity, ranging from 1.18 µg/mL to 280 µg/mL. As expected, *Piper betle* L. with the highest TPC and TFC showed the best inhibitory activity of the XO inhibitory activity with the lowest value of IC_{50} (1.18 µg/mL) which was lower than allopurinol (1.57 µg/mL). The results agree with one study reported on the XO that the IC_{50} value of *Piper betle* L. was 16.5 µg/mL compared to the value of allopurinol, 6.16 µg/mL [26]. *Artocarpus Altilis* P. showed a value of IC_{50} of 32.31 µg/mL which

Table 2. TPC, TFC, and XO inhibitory activity of ten plants

Medicinal plants	TPC (mg GAE/g)	TFC (mg QUE/g)	IC_{50} (µg/mL)
Piper lolot C.DC.	73.56 ± 3.98	69.59 ± 1.82	<10% × 300 µM[a]
Pandanus amaryllifolius R.	41.15 ± 0.54	50.34 ± 0.63	<50% × 300 µM[a]
Brassica juncea L.	32.13 ± 0.49	78.68 ± 2.10	<20% × 300 µM[a]
Piper betle L.	427.89 ± 3.52	605.81 ± 11.60	1.18 ± 0.02
Perilla frutescens L.	104.62 ± 0.20	137.75 ± 3.01	88.04 ± 2.83
Anacardium occidentale L.	122.78 ± 0.89	150.52 ± 3.99	81.21 ± 1.55
Polygonum barbatum L.	70.45 ± 0.71	85.52 ± 2.93	113.94 ± 7.99
Artocarpus altilis P.	140.60 ± 0.42	309.53 ± 1.58	32.31 ± 1.08
Vitex negundo L.	75.80 ± 0.62	82.11 ± 2.90	280.00 ± 10.78
Annona squamosal L.	100.20 ± 0.94	223.06 ± 4.75	72.03 ± 1.58
Allopurinol	–	–	1.57 ± 0.01

[a]Inhibitory activity (%) at the highest tested concentration

was about 30 times higher than *Piper betle* L. one when the TPC and TFC values were approximately 2.7 and 1.7 times, respectively, lower than those of the *Piper betle* L. In addition, *Annona squamosal* L., *Perilla frutescens* L., *Anacardium occidentale* L., had IC_{50} values ranging from 72.03 to 88.04 μg/mL. *Polygonum Barbatum* L. and *Vitex negundo* L. had a value of IC_{50} greater than 100 μg/mL, which was in accordance with their low TPC and TFC (<100 mg/g). *Piper lolot* C.DC., *Pandanus amaryllifolius* R., and *Brassica juncea* L. showed no inhibitory activity with the inhibition percentage lower than 50% at 0.3 mg/mL. The strong inhibitory activity of *Piper betle* L. XO was predicted to be related to hydroxychavicol, which was a phenolic compound identified in *Piper betle* L. and found to be a more potent XO inhibitor than allopurinol [27]. Out of ten plants, *Piper betle* L. was selected as the most promising medicinal plant in XO inhibitory activity, and the different parts of *Piper betle* L. were surveyed for more details.

Study on the Piper betle L. different parts

In this study, the TPC, TFC, XO inhibitory activity, and antioxidant activity of different parts (stem, leaves, and whole plant) of the *Piper betle* L. were determined to provide the basis for further investigation of this plant (see Figure). The TPC and TFC of leaf extract were the highest values of 437.12 mg GAE/g and 668.18 mg QUE/g, respectively, and the stem extract had the lowest values of TPC and TFC. Thus, the leaf extract showed the best ability to inhibit XO with an IC_{50} value of 0.82 μg/mL when compared to the value of the stem extract and the whole plant extract, 2.62 μg/mL and 1.18 μg/mL, respectively, and compared to allopurinol (1.57 μg/mL). Moreover, the leaf extract also showed the most potent antioxidant activity with the lowest value of IC_{50} of 4.21 μg/mL, which is lower than that of ascorbic acid (5.92 μg/mL). In addition, several studies showed similar results that the total amounts of polyphenols and flavonoids of leaf extract were higher than those of stem extract, and the antioxidant activity of leaf extract was also better [28, 29]. Clinical evidence suggests that hyperuricemia could be a significant risk factor for diabetes in gout patients and therefore the pathogenesis of both acute and chronic pancreatitis can be related to oxidative stress [30, 31]. From these results, the leaf extract of *Piper betle* L. promises to be a potential anti-gout agent and used for the treatment of gout and its complications.

