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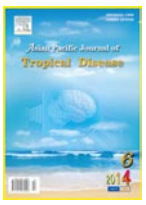

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Xanthine oxidase inhibitory activity of some Leguminosae plants

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PEER REVIEW

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Comments

This is an interesting report on ethnopharmacological study that can be further implied for practical use in medical practice. There are not much related reports. Hence, the work is acceptable in concept and the work remains originality.
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ABSTRACT

Objective: To evaluate the xanthine oxidase inhibitory activity of the methanol leaf extracts of following *Cassia javanica*, *Cynometra ramiflora*, *Cassia fistula*, *Senna siamea*, *Tamarindus indicus*, *Intsia bijuga*, *Cassia spectabilis*, *Saraca thaipingensis* (*S. thaipingensis*), *Caesalpinia pulcherrima* (*C. pulcherrima*) and *Bauhinia purpurea*.

Method: The xanthine oxidase inhibitory activity was tested spectrophotometically under aerobic conditions. Absorption increments was monitored every 30 seconds for 10 min at 295 nm indicating the formation of uric acid. The extracts were also tested for the presence of terpenoids, saponins, tannins, flavonoids, steroids, phenolic compounds, alkaloids and cardiac glycosides.

Results: All the extracts inhibited the action of xanthine oxidase. *S. thaipingensis* and *C. pulcherrima* exhibited higher than 50% at a concentration 0.05 mg/mL. The IC_{50} of *S. thaipingensis* and *C. pulcherrima* were determined as 0.033 mg/ml and 0.053 mg/mL, respectively while that of allopurinol is 0.004 mg/mL.

Conclusion: *S. thaipingensis* and *C. pulcherrima* are potentially good sources of xanthine oxidase inhibitors.

KEYWORDS

Gout, Hyperurecemia, Medicinal plants, Plant extracts

1. Introduction

Gout is an inflammatory arthritis resulting from the deposition of monosodium urate crystals in joints and other tissues[1]. Urate-lowering treatment is needed since mammals cannot further metabolize uric acid due to the absence of uricase[2]. Increase uric acid concentration is associated with hyperuricemia; however, not all patients with hyperuricemia have gout[3]. The estimated number of people suffering from gout might be an underrepresentation since the condition is underdiagnosed[4]. It has a higher prevalence in men older than 30 years old and in women older than 50 years old[5].

Relief of pain and disability is the main therapeutic goal for gout. Urate-lowering agents include xanthine oxidase inhibitors[6], uricosuric agents[7] and uricase agents[8]. Xanthine oxidase catalyzes the oxidation of hypoxathine to xanthine and of xanthine to uric acid. Thus, inhibitors block the synthesis of uric acid. Allopurinol is the most commonly prescribed xanthine oxidase inhibitor[9]. It alleviates symptoms in most patients; however, it is poorly tolerated in some and leads to hypersensitivity reactions[10].

Plant extracts have been evaluated as potential sources of xanthine oxidase inhibitors. Several plants from

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Australia[11], China[12], Czech Republic[13], and North America[14], have shown inhibitory effects on xanthine oxidase. In our previous efforts, a non-competitive inhibitor of xanthine oxidase was isolated and identified from a Leguminosae plant. In this study, we evaluated the xanthine oxidase inhibitory activity of several plants belonging to the same family. To the best of our knowledge *Cassia javanica* (*C. javanica*), *Cynometra ramiflora* (*C. ramiflora*), *Cassia fistula* (*C. fistula*), *Senna siamea* (*S. siamea*), *Tamarindus indicus* (*T. indicus*), *Intsia bijuga* (*I. bijuga*), *Cassia spectabilis* (*C. spectabilis*), *Saraca thaipingensis* (*S. thaipingensis*), *Caesalpinia pulcherrima* (*C. pulcherrima*) and *Bauhinia purpurea* (*B. purpurea*) have not been evaluated for their xanthine oxidase inhibitory activity.

