IN VITRO EVALUATION OF XANTHINE OXIDASE INHIBITORY ACTIVITY OF SONCHUS ARvensis LEAVES

RINI HENDRIANI1,2, ELIN YULINAH SUKANDAR3, KUSNANDARANGGADIREDJA4, SUKASNO5

1School of Pharmacy, Universitas Padjadjaran, Sumedang, Jatinangor, Indonesia; 2Faculty of Pharmacy, Universitas Padjadjaran, Jalan By Bandung-Sumedang, Jatinangor. Email: rhendriani@yahoo.com

Received: 01 Feb 2014, Revised and Accepted: 12 Mar 2014

ABSTRACT

Objective: This study examined the inhibitory effects of different extracts of S. arvensis leaves on the activity of xanthine oxidase, an essential enzyme for uric acid synthesis.

Methods: Activity test was conducted in vitro by measuring the activity of xanthine oxidase using UV spectrophotometry.

Results: The IC50 of ethanolic extract of S. arvensis leaves was 23.64 μg/mL. Meanwhile, the n-hexane, ethylacetate and water fraction of the extract had respective IC50 of 26.319, 16.20 and 141.80 μg/mL. When the leaves were extracted with ethyl acetate, the extract thus obtained showed an IC50 of 15.29 μg/mL. Allopurinol, as reference drug, had IC50 of 4.84 μg/mL.

Conclusion: Ethyl acetate extract of S. arvensis leaves potential to be developed as agent to treat hyperuricemia and gout.

Keywords: Sonchus arvensis, Gout, Xanthine oxidase, Inhibitory effect, In vitro.

INTRODUCTION

Gout is a painful inflammatory arthritis which can lead to decrease in quality of life [1]. The disease occurs when body fluids are saturated due to high levels of uric acid that eventually settles in the joints. Gout could be overcome by lowering the levels of uric acid in the blood to normal limits. Uricosurics and uricosuric therapies are drugs commonly used for this purpose. Uricosuric drugs work by inhibiting the excretion of uric acid from the kidney as in the case of probenecid, and so that the removal of uric acid through the kidneys can be improved. Uricostatic drugs inhibit the work of xanthine oxidase enzyme that converts hypoxanthine into xanthine and xanthine into uric acid, thus reducing uric acid production. Uric acid is the final product of purine metabolism in human body, that exits the body through urinary excretion. This substance has very low solubility and tend to form crystals. Uric acid accumulated in the joints commonly found in the form of monosodium uric crystals, that induce inflammatory reactions [2,3]. A representative drug widely used to treat hyperuricemia and gout is allopurinol. Allopurinol, which works by preventing the formation of uric acid through the inhibition of the enzyme xanthine oxidase, is particularly useful in dealing with chronic gout characterized by tophi formed by deposits of uric acid crystals in the joints, kidneys, and soft tissue. The tophaceous bodies will eventually impaired renal function [4]. Apart from its efficacy, allopurinol can cause hazardous side effects such as nephropathy, allergic reactions and increase in toxicity of 6-mercaptopurine [5]. Furthermore, the drug can also induce hapatotoxic and allergic reactions [6]. This fact warrants the search for new alternatives to lower uric acid with minimal side effects. Many has now turned to plant-derived substances as the source of medication. This research was conducted in an attempt to develop substances with xanthine oxidase inhibiting activity derived from the plant Sonchus arvensis. This plant has been traditionally used in Indonesia as gout remedy [7]. In the previous studies the species of sonchus has been known for its diverse activities and has been used in relieving disorders such as hepatotoxicity [8], nephrotoxicity [9], cardiotoxicity [10], asthma [11], and oxidative stress [12]. As to its effect on xanthine oxidase, however, no in depth studies has been conducted.

MATERIALS AND METHODS

Plant materials

S. arvensis leaves, was collected during the period of December 2012 through March 2013 from the botanical garden of Manoko in Lembang, West Java, Indonesia. The plant materials were authenticated at the Herbarium Bandungense, the School of Life Sciences and Technology, Bandung Institute of Technology.

Plant Extraction

This study used the cold maceration method for extraction, with 96% ethanol and ethyl acetaete as extracting solvents for the dried ground plant materials. The macerated was collected once everyday, followed by soaking the residue with the same solvent system. This procedure was repeated for three consecutive days. The macerate thus accumulated was concentrated using rotary evaporator under reduced pressure. This was followed by fractionation of the ethanolic extract using n-hexane, ethyl acetate and water.

Xanthine oxidase inhibitory assay

Activity test was conducted in vitro by measuring the activity of the enzyme xanthine oxidase using UV spectrophotometry [6,13,14,15,16,17] with the slight modification. Xanthine oxidase enzyme from bovine milk was prepared by dilution of the enzyme to a final concentration 2 Units/mL. 1 mM xanthine substrate solution was made by adding 5 drops of 1.0 M NaOH to increase the solubility of xanthine. Ethanol and ethyl acetate extracts and also fractions of ethanol extract were dissolved in 1% dimethyl sulfoxide (DMSO) and made the test concentration at 50, 100 and 200 mg/mL. Allopurinol used as positive control.

Total volume of the assay mixture was 3.2 mL consisting of 1 mL sample test plant studied at various concentration, 1 mL of 0.15 M phosphate buffer (pH 7.8), 100 μL of solution of the enzyme xanthine oxidase. After preincubation of the test solution at 37 °C for 15 min, the reaction was initiated by addition of 100 μL of xanthine substrate solution and incubated at 37 °C for 30 min.

