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Research Article

XANTHINE OXIDASE INHIBITORY ACTIVITY OF *RHUS MYSORENSIS* LEAVES

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ABSTRACT

Objective: The objective of this study was to assess the *in vitro* xanthine oxidase (XO) inhibiting activity of the methanolic leaf extract of the *Rhusmysorensis*.

Methods: Uric acid is synthesized in the presence of XO which is an essential enzyme. Various concentrations of the leaf extract were taken. XO inhibiting activity was spectrophotometrically assayed. By increasing absorbance at 295 nm, the degree of enzyme spontaneity was determined.

Results: Inhibition concentration 50 (IC50) of the methanolic extract of *R. mysorensis* leaves was 45.24±0.34 µg/ml, compared with IC50 value of the standard allopurinol of 7.8±1.28 µg/ml.

Conclusion: Methanol extract of *R. mysorensis* can be used to treat hyperuricemia and gout after proper preclinical and clinical studies.

Keywords: *Rhus mysorensis*, Inhibitory effect, Gout, Hyperuricemia, Xanthine oxidase.

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INTRODUCTION

Gout is a known metabolic disorder distressing humans and its morbidity is present keeping on growing over the past years. The gout prevalence is diagnosed by increased level of uric acid that accumulates in joints and kidneys, causing gouty arthritis and nephrolithiasis [1,2]. An enzyme xanthine oxidase (XO) which catalyzes the hydroxylation of hypoxanthine which converts xanthine into uric acid. Then, the uric acid is excreted by kidneys. Production of excessive otherwise uric acid elimination is decreased which results into hyperuricemia [3]. The drugs used for this purpose are uricosurics and uricostatics. Uricosuric drugs work by inhibiting the absorption of uric acid in the kidney tubules, whereas uricostatic drug inhibits XO enzyme which converts hypoxanthine into xanthine and it is converted into uric acid, thereby decreases the production of uric acid. The World Health Organization reveals that medicinal plants would be the best source of drugs. Before using it in humans every plants should be investigated for its safety and efficacy.

R. mysorensis belonging to the family of Anacardiaceae is a shrub with thorny branches found in hot dry places and stony regions in India. Reports suggest that the plant contains alkaloids, glycosides, flavonoids, saponins, tannins, sterols, phenols, amino acids, and protein. Earlier studies of the plant claim that it has potential hepatoprotective, antimicrobial, antiurolithiatic, antidiabetic, hypolipidemic, and antioxidant activities. Folk usage of the plant suggested that its use in diabetes, antifertility, psoriasis, and diarrhea problems [4]. However there is no relavant study about inhibiting activity of xanthine oxidase of *R. mysorensis*. Hence, the inhibitory activity for XO in this study was analyzed using methanolic extract of the leaves of *R. mysorensis*.

MATERIALS AND METHODS

Plant material collection

The whole plant *R. mysorensis* was collected from hilly Tirupati region, Andhra Pradesh. Authentication for the plant is done by Dr. Madhav Chetty. K, Department of Botany, Sri Venkateswara University, Tirupati.

Extraction of plant material

Leaves were removed and air dried under shade, powdered and it was stored in an airtight container. Weigh 500 g of the powdered material was extracted with methanol (80%) by the process of soxhlation. The filtrate was concentrated at reduced pressure by rotary flash vacuum evaporator [5].

Drugs and chemicals

XO, xanthine, and allopurinol were obtained from HiMedia Labs, Mumbai, India. The drugs and chemicals used for this study were acquired with commercial and diagnostic category.

XO Inhibiting (XOI) activity [6]

Inhibiting activity of XO was spectrophotometrically assayed in aerobic environments by means of substrate - xanthine. The standard drug allopurinol (1 mg/ml) and the extract were prepared by dissolving in dimethyl sulfoxide initially (not >5% of total volume) and then made up to the required volume using potassium dihydrogen phosphate buffer with pH of 7.5. Then, assay mixture consisting 1 ml of extract at different concentrations (5–100 µg/ml), 2.9 ml potassium dihydrogen phosphate buffer with pH of 7.5, and 0.1 ml XO enzyme solution (0.1 U/ml in potassium dihydrogen phosphate buffer at pH of 7.5 is prepared instantly previous to work). After preincubation for 15 min at about 25°C, 2 ml of substrate solution was added to initiate the reaction. The assay mixture was incubated at 25°C for 30 min, by the addition of 1 N HCl, the reaction was then stopped. At 290 nm, absorbance is measured against blank. Allopurinol (5–100 µg/ml), recognized inhibitor of XO, is selected as a positive control. XO 1 unit is the quantity needed to yield 1 mmol of uric acid per minute by 25°C[6,7]. XO inhibiting activity was calculated as percentage inhibition as given below.

$$\text{Percentage inhibition} = \{(A-B)-(C-D)/(A-B)\} \times 100$$

Here, A - is the enzyme activity without fraction,
B - is the control for A without fraction and enzyme,
C and D were the fraction activity with or without XO.

Table 1: *In vitro* XO inhibitory activity of *R. mysorensis* leaves

Drugs	Percentage XO inhibition					IC_{50} (µg/ml)
	5 µg/ml	10 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	
R. mysorensis leaves extract	27.54±0.96	33.71±0.29	41.25±0.53	51.44±0.89	63.52±0.64	45.24±0.34
Allopurinol (Std.)	31.87±0.32	63.38±0.55	78.63±0.84	86.61±0.55	93.02±1.28	7.8±1.28

Values are mean±SEM of three parallel measurements, *R. mysorensis: Rhus mysorensis*, XO: Xanthine oxidase

In triplicates, the assay is made and the values for inhibition concentration 50 (IC50) were calculated based on the inhibitory activity.

RESULTS AND DISCUSSION

The present study is conducted to examine XO inhibiting activity for *R. mysorensis* leaf extract that might have the perspective to treat increased amount of uric acid and gout. The synthesis of uric acid by the conversion of hypoxanthine into xanthine, the oxidative pathway which occurs in the presence of enzymes XO and guanase, continued with uric acid oxidized from xanthine which is catalyzed by XO. Inhibiting the XO is thus very essential and necessary as pharmacological interference for gout and hyperuricemia [7].

Various concentrations of the leaf extract were taken and XOI activity was assayed by spectrophotometrically. The enzyme inhibitory activity was calculated by raising the absorbance to about 295 nm. The IC50 of the methanolic extract of *R. mysorensis* leaves was 45.24±0.34 µg/ml, compared with IC50 value of the standard allopurinol of 7.8±1.28 µg/ml. The results are given in Table 1.

CONCLUSION

We concluded that the methanolic extract of *R. Mysorensis* leaves processes and having a good XO inhibiting activity, thereby it has the bioactive elements helpful for the management of diseases occurred by XO. Hence, further in future, it is necessary to make studies on isolation of biologically active substances those effects with XO inhibiting activity.

CONFLICTS OF INTEREST

All the authors declared that this research work does not have any conflicts of interest.

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