XANTHINE OXIDASE INHIBITORY ACTIVITY OF METHANOL EXTRACT FRACTIONS OF VARIOUS INDONESIAN ETHNOPHARMACOLOGICAL PLANTS

ADITYA SINDU SAKTI, HARNANDA WIDYASTANTO, ASTRI MAULIDINA, DIAN MITASARI, DIYAH SANTI ERIYANI, ABDUL MUN’IM*

Department of Pharmacognosy Phytochemistry, Faculty of Pharmacy, Universitas Indonesia, Depok 16424, Indonesia.
Email: munim@farmasi.ui.ac.id

Received: 02 October 2019, Revised and Accepted: 24 December 2019

ABSTRACT

Objective: Hyperuricemia involves an increase in serum uric acid levels, resulting in kidney damage, increased mortality, and reduced quality of life. Inhibitors of xanthine oxidase, which catalyzes the last step in uric acid synthesis, are targets for therapeutic intervention.

Methods: An ethnopharmacological approach, screening four native Indonesian herbal medicinal plants with reported activity against hyperuricemia, was used for preliminary studies, fractionating methanolic extracts by solvent partitioning. Fractions were then tested in vitro for xanthine oxidase inhibitory activity, and the most active fraction was then subjected to preliminary phytochemical screening.

Results: The target tissue of the four herbal medicinal plants investigated was Indian bay leaf (Syzygium polyanthum Wight.), God’s crown fruit (Phaleria macrocarpa Boerl.), snake fruit peel (Salacca edulis Reimw.), and Job’s tears tuber (Cyperus rotundus Linn.). Each sample was extracted by maceration with 80% methanol. The concentrated extract was then fractionated by the liquid-liquid partition method (1:1 v/v) using n-hexane, ethyl acetate, butanol, and methanol sequentially as solvents. The results revealed that the ethyl acetate fraction was the most active fraction. S. polyanthum leaf and C. rotundus tuber showed the greatest potential in inhibiting xanthine oxidase, with half-maximal inhibitory concentrations of 18.43 and 10.50 μg/ml, respectively. Enzyme kinetics analysis shows that each plant fraction works as a competitive inhibitor of xanthine oxidase.

Conclusion: Preliminary screening identified the ethyl acetate fractions of two native Indonesian herbal medicinal plants as showing potential for anti-hyperuricemia activity.

Keywords: Ethnopharmacological, Hyperuricemia, Phytochemical screening, Xanthine oxidase.

INTRODUCTION

Hyperuricemia is a pathological condition where uric acid levels in the blood are elevated above the normal range [1]. This condition can persist for a long time without any symptoms. During the asymptomatic period, the deposition of uric acid crystals may lead to chronic pain, causing joint damage in some patients [2]. In young patients, hyperuricemia stimulates oxidative stress, inflammation, and inflammatory response as feedback from the oxidative stress triggered by the high uric acid concentration itself [3]. Acute and chronic inflammation due to crystal deposition in joints and soft tissues is a consequence that occurs when hyperuricemia is not immediately treated [4].

Uric acid is the final product of the catabolism of purine nucleotides. There are two main sources of purines. The first one is endogenous, originating from the synthesis of purines de novo and their subsequent breakdown as part of nucleic acid turnover. Purines can also come from exogenous sources, such as food [5]. Many enzymes are involved in the purine catabolism pathway, where eventually adenine will be converted into hypoxanthine and guanine will be converted to xanthine. Hypoxanthine will be oxidized to xanthine, and finally, xanthine is oxidized again by xanthine oxidase to form uric acid [1]. Xanthine oxidase inhibitors, such as purine analogs, are used to treat hyperuricemia and gout, and there is pharmaceutical interest in the exploitation of natural plant xanthine oxidase inhibitors such as inositol and flavonoids.

An ethnopharmacological approach investigates the basis of the traditional use by native people of natural materials, such as plants, animals, fungi, microorganisms, and minerals, in treating specific medical conditions [6]. Many modern pharmaceuticals originated from traditional medicine and ethnopharmacology [7], either directly, using natural compounds as drugs, or as lead compounds in drug development.

Tubers of rumput teli or Job’s tears (Cyperus rotundus Linn.) have been widely used in Indonesia as a traditional medicine to treat dysentery and painful joints, and as a diuretic [8], whereas leaves of the Indian bay leaf (Syzygium polyanthum Wight.) are used as a traditional medicine to treat muscle pain and uric acid accumulation [9]; a water extract of S. polyanthum has also been reported to significantly reduce blood plasma uric acid concentrations [10]. God’s crown (Phaleria macrocarpa Berl.) is a native plant from Papua, Indonesia, the fruit of which has been used traditionally for a number of medicinal uses, including the treatment of kidney disease [11]. Snake fruit (Salacca edulis Reimw.) peel is traditionally used to reduce uric acid levels and has been shown to reduce uric acid levels in the Wistar rat model [12]. The aim of the current study was to investigate the potential of these ethnopharmacological plants to reduce uric acid levels through inhibition of the xanthine oxidase pathway.