The reaction was stopped by adding 1 mL of 1N HCL Spectrophotometer absorbance at 295 nm, suggesting the formation of uric acid. Percent of inhibition of xanthine oxidase activity of the test sample was determined by measuring the absorbance of uric acid from the mixture without test extracts (blank samples) compared with the absorbance of a mixture of test extracts. IC50 values were obtained by linear regression analysis of a plot of a series of different sample concentrations against percent inhibition.

RESULTS

As shown in Table 1, Results of inhibitory test on xanthine oxidase activity showed that the ethanolic extract of S. arvensis leaves had IC50 of 23.64 μg/mL, and the ethyl acetate extract gave an IC50 value of...
15.29 µg/mL. Meanwhile, the fractions of n-hexane, ethylacetate and water of ethanolic extract had the respective IC50 of 623.19, 16.20 and 141.80 µg/mL. Table 1 further shows that, used as reference drug, allopurinol had IC50 value of 4.84 µg/mL.

Table 1: It shows that xanthine oxidase inhibitory activity of Sonchus Arvensis leaves. The leaves were firstly extracted with ethanol and ethyl acetate. The ethanic extract was further fractionated with n-hexane, ethyl acetate and water. All extracts and fractions were tested for their xanthine oxidase inhibitory activity using UV spectrophotometry.

<table>
<thead>
<tr>
<th>Plant material</th>
<th>Concentration (µg/mL)</th>
<th>IC50</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>Ethyl acetate extract</td>
<td>54.07 ± 14.65</td>
<td>72.25 ± 17.43</td>
<td>86.75 ± 18.51</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>47.37 ± 7.08</td>
<td>82.18 ± 8.86</td>
<td>88.45 ± 14.65</td>
</tr>
<tr>
<td>Ethyl acetate fraction of Ethanol extract</td>
<td>52.06 ± 14.79</td>
<td>72.80 ± 17.82</td>
<td>83.16 ± 17.91</td>
</tr>
<tr>
<td>n-Hexane fraction of Ethanol extract</td>
<td>8.19 ± 1.71</td>
<td>15.46 ± 5.61</td>
<td>37.84 ± 10.97</td>
</tr>
<tr>
<td>Water fraction of Ethanol extract</td>
<td>17.82 ± 7.59</td>
<td>28.20 ± 6.10</td>
<td>74.55 ± 12.64</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>59.14 ± 3.26</td>
<td>71.38 ± 7.01</td>
<td>91.75 ± 11.99</td>
</tr>
</tbody>
</table>

p values were obtained from comparison of treatment groups to Allopurinol.

Results of phytochemical screening, as shown in Table 2, demonstrated that the drug contained flavonoid and steroids/triterpenoids.

Table 2: It shows results of phytochemical screening of Sonchus arvensis leaves and its ethanolic and ethyl acetate extracts.

<table>
<thead>
<tr>
<th>Chemical Class</th>
<th>Ground Leaves</th>
<th>Ethanolic extract</th>
<th>Ethyl acetate extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids/Triterpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

DISCUSSION

The present study was carried out to investigate the xanthine oxidase inhibitory activity of S. arvensis leaves extracts and fractions in search for substances that might have potential as alternatives to treat hyperuricemia and gout.

Extraction was done on ground leaves of S. arvensis with cold maceration followed by low pressure evaporation at minimal heating to preserve active substances contained, which are mostly phenolic and flavonoid such as kaempferol, quercetin, orientin, rutin, hyperoside, catechin and myricetin [18]. Flavonoids have been known to have blood uric acid lowering activity through inhibition of xanthine oxidase. Kaempferol is a xanthine oxidase inhibitor without any additional superoxide scavenging activity, while quercetin and myricetin are inhibitors with additional superoxide scavenging activity [19].

The synthesis of uric acid in the oxidative pathway occurs via the conversion of hypoxanthine into xanthine, catalyzed by the enzymes xanthine oxidase and guanase. This is followed by oxidation of xanthine into uric acid, which is also catalyzed by xanthine oxidase. Inhibition of xanthine oxidase is thus essential as pharmacological intervention for hyperuricemia and gout [2].

Results showed that the ethyl acetate fraction gave the highest inhibition among the fractions tested. However, ethyl acetate extract obtained from direct extraction of the leaves was shown to be superior in terms of activity. These findings indicated that more bioactive components were extracted with direct extraction with ethyl acetate. Many biological activities exerted by plant extracts have been associated with flavonoid content, known for effective reactive oxygen scavenging activity [18]. As shown by the result of phytochemical screening, the crude drug ethanolic as well as ethyl acetate extract showed positive results for flavonoid. Further study is needed to investigate whether flavonoid is the one responsible for the xanthine oxidase-inhibiting activity.

CONCLUSION

S. arvensis under investigation exhibit xanthine oxidase inhibitory activity. Results of present study suggest that ethyl acetate extract of S. arvensis leaves has the most potent activity in inhibiting xanthine oxidase activity, indicating its potential to be developed as agent for treating hyperuricemia and gout.

ACKNOWLEDGEMENT

We gratefully acknowledge the financial assistance received from Bandung Institute of Technology through research grant funding scheme to carry out this study.

REFERENCES

5. Zhao X, Zhu JX, Mo SF, Pan Y, Kong L.D. Effects of cassia oil on serum and hepatic uric acid levels in o xo nate-induced mice and xanthine dehydrogenase and xanthine oxidase activities in mouse liver. JEthnopharmacol. 2006; 103(3): 357-65
11. Khan RA, Khan MR, Sabreen S. Protective effect of Sonchus asper extracts against experimentally-induced lung injuries in...


