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Xanthine Oxidase Inhibitory Activity of Some Malaysian Plants

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ABSTRACT Inhibition of xanthine oxidase (XO) is an effective therapeutic approach for treating hyperuricemia that causes gout. Methanol extracts of nine Malaysian medicinal plants, namely *Blumea balsamifera, Orthosiphon stamineus*, *Alyxia lucida*, *Andrographis paniculata, Eurycoma longifolia, Ardisia crispa, Smilax myosotiflora, Zebrina pendula* and *Tinospora crispa* were assayed for XO inhibitory activity. The enzymatic activity was estimated by measuring the increase in absorbance at 292 nm due to uric acid formation. Allopurinol, a known inhibitor of xanthine oxidase, was used to validate the method and was adopted as positive control in the studies. The accuracy of the enzymatic assay method was found to be satisfactory (CV < 12.25 %) and IC_{50} of allopurinol was 0.022 μg/ml. Of the plants tested, five were found to have more than 25 % inhibition at a concentration of 100 μg/ml in the assay mixture. *Blumea balsamifera* exhibited the highest activity (IC_{50} = 1.15 μg/ml). The IC_{50} values of *O. stamineus*, *A. lucida*, *A. paniculata* and *T. crispa* were 30.79, 63.19, 230.18 and 370.35 μg/ml respectively.

Keywords: xanthine oxidase, allopurinol, Malaysian medicinal plants

ABSTRAK Perencatan xanthin oksidase adalah pendekatan terapeutik yang berkesan untuk merawat hiperuricemia yang mengakibatkan penyakit gout. Ekstrak metanol dari sembilan tumbuhan ubatan Malaysia iaitu, *Blumea balsamifera, Orthosiphon stamineus*, *Alyxia lucida*, *Andrographis paniculata, Eurycoma longifolia, Ardisia crispa, Smilax myosotiflora, Zebrina pendula* dan *Tinospora crispa* telah dikaji kesan perencatan terhadap xanthin oksidase. Aktiviti enzim ditentukan dengan mengukur peningkatan penyerapan pada 292nm akibat pembentukan asid urik. Allopurinol, suatu perencat xanthin oksidase telah digunakan bagi validasi kaedah dan digunakan sebagai kawalan positif dalam kajian ini. Ketepatan kaedah enzimatik ini didapati memuaskan(CV < 12.25 %) dan IC_{50} bagi allopurinol adalah 0.022 μg/ml. Dari tumbuhan ubatan yang dikaji, lima memberi perencatan lebih dari 25 % pada kepekatan 100 μg/ml. *Blumea balsamifera* menunjukan aktiviti tertinggi (IC_{50} = 1.15 μg/ml). Nilai IC_{50} bagi *O. stamineus*, *A. lucida*, *A. paniculata* dan *T. crispa* adalah 30.79, 63.19, 230.18 dan 370.35 μg/ml masing-masing.

INTRODUCTION

Hyperuricemia, associated with gout, results from the overproduction or under excretion of uric acid and is greatly influenced by a high dietary intake of food rich in nucleic acids. The catalysis of xanthine by the xanthine oxidase (XO) (EC 1.2.3.2) can lead to the accumulation of uric acid, and ultimately causes gout [1]. Accordingly, one of the therapeutic approaches to treat gout is the use of XO inhibitors that block the production of uric acid [2]. Allopurinol, the sole XO inhibitor prescribed for chronic gout, acts as a substrate for the competitive inhibition of the enzyme, but at higher concentrations, is a noncompetitive inhibitor [3]. However, this drug gives inevitably severe adverse effects such as hepatitis, nephropathy and allergic reactions [2]. Therefore, there is a need to search for new XO inhibitors. Plants have been used by indigenous people for the treatment of gout, or diseases with associated symptomologies such as rheumatism or arthritis, and they may contain XO inhibitors [4-5]. The objective of this study is therefore to determine the validity of some local plant remedies used for gout or diseases with associated symptomologies by examining their xanthine oxidase inhibitory activity.

MATERIALS AND METHODS

Plant Material

Alyxia lucida, Ardisia crispa and *Smilax myosotiflora* were obtained from Alor Setar, Kedah. *Orthosiphon stamineus* was obtained from Kepala Batas, Penang. *Blumea balsamifera* was collected from Botanical Garden, Penang. *Andrographis paniculata* was obtained from Malacca. *Eurycoma longifolia, Tinospora crispa* and *Zebrina pendula* were obtained from Kuala Lumpur.

Extraction

Air-dried plants samples were ground and solvent extracted at 45° C in methanol, for five to seven days. The extracts were then filtered and the filtrate concentrated to dryness *in vacuo*.

Enzyme and Chemicals

Xanthine oxidase, from buttermilk, and xanthine were purchased from Sigma (USA). Dimethyl sulphoxide (DMSO) was purchased from Riedel-de Haen, Germany. All other reagents used were of analytical grade. The buffer used was 50 mM potassium phosphate buffer, pH 7.8. The substrate solution, 0.15 mM xanthine in water was prepared immediately before use. Enzyme solution containing 0.04 units per ml in 50 mM phosphate buffer, pH 7.8 was prepared immediately before use.

Test Solution

The test samples were dissolved in DMSO and diluted with phosphate buffer, pH 7.8 to give final concentration of 1 % in the assay mixture. Extracts were initially screened for XO at 100 μg/ml in the assay mixture. For the IC_{50} determination, the extracts were examined at six concentrations.