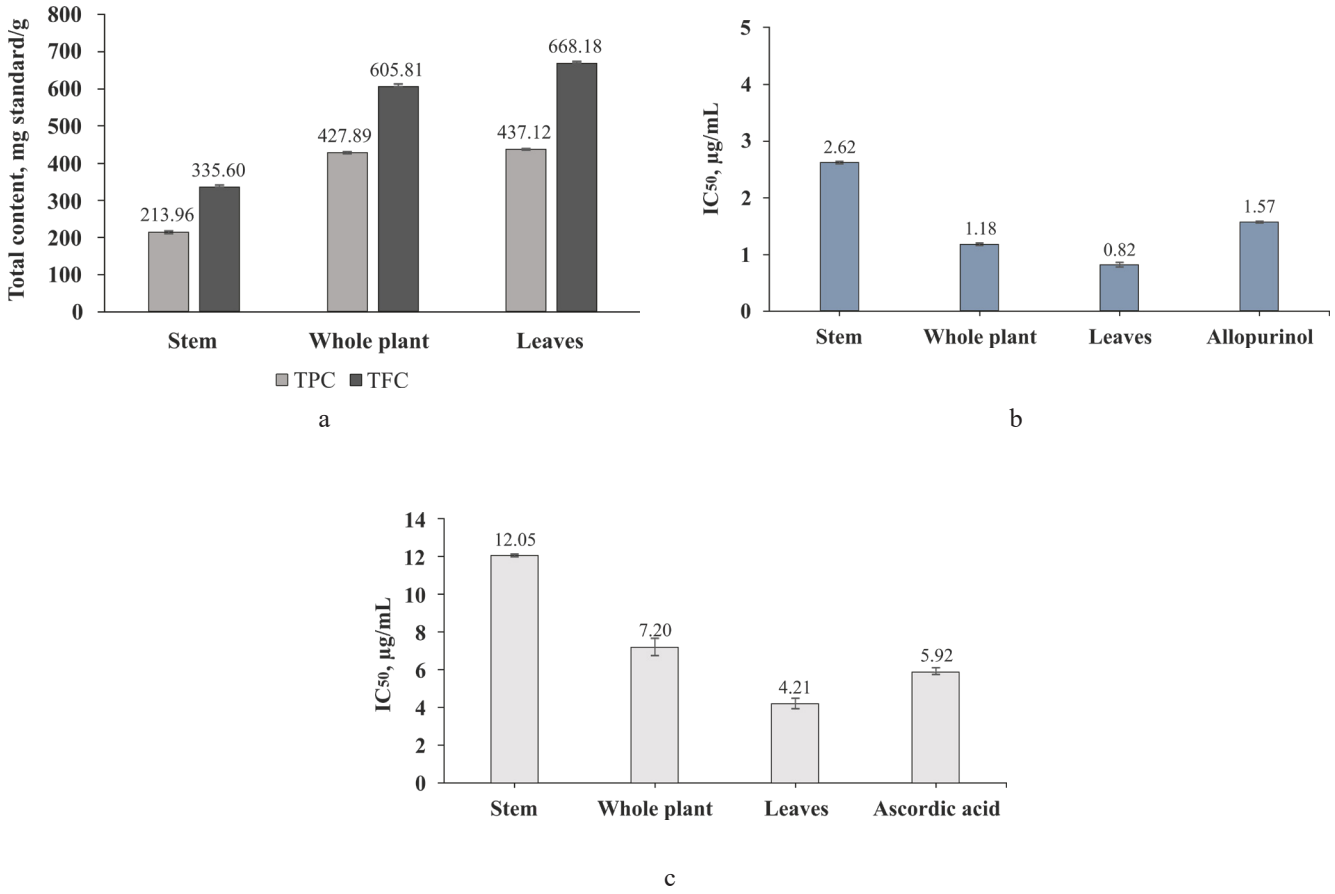

Figure. (a) TPC and TFC, (b) XO inhibitory activity, and (c) antioxidant activity of different parts of *Piper betle* L.

CONCLUSIONS

The present work evaluated and screened medicinal plants for XO inhibitory activity. The extract of *Piper betle* L. showed strong XO inhibitory activity with an IC_{50} value lower than allopurinol (1.57 μg/mL) with high TPC and TFC. The *Piper betle* L. leaf was the part of the *Piper betle* L. that showed the best inhibitory activity and also antioxidant activity with an IC_{50} value of 0.82 μg/mL and 4.21 μg/mL, respectively. In summary, *Piper betle* L. leaf could be a good candidate for future studies of this plant on the treatment of gout and its complications.

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Author's contributions

Anh C. Ha – developing the concept and methodology of the study, supervising, writing and editing the text of the article.

Chinh D.P. Nguyen – conducting research, writing the literature review, and editing the text of the article;

Tan M. Le – developing the concept, formal analysis, conducting research, and writing the text of the article;

The authors declare no conflicts of interest.

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