2. Materials and methods

2.1. Collection of samples

C. javanica, *C. ramiflora*, *C. fistula*, *S. siamea*, *T. indicus*, *I. bijuga*, *C. spectabilis*, *S. thaipingensis*, *C. pulcherrima* and *B. purpurea* leaves were collected and submitted to the University of the Philippines Herbarium for identification and authentication.

2.2. Crude extraction

The plant samples were washed with running water and allowed to drip dry. The dried samples were weighed then homogenized for overnight soaking in methanol using clean glass jars. The extracts were filtered using a cotton plug and concentrated *in vacuo* using a rotary evaporator (Heidolph) to yield the methanolic extract.

2.3. Solvent extraction

The solvents used for extraction and fractionation were single distilled, technical grade reagents.

2.4. Assay

The xanthine oxidase activity measured spectrophotometrically using the procedure of Owens and Johns with the following modifications[14]. The positive control, allopurinol (Sigma A8003) solution, was prepared by dissolving 5.0 mg in 5.0 mL of 0.15 mol/L phosphate buffer (pH 7.5). Xanthine oxidase from bovine milk was purchased from Sigma (Sigma X4500). The enzyme solution was prepared by diluting 30 μL of a 5.0 U/0.2 mL xanthine oxidase solution to a final volume of 3.0 mL. The substrate was prepared by adding 5 drops of 1.0 mol/L NaOH to 22.7 mg of xanthine (Sigma X7375) to aid its dissolution with deionized water to a final volume of 250 mL. The plant extracts were dissolved in 1% dimethyl sulfoxide to a final concentration of 1 mg/mL. All solutions were prepared immediately before use.

Total volume of the assay mixture was 3.4 mL with the plant extract under study (1.0 mg/mL), 0.15 mol/L phosphate buffer (pH 7.5) and 100 μL of 0.03 U/mL xanthine oxidase enzyme solution. After preincubation of the test solution at 25 °C for 10 min, the reaction was initiated by addition of 1 mL of 0.6 mmol/L substrate solution of xanthine. The reaction was monitored by reading absorption (Shimadzu UV-1700 series spectrophotometer) increments of 30 seconds for 10 min at 295 nm indicating the formation of uric acid. Final concentration of allopurinol was 30 μg/mL. The percent xanthine oxidase inhibitory activity of the assayed samples was determined through the slope of the plot of absorbance against time (seconds). IC_{50} values were obtained through linear regression analysis of the plot of concentration (0.05-0.30 mg/mL) against percent inhibition.

2.5. Phytochemical screening

Phytochemical screening on the crude methanolic plant extracts was done to correlate the presence of secondary metabolites with the inhibition of xanthine oxidase, if any, of the extracts. The analysis was done using modified methods employed by Edeoga and Harborne[15,16].

2.6. Data analysis

From the absorbance versus time plot, the slope which represented the enzyme activity was obtained. The percent xanthine oxidase inhibitory was then computed using the equation below:

$$\text{Activity}=\frac{\left(\frac{\Delta A1}{\Delta t}\right)-\left(\frac{\Delta A2}{\Delta t}\right)}{\left(\frac{\Delta A1}{\Delta t}\right)}\times 100$$

where $\frac{\Delta A1}{\Delta t}$ and $\frac{\Delta A2}{\Delta t}$ are the slopes of the solvent control and test samples respectively.

3. Results

The methanol extracts were evaluated for their xanthine oxidase inhibitory activity as shown in Figure 1. All extracts showed significant inhibition with *S. thaipingensis* exhibiting the highest activity at 66.7%. The methanol extracts were then qualitatively tested for the various phytochemicals they contained. *S. thaipingensis* and *C. pulcherrima* tested positive for flavonoids, tannins, terpenoids, phenolics, saponins and steroids.