Assay of Xanthine Oxidase Activity

The XO activities with xanthine as the substrate were measured spectrophotometrically by method of Noro [6] with modification. The assay mixture consisted of 0.1 ml of test solution, 2.9 ml of 50 mM phosphate buffer (pH 7.8) and 0.1 ml of enzyme solution. After preincubation of the mixture at 37° C for 10 min, the reaction was initiated by adding 2.0 ml of the substrate solution. This assay mixture was incubated at 37° C for 30 min. The reaction was stopped by adding 1.0 ml of 1 M HCl, and the absorbance of the assay mixture was measured at 292 nm. A blank was prepared in the same way, but the enzyme solution was added to the assay mixture after adding 1 N HCl.

Estimation of Xanthine Oxidase Inhibitory Activity

XO inhibitory activity was expressed as the percentage inhibition of XO in the above assay system, calculated as $(1-B/A) \times 100$, where A is the activity of the enzyme without test material and B is the activity of the enzyme with test material.

Assay Validation

Allopurinol, a known inhibitor of xanthine oxidase, was used to validate the method and was adopted as positive control in the studies. Six concentrations of allopurinol (0.00625-0.2 μg/ml) were used to determine the between-day and within-day accuracy (n=6). Each test was carried out in triplicate.

RESULTS AND DISCUSSION

The between-day and within-day accuracies of the enzymatic assay method are shown in Table 1 and Table 2. The coefficient of variation (C.V.) of both within-day and between-day accuracy was found to be satisfactory with values less than 12.25 %. The IC_{50} of allopurinol was found to be 0.022 μg/ml (0.162 μM) (Figure 1). This value is comparable to the reported values from literature [7-10].

For all the nine extracts tested, their IC_{50} are shown in Table 3. Two plants, namely *B. balsamifera* and *O. stamineus* exhibited IC_{50} values less than 50 μg/ml. It has been reported that extracts causing more than 50 % enzyme inhibition at a test concentration of 50 μg/ml warranted further investigation [11]. The most active of the plant examined was *B.balsamifera* with IC_{50} of 1.15 μg/ml. *B. balsamifera* or locally known as 'daun capa' or 'telinga kerbau' from the Compositae family has been reported to be used to treat diseases such as rheumatism or arthritis [4,5,12]. *O. stamineus* on the other hand, also has been reported to treat rheumatism in Indonesia and Vietnam [13]. This study has demonstrated that the effects of the two medicinal plants against gout or diseases with associated symptomologies such as rheumatism or arthritis may, at least in part, be due to the xanthine oxidase inhibitory action.

CONCLUSION

From the results obtained in this study, it may be concluded that *B. balsamifera* and *O. stamineus* are promising plants with high XO inhibitory activity suitable for the isolation of their active constituents through a bioassay guided fractionation.

Figure 1. The IC_{50} of Allopurinol

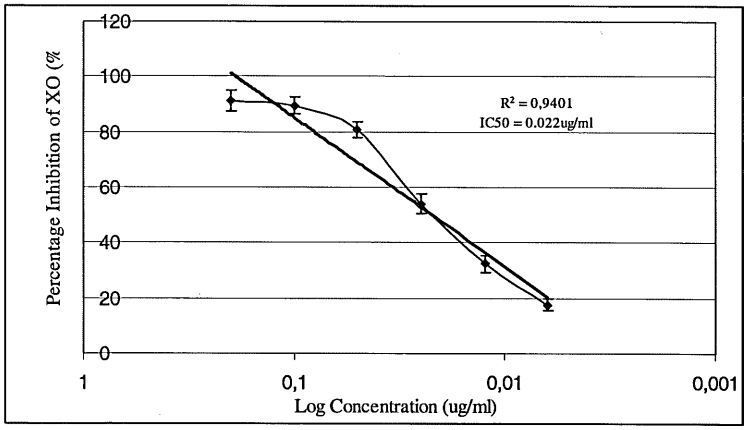

Table 1. Within-day assay accuracy of the XO enzyme inhibitory method

Concentration (µg/ml)	Mean (% Inhibition)	SD	C.V. (%)
0.00625	15.54	1.06	6.74
0.01250	33.82	2.20	6.49
0.025	49.49	1.93	3.91
0.05	89.52	4.53	5.06
0.1	91.19	4.90	5.37
0.2	92.48	2.10	2.27

Table 2. Between-day assay accuracy of the XO enzyme inhibitory method

Concentration (µg/ml)	Mean (% Inhibition)	SD	C.V.(%)
0.00625	17.59	2.15	12.25
0.01250	32.44	3.01	9.30
0.025	53.88	3.53	6.54
0.05	80.77	3.02	3.74
0.1	89.51	3.24	3.63
0.2	91.04	3.74	4.11

Table 3. The IC_{50} of methanolic extracts from selected Malaysian medicinal plants

Plant	Part Used	IC_{50} Values (μg/ml)
Alyxia lucida	Root	63.19
Andrographis paniculata	Leaf	230.18
Ardisia crispa	Root	> 1000
Blumea balsamifera	Leaf	1.15
Eurycoma longifolia	Root	>1000
Orthosiphon stamineus	Leaf	30.79
Smilax myosotiflora	Root	>1000
Tinospora crispa	Root	370.35
Zebrina pendula	Aerial part	>1000

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