PHYTOCHEMICAL SCREENING, TOTAL POLYPHENOL AND FLAVONOID CONTENT, AND XANTHINE OXIDASE INHIBITORY ACTIVITY OF VIETNAMESE MEDICINAL PLANT EXTRACTS

● HA CAM ANH - LE MINH TAN - NGUYEN PHAM DUC CHINH

ABSTRACT:
This study investigates gout inhibitory activity of five plant extracts including Artemisia vulgaris L., Blumea balsamifera (L.) DC., Cynara cardunculus L., Nelumbo nucifera G., and Phyllanthus urinaria L. The anti-gout activity of these extracts was evaluated by xanthine oxidase inhibition assay. The total phenolic and flavonoid contents are also determined. The Artemisia vulgaris L. are exhibited the xanthine oxidase inhibitory activity with an IC₅₀ value of 87.35 μg/mL. Blumea balsamifera (L.) DC. has the highest total polyphenol content of 170.49 mg GAE/g extract, while Phyllanthus urinaria L. has the highest total flavonoid content of 153.02 mgQUE/g extract. Furthermore, the amount of total polyphenol and flavonoid content of Artemisia vulgaris L. is 132.36 mg GAE/g extract and 137.75 mg QUE/g extract respectively.

Keywords: gout, xanthine oxidase inhibitors, screening, medicinal plants.

1. Introduction
Gout is inflammatory arthritis caused by the crystallization of monosodium urate monohydrate (MSU) in articular joints and periarticular tissues. The disease's prevalence has risen annually, possibly as a result of dietary and lifestyle changes [1]. Gout is most commonly associated with recurring bouts of acute arthritis and tophi depositions, as well as other chronic illnesses such as typical complications of renal, metabolic syndrome, hypertension, and cardiovascular disorders [2]. As a result, gout treatment has become a severe issue that must be addressed. Xanthine oxidase (XO), a key enzyme in the development of gout, catalysed for oxidation of xanthine into uric acid and releasing peroxide anion. Uric acid is also the main risk factor for gout. Thus, it is important to inhibit the activity of the xanthine oxidase enzyme to control uric acid accumulation and deposit. Currently, Gout and hyperuricemia are being treated using a variety of anti-gout medications [3]. However, few drugs have more side effects including gastric damage, renal toxicity, and hypersensitivity, thus limiting their clinical uses [4]. Previous pharmacological studies had attributed an anti-gout effect of these herbal extracts to inhibit xanthine oxidase [5]. It showed that the herbal extracts have a significant curative effect on gout and its applications. It is beneficial to search XO inhibitors and herbs for the prevention of hyperuricemia and gout. Therefore, the aim of this study is to investigate and evaluate the xanthine oxidase inhibitory effect of 5 plants, potent in gout treatment, including, Artemisia...
2. Materials and Methods

2.1. Materials and Chemicals

Plants were collected on 02/2021 in Ho Chi Minh City before washing and drying under shade until the moisture content less than 15%. The dry leaves of 5 herbs were ground 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 - Ho Chi Minh City.

Chemicals were purchased from commercial suppliers in analytical grade, as follows: ethanol (C₂H₅OH), methanol (CH₃OH), sodium nitrite (NaNO₂), sodium carbonate (Na₂CO₃), sodium hydroxide (NaOH), aluminum chloride (AlCl₃), dimethyl sulfoxide (DMSO), ferric chloride (FeCl₃), Folin-Ciocâlteu reagent, quercetin, xanthine oxidase (4.5U/ml, from bovine milk), gallic acid.

2.2. Experimental protocol

A total of 20g of plant powders was immersed with 200mL of ethanol 96% at 50°C for 45 min two times. The ethanolic extract was filtered using vacuum filtration before solvent removal by rotary evaporator. The phytochemical screening of the ethanolic extracts was used to determine the presence of bioactive compounds: polyphenols, flavonoids, alkaloids, tannins, and carotenoids.

2.3. Determination of Total Polyphenol Content (TPC)

In this study, the TPC of extracts was measured by the Folin-Ciocâlteu assay, as described by Le et al. [6]. In brief, 40 µL of each extract dissolved in DMSO and 200 µL of Folin-Ciocâlteu reagent was mixed at ambient temperature for 5 min. The mixture was added 600 µL of Na₂CO₃ 20% and 3160 µL of distilled water. The absorbance of the mixture was measured at the wavelength of 760 nm by UV-Vis Spectrophotometer. The TPC was expressed as mg of gallic acid equivalent per gram of sample (mg GAE/g).

2.4. Determination of Total Flavonoids Content (TFC)

The TFC in each extract were determined by the aluminium-chloride colorimetric assay method, as described by Do et al. [7]. A total of 10 mg of extract dissolved in 1 mL of methanol in various concentrations was mixed with 0.15 mL of NaNO₂ 5% and 2 mL of distilled water. The mixture was reacted in 5 minutes and then added 0.15 mL of AlCl₃ 10%. In the next one minute, 1 mL of NaOH 1M and 1.2 mL of distilled water were added to stop the reaction. The mixture was measured absorbance at the wavelength of 425 nm. Quercetin was used as standard to illustrate the calibration graph and the TFC was express as milligram of Quercetin equivalents per gram of sample (mg QUE/g).

2.5. In vitro xanthine oxidase inhibition activity assay

The XO inhibitory activity of the extracts was determined using in vitro assay as described by Duong et al. [8]. In brief, 250 µL extract in DMSO, 5% 175µL sodium phosphate buffer (pH 7.5), and 150 µL enzyme (0.2 units/mL of xanthine oxidase in phosphate buffer) was added into 96-well plate. The mixture was incubated for 15 minutes at 37°C before adding 300 µL of xanthine (mM). The reaction was continued for 30 minutes at 37°C and then stopped by adding 125 µL HCl 1M. The absorbance of the mixture was measured at 290nm using UV-Vis Spectrophotometer. Allopurinol was used as the positive control and the mixture without sample was used as the negative control. The XO inhibitory activity was expressed as the inhibitory percentage calculated as using the formula:

\[
\text{% XO inhibition} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100%
\]

with \(A_{\text{blank}}\) is the absorbance at 290 nm of blank; \(A_{\text{sample}}\) is the absorbance at 290 nm of the sample.

3. Results and Discussion

3.1. Phytochemical screening of the extracts

The qualitative chemical screening of 5 plant extractions were conducted to identify the bioactive
Table 1: Phytochemical screening results of leaf extract of ten plants.

<table>
<thead>
<tr>
<th>Bioactive compounds</th>
<th>Test</th>
<th>Examinations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A. vulgaris</td>
</tr>
<tr>
<td>Polyphenol</td>
<td>FeCl₃</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>Mg/HCl</td>
<td>++</td>
</tr>
<tr>
<td>Tannin</td>
<td>Gelatine</td>
<td>++</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>Drageidroff</td>
<td>++</td>
</tr>
<tr>
<td>Carotenoid</td>
<td>H₂SO₄</td>
<td>+</td>
</tr>
</tbody>
</table>

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

Source: Authors' calculation

Compounds and the results were shown in Table 1. All extracts have the presence of polyphenols, which flavonoids only exist in A. vulgaris, C. cardunculus, N. nucifera. The presence of polyphenol and flavonoid indicated that the plant extract have potent in anti-xanthine oxidase. Polyphenols, which are secondary metabolites generated by higher plants, have a wide range of biological effects, including antioxidant, anti-inflammatory, anti-carcinogenic, and anti-gout properties [1]. Flavonoids reduce the activity of many enzymes involved in the generation of free radicals, such as xanthine oxidase, peroxidase, and nitric oxide synthase, resulting in less oxidative damage to macromolecules [9]. Tannins, which are water-soluble polyphenols, also contain a number of in vitro bioactivities, the most well-studied of which are antioxidant and antibacterial characteristics [10]. Alkaloids have powerful biological effects on human beings, including anti-inflammatory, anticancer, and anti-gout properties [11].

3.2. TPC, TFC, and xanthine oxidase inhibitory activity of the extracts

Phenolic and flavonoids compounds are commonly found in both edible and inedible plants, and have been reported to have multiple biological effects, including xanthine oxidase inhibition activity. Total polyphenols and flavonoids content, and xanthine oxidase inhibitory activity of 5 plant extracts were determined and the results were described in Table 2. The TPC of these extracts ranged from 38 mg GAE/g to 170.49 mg GA/g, while the TFC is from 35.88 mg QUE/g to 153.02 mg QUE/g. B. balsamifera has the highest value of TFC (153.02 mg QUE/g) and P. urinaria has the highest value of TPC (170.49 mg GAE/g). Besides, most medicinal plants have xanthine oxidase inhibitory activity. The A. vulgaris shows the best xanthine oxidase inhibitory activity with IC₅₀ value of 87.35 µg/mL which was lower than the positive control allopurinol (1.57 µg/mL). Xanthine oxidase inhibition of the plant extracts may relate to TPC, TFC, and the chemical structures of individual phenolic. As expected,

Table 2: TPC, TFC, and xanthine oxidase inhibitory activity of ten plants

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>TPC (mgGAE/g)</th>
<th>TFC (mgQUE/g)</th>
<th>XOI* (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. vulgaris</td>
<td>132.36 ± 2.33</td>
<td>137.75 ± 1.33</td>
<td>87.35 ± 0.43</td>
</tr>
<tr>
<td>B. balsamifera</td>
<td>110.05 ± 1.68</td>
<td>153.02 ± 2.77</td>
<td>377.34 ± 0.56</td>
</tr>
<tr>
<td>C. cardunculus</td>
<td>38.26 ± 0.15</td>
<td>35.88 ± 0.89</td>
<td>205.02 ± 1.42</td>
</tr>
<tr>
<td>N. nucifera</td>
<td>86.21 ± 1.21</td>
<td>146.04 ± 1.47</td>
<td>&lt;31.57% * 1000 µM³</td>
</tr>
<tr>
<td>P. urinaria</td>
<td>170.49 ± 2.54</td>
<td>116.81 ± 2.13</td>
<td>88.47 ± 1.18</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>-</td>
<td>-</td>
<td>1.32 ± 0.04</td>
</tr>
</tbody>
</table>

*IC₅₀ value

Source: Authors' calculation
Piper betle with the highest TPC and TFC shows the best inhibition activity. Followed by Artocarpus Altilis, the value of IC$_{50}$ was 32.3 µg/mL which was higher 20 times than the positive control. Moreover, Annona squamosal, Anacardium occidentale. Perilla frutescens range from 71.54 to 93.96 µg/mL.

4. Conclusions

In the present work, the inhibitory ability of 5 herbal extracts on inhibiting XOD activity were estimated. The results show that the inhibitory activity of A. vulgaris on inhibiting XOD was stronger than the other herbal extracts with IC$_{50}$ value of 87.35 µg/mL. Furthermore, total flavonoid and polyphenol content of Artemisia vulgaris L. reached the high content of 132.36 mg GAE/g extract and 137.75 mg QUE/g extract, respectively. The results could provide a theoretical basis for the further development of natural medicines to treat gout by medicinal plants.

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REFERENCES:

10. E. M. C. Alexandre et al. (2017). High-pressure assisted extraction of bioactive compounds from industrial fermented fig by-product. Journal of Food Science and Technology. 54(8), 2519-2531.
ĐỊNH TÍNH SỞ BỘ, TỔNG HÀM LƯỢNG POLYPHENOL VÀ FLAVONOID, VÀ HOẠT TÍNH ỨC CHẾ XANTHINE OXIDASE CỦA MỘT SỐ CHIẾT XUẤT DƯỢC LIỆU Ở VIỆT NAM

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TÓM TẮT:
Nghiên cứu đã khảo sát hoạt tính kháng gout của năm dịch chiết từ thực vật bao gồm Artemisia vulgaris L., Blumea balsamifera (L.) DC., Cynara cardunculus L., Nelumbo nucifera G., Phyllanthus urinaria L. Hoạt tính kháng gout được xác định thông qua phương pháp ức chế enzyme xanthine oxidase. Bên cạnh đó, tổng hàm lượng polyphenol và flavonoid cũng được xác định trong nghiên cứu này. Kết quả cho thấy Artemisia vulgaris L. có hoạt tính ức chế xanthine oxidase cao nhất với giá trị IC₅₀ là 87,35 µg/mL. Blumea balsamifera (L.) DC. có tổng hàm lượng polyphenol cao nhất là 170,49 mg GAE/g chiết xuất, trong khi Phyllanthus urinaria L. có tổng hàm lượng flavonoid cao nhất là 153,02 mg QUE/g chiết xuất. Hơn nữa, tổng hàm lượng polyphenol và flavonoid của Artemisia vulgaris L. lần lượt là 132,36 mg GAE/g chiết xuất và 137,75 mg QUE/g chiết xuất.

Keywords: polyphenol, tối ưu hóa, RSM, tyrosinase, α-glucosidase.