MATERIALS AND METHODS

Plant material
Indian bay leaf (S. polyanthum) and God’s crown fruit (P. macrocarpa) were obtained from a local market in West Java, Indonesia. Fruit of snake fruit (S. edulis) was obtained from Magelang, Indonesia and tubers of Job’s tears (C. rotundus) were obtained from Bogor, Indonesia. Each of the samples was authenticated by Herbarium Bogoriense. The samples were cleaned, impurities were removed, and each sample was
then dried at 60°C. The dried samples were powdered and stored in desiccator cabinets at room temperature until further analysis.

**Preparation of extract**

The preparation of extract was performed based on Yanti et al. with a number of modifications [13]. Each sample powder (1 kg) was extracted using the maceration method with 80% (v/v) methanol as the solvent. The powder was allowed to extract in the methanol for approximately 24 h, before being filtered. The solid was re-extracted 3 more times, and the filtrates obtained were combined and the pooled filtrate was then evaporated to dryness using a rotary vacuum evaporator at 40°C. This crude extract was stored at −20°C before use for further analysis.

**Procedure of fractionation**

The extract obtained was dissolved in 200 ml dH2O, and then fractionated by liquid-liquid partitioning (1:1 v/v), using a sequence of solvents, from non-polar to polar, namely, n-hexane, ethyl acetate, butanol, and methanol. Partitioning was carried out 3 times in a separating funnel, each for 1 h. The fractions from one extract were combined and dried with a rotary vacuum evaporator to produce a concentrated extract fraction [14].

**Identification of glycosides**

Identification of glycosides was performed using Molisch’s test methods. A sample (500 mg) of the fraction was put in a test tube, to which was added 10 ml of hot distilled water, and the tube was shaken vigorously for 10 s. The formation of red color at point of the addition of 1 ml of 2N HCl indicated the presence of glycosides [17].

**Identification of saponins**

Identification of saponins was carried out by the simple froth test method. A sample (500 mg) of the extract fraction was put in a test tube, to which was added 10 ml of hot distilled water, and the tube was shaken vigorously for 5 min and that did not disappear with the addition of 1 ml of 2N HCl, indicated the presence of saponin compounds [17].

**Identification of flavonoids**

Flavonoid identification was performed using Shinoda test methods. A sample (500 mg) of the fraction was dissolved in 2 ml of ethanol, to which was added 500 mg of Zn powder and 2 ml 2N HCl, following which the reaction mixture was allowed to stand for 1 min. Following the addition of 10 ml concentrated HCl, the appearance of a red color after 2–5 min incubation indicated the presence of flavonoids. The same method was used with 100 mg of Mg powder replacing the Zn powder to identify the presence of flavones, chalcones, and aurones. The appearance of a yellow to orange color indicated the presence of compounds of flavones, chalcones, or aurones [16].

**Identification of tannins**

A sample (200 mg) of the fraction was dissolved in 5 ml of hot distilled water, to which was added 10% NaCl, and the solution was filtered. The filtrate (TS) was then tested using the gelatin test and FeCl3 test methods. To 1 ml of TS was added 3 ml 10% gelatin solution, with the formation of a white precipitate, indicating the presence of tannins. The ferric chloride test was performed by added 2 ml 3% FeCl3 to 1 ml of TS, with a change in coloration to violet-green indicating that the sample contained tannins [17].

**Identification of terpenes**

A sample (200 mg) of the fraction was dissolved in a solution of concentrated acetic acid:concentrated sulfuric acid (2:1 v/v), at which point the formation of a greenish-red or violet-blue color indicated the presence of terpenes. To confirm the result, the procedure continued by spraying a sample with a solution of p-anisaldehyde in concentrated sulfuric acid, when dark-blue, green, red, or brown fluorescence at 366 nm ultraviolet light indicated the presence of terpenes [17].

**RESULTS AND DISCUSSION**

**Xanthine oxidase inhibitory activity**

The data obtained showed that the ethyl acetate fraction from each sample showed the highest inhibitory activity against xanthine oxidase. Phytochemical screening was performed for a number of chemical groups, namely, alkaloids, glycosides, saponins, flavonoids, tannins, and terpenes.

**Identification of alkaloids**

A sample (300 mg) of extract fraction was dissolved in 5 ml of 0.2 N aqueous HCl, and then heated for 2 min. The mixture was filtered, and the filtrate was used as the test solution (TS). Each 1 ml aliquot of TS was reacted with 2 ml of various test reagents. Bouchardat’s reagent resulted in a dark-brown precipitate in the presence of alkaloids, whereas Mayer’s reagent resulted in a white precipitate for a positive reaction, and Dragendorff’s test showed a reddish-orange precipitate as a positive result [16].

**Identification of flavonoids**

A sample (300 mg) was dissolved in 15 ml of 1% HCl, then filtered. The filtrate obtained was washed with ether 3 times, and the resulting filtrate was evaporated to dryness at 40°C. To the filtrate were then added 2 ml methanol, this solution being used as the TS. An aliquot (1 ml) of TS was evaporated to dryness and dissolved in 20 ml concentrated acetic acid and 1 ml concentrated sulfuric acid. A green or blue color indicated the presence of glycosides. Identification with Molisch’s test was performed by dissolving the evaporated TS in 2 ml dH2O and 5 ml Molisch’s reagent. To the solution, 2 ml sulfuric acid was added carefully. The formation of a reddish-purple colored ring at the junction between the two layers indicated the presence of glycosides [17].