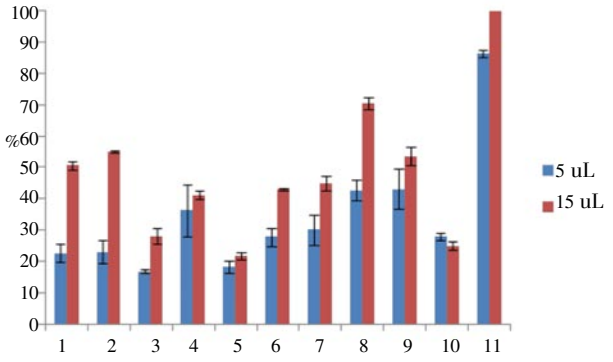

Figure 1. Percent inhibition of the different samples.
All samples were prepared in 1mg/mL concentration. (n=5). 1: *C. javanica*, 2: *C. ramiflora*, 3: *C. fistula*, 4: *S. siamea*, 5: *T. indica*, 6: *I. bijuga*, 7: *C. spectabilis*, 8: *S. thaipengensis*, 9: *C. pulcherrima*, 10: *B. purpurea*, 11: Allopurinol.

4. Discussion

Allopurinol is rapidly oxidized by xanthine oxidase *in vivo* to its active metabolite oxypurinol, which also inhibits xanthine oxidase. However, it induces serious side effects such as renal failure, impaired liver function and allergic reactions[17]. Hence, there is a need to look for new xanthine oxidase inhibitors. Evaluation of ten Leguminosae plants revealed varying activities of xanthine oxidase inhibition. All extracts showed significant inhibition with *S. thaipingensis* exhibiting the highest activity at 66.7% followed by *C. pulcherrima* with an activity of 57.9% at concentrations of 0.05 mg/mL. The results showed consistent dose-dependence effect on percent inhibition; all samples at 0.0167 mg/mL concentration exhibited lower activity than those samples tested at 0.05 mg/mL. Plants with higher than 50% inhibition activity were further tested and their IC_{50} were determined. The IC_{50} of *S. thaipingensis* and *C. pulcherrima* were 0.033 mg/mL and 0.053 mg/mL, respectively; while that of allopurinol is 0.004 mg/mL. It is possible that isolation of the bioactive compound from *S. thaipingensis* and *C. pulcherrima* would exhibit lower IC_{50}.

Flavonoids were found to inhibit xanthine oxidase activity[18-20]. Natural and synthetic derivatives have been tested to investigate new sources of inhibitors[21-23]. Xanthine oxidase converts xanthine to uric by transferring an oxygen atom to xanthine from the enzyme's molybdenum center. Flavonoids work by competitively binding at the binding site of xanthine[24]. *S. thaipingensis* and *C. pulcherrima* tested positive for the presence of flavonoids and it is possible that these secondary metabolites are responsible for the observed bioactivity. This is the initial report of xanthine oxidase inhibitory action for both plants.

To the best of our knowledge, this is the first report of the evaluation of the xanthine oxidase inhibitory activity of the tested medicinal plant extracts. We have proven that other Leguminosae plants are also potential sources of xanthine oxidase inhibitory agents. Future work will be done to isolate and identify the structure of the compounds with potential xanthine oxidase inhibitory activity.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

This is an interesting report on ethnopharmacological study that can be further implied for practical use in medical practice. At least, it can be applied into the tropical Ayurveda practice.

Research frontiers

This work is a good model in ethnopharmacology study. Although it does not directly contribute to tropical biomedicine but it partial involves since the work studies on the local plant in tropical area.

Related reports

There are not much related reports. Hence, the work is acceptable in concept and the work remains original. The admiration should be given to the authors since the work makes use of local wisdom that can further help reduce cost

in public health for management of the important metabolic disease, gout.

Innovations & breakthroughs

The further concern is to elaborate the possible economical usefulness of the new plant regimen in pharmaceutical scale.

Applications

As noted, the work can be further applied in pharmaceutical science. The application can be expected but it needs further validation and further study to confirm the effectiveness, efficacy and safety of the developed ethnopharmacological regimens.

Peer review

This is an interesting report on ethnopharmacological study that can be further implied for practical use in medical practice. There are not much related reports. Hence, the work is acceptable in concept and the work is original.

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