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Xanthine oxidase inhibition by using different extracts of selected medicinal plants

Dhole GA* and Bodke SS

Department of Botany, Yeshwant Mahavidyalaya, Nanded-431802, Nanded, Maharashtra State, India
*Email: gadhole2010@gmail.com

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ABSTRACT

The inhibition of xanthine oxidase by various solvent extracts of selected medicinal plants was investigated. The different solvents plant extracts from *Annona squamosa*, *Pergularia daemia*, *Bauhinia racemose*, *Cissus quadrangularis*, and *Albizia lebbeck* were tested for their ability to inhibit xanthine oxidase. The maximum xanthine oxidase enzyme inhibition was found in *Cissus quadrangularis* chloroform extract (IC_{50} = 0.197 mg/ml), whereas the lowest activity was recorded in *Annona squamosa* water extract (IC_{50} = 0.672 mg/ml). The different solvent extracts of *Pergularia daemia*, *Bauhinia racemose*, and *Albizia lebbeck* showed moderate xanthine oxidase inhibitory activity. The ethanolic extract (83.72 mg/g) of *Pergularia daemia* showed the maximum amount of phenolic content followed by the ethanolic extract (77.11 mg/g) of *Cissus quadrangularis*.

Keywords: *Pergularia daemia*, *Cissus quadrangularis*, *Xanthine oxidase*, Enzyme inhibition, arthritis, total phenolic content

INTRODUCTION

The increasing interest in natural healing procedures and the use of natural product remedies has led to an increase in interest in medicinal plants and traditional plant extract production procedures. The plants utilized in this study were chosen based on several factors, the most important of which is their historic usage as anti-inflammatory remedies for the treatment of arthritis by traditional peoples. The enzyme xanthine oxidase (XO) is essential for the formation of uric acid from the purines hypoxanthine and xanthine and is associated with the medical disease gout. Gout is characterized by the production of uric acid in the joints, resulting in severe inflammation; inhibiting XO causes gout must go away (Chiang *et al*, 1994). Medicinal plants contain several phytochemicals that are responsible for inhibiting the activity of xanthine oxidase which is useful in the treatment of gout inflammation and related arthritis complications.

MATERIALS AND METHODS

Plant material and preparation of plant extracts:

The plants *Annona squamosa*, *Pergularia daemia*, *Bauhinia racemose*, *Cissus quadrangularis*, and *Albizia lebbeck* were collected from the various places in Nanded district, Maharashtra, and identified and certified by a taxonomist from the Department of Botany, Yeshwant Mahavidyalaya, Nanded-431602, Maharashtra.

The root bark of the *Annona squamosa*, *Albizia lebbeck, Bauhinia racemose*, and the whole plant of *Pergularia daemia*, *Cissus quadrangularis* were obtained and dry in the shadow. A mixture grinder was used to crush the dried plant material and whole plants into a fine powder. The plant's fine powder was extracted using the Soxhlet equipment and in a variety of solvents such as water, ethanol, and chloroform. Finally, the filtered extract was concentrated and kept in a refrigerator to use for a variety of experiments.

Xanthine oxidase inhibition:

For the in vitro Xanthine oxidase inhibitory activity, all the extracts were tested. Using a conventional approach, the activity was measured spectrophotometrically (Owen and Johns, 1999). 1 ml test solution (different concentrations of extract), 2.9 ml phosphate buffer (pH 7.5), and 0.1 ml enzyme solution (0.01 units/ml in phosphate buffer, pH 7.5) were used for the assay. The reaction was started by adding 2 ml of substrate solution after a 15-minute preincubation at 25°C (150 mM xanthine in the same buffer). For 30 minutes, the assay mixture was incubated at 25°C. The reaction was then terminated with 1 ml of 1N hydrochloric acid, and the absorbance was measured with a UV spectrophotometer at 290 nm. A blank was made in the same way, but after adding 1N hydrochloric acid, the enzyme solution was added to the assay mixture. The test was repeated three times. At 25 degrees Celsius, one unit of xanthine oxidase equals the quantity of enzyme required to create 1 mmol of uric acid per minute.

Estimation of total phenols:

The concentration of total phenols in various extracts of chosen medicinal plants was determined using the standard method (Bray and Thorpe, 1954). The theory behind this method is that phenols react with the oxidizing agent phosphomolybdate (Folin-Ciocalteau reagent), forming a blue-colored complex with a maximum absorbance of 660nm. For the creation of a standard curve, catechol (500 mg/ml) was utilized as a standard phenol. Polyphenol concentrations were measured in milligrams per gram of material.

RESULTS AND DISCUSSIONS:

The xanthine oxidase enzyme inhibition of selected medicinal plants was shown in Table 1.

Table 1. Xanthine oxidase inhibition by using selected medicinal plants

Sr. No.	Name of plant	Extract	IC_{50} (mg/ml)
1	*Annona squamosa*	(W.)	0.672
		(E.)	0.568
		(Ch.)	0.540
2	*Bauhinia racemose*	(W.)	0.530
		(E.)	0.507
		(Ch.)	0.498
3	*Pergularia daemia*	(W.)	0.428
		(E.)	0.410
		(Ch.)	0.379
4	*Cissus quadrangularis*	(W.)	0.472
		(E.)	0.307
		(Ch.)	0.197
5	*Albizia lebbeck*	(W.)	0.647
		(E.)	0.539
		(Ch.)	0.512
6	Allopurinol	--	0.102

(W.) – water extract, (E.) – ethanolic extract, (Ch.) – chloroform extract

The results summarized are the mean values of two parallel experiments.

Table 2. Phenolic content of the selected medicinal plants

Sr. No.	Name of plant	Extract	IC_{50} (mg/g)
1	*Annona squamosa*	(W.)	13.08
		(E.)	53.21
		(Ch.)	22.10
2	*Bauhinia racemose*	(W.)	7.50
		(E.)	34.00
		(Ch.)	30.70
3	*Pergularia daemia*	(W.)	54.33
		(E.)	83.72
		(Ch.)	68.30
4	*Cissus quadrangularis*	(W.)	43.50
		(E.)	77.11
		(Ch.)	59.80
5	*Albizia lebbeck*	(W.)	32.40
		(E.)	46.22
		(Ch.)	47.20

(W.) – water extract, (E.) – ethanolic extract, (Ch.) – chloroform extract.
The results summarized are the mean values of two parallel experiments.

The chloroform extract (IC_{50} = 0.197 mg/ml), ethanol extract (IC_{50} = 0.307 mg/ml) and water extract (IC_{50} = 0.472 mg/ml) of *Cissus quadrangularis* showed maximum inhibition of xanthine oxidase enzyme. The chloroform extract (