**Identification of saponins**

Identification of saponins was carried out by the simple froth test method. A sample (500 mg) of the extract fraction was put in a test tube, to which was added 10 ml of hot distilled water, and the tube was shaken vigorously for 10 s. The formation of red color after 2–5 min incubation indicated the presence of flavonoids. The same method was used with 100 mg of Mg powder replacing the Zn powder to identify the presence of flavones, chalcones, and aurones. The appearance of a yellow to orange color indicated the presence of compounds of flavones, chalcones, or aurones [16].

**Identification of tannins**

A sample (200 mg) of the fraction was dissolved in 5 ml of hot distilled water, to which was added 10% NaCl, and the solution was filtered. The filtrate (TS) was then tested using the gelatin test and FeCl3 test methods. To 1 ml of TS was added 3 ml 10% gelatin solution, with the formation of a white precipitate, indicating the presence of tannins. The ferric chloride test was performed by added 2 ml 3% FeCl3 to 1 ml of TS, with a change in coloration to violet-green indicating that the sample contained tannins [17].

**Identification of terpenes**

A sample (200 mg) of the fraction was dissolved in a solution of concentrated acetic acid:concentrated sulfuric acid (2:1 v/v), at which point the formation of a greenish-red or violet-blue color indicated the presence of terpenes. To confirm the result, the procedure continued by spraying a sample with a solution of p-anisaldehyde in concentrated sulfuric acid, when dark-blue, green, red, or brown fluorescence at 366 nm ultraviolet light indicated the presence of terpenes [17].

**RESULTS AND DISCUSSION**

Xanthine oxidase inhibitory activity

The data obtained showed that the ethyl acetate fraction from each sample showed the highest inhibitory activity against xanthine oxidase.
Terpene−Glycoside

Fig 1, leaf methanolic extract, including α-pinene, linalool, and terpenes. Table 1, all of the ethyl acetate fractions contained flavonoids and tannins [24,25]. The Abd Rahim et al. [22], although this tentative identification requires confirmation. The presence of flavonoid compounds in the C. rotundus fraction, which we identified, was also reported by Kilani et al. [23]. They described that the ethyl acetate fraction of C. rotundus contained flavonoid compounds such as asfalechin, catechin, quercetin, and luteolin.

We reported that the ethyl acetate fraction of S. edulis contained a number of phytochemical constituents such as glycosides, flavonoids, and tannins (Table 1). Afrani et al. discovered that the ethyl acetate fraction of S. edulis contained a number of phytochemical compounds such as 3-hydroxyxystigman-5(6)-en and pyrrole-2,4-dicarboxylic acid, methyl ester [24], whereas another phytochemical screening study also reported that S. edulis contained flavonoids and tannins [24,25]. The positive results for glycosides detected in this current research are expected to be associated with the high sugar content in S. edulis.

Based on Table 1, the phytochemical compounds present in the ethyl acetate fraction of P. macrocarpa include glycosides, saponins, flavonoids, tannins, and terpenes. These data are similar to those in an article reviewing data reported by Alara et al. The positive results for glycosides, saponins, and terpenes could have been due to the presence of fevicordin in P. macrocarpa, which is classified as terpenoid. In the form of sugar conjugates, fevicordin would give positive results for glycoside as well as saponin. It was also reported that P. macrocarpa contains flavonoids, tannins, and gallic acid [26].

CONCLUSION
Various plants used in ethnomedicine have been scientifically proven to have beneficial effects in treating hyperuricemia through a competitive inhibitory mechanism against xanthine oxidase. In the current study, C. rotundus tuber was the herbal medicinal plant with the greatest potential for development as a hyperuricemia treatment by inhibiting xanthine oxidase (IC₅₀= 0.50 μg/ml).

ACKNOWLEDGMENTS
This study was supported by the Directorate of Research and Community Engagement Universitas Indonesia through Hibah PITTA 2017. that are

REFERENCES

The 4th International Conference on Global Health 2019

<table>
<thead>
<tr>
<th>Samples</th>
<th>Phytocompounds</th>
<th>Saponin</th>
<th>Flavonoid</th>
<th>Tannin</th>
<th>Terpene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syzygium polyanthum</td>
<td>Alkaloid: +, Glycoside: +, Saponin: +, Flavonoid: +, Tannin: +, Terpene: +</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyperus rotundus</td>
<td>Alkaloid: +, Glycoside: +, Saponin: +, Flavonoid: +, Tannin: +, Terpene: +</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salacca edulis</td>
<td>Alkaloid: +, Glycoside: +, Saponin: +, Flavonoid: +, Tannin: +, Terpene: +</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phaleria macrocarpa</td>
<td>Alkaloid: +, Glycoside: +, Saponin: +, Flavonoid: +, Tannin: +, Terpene: +</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(+ present, - not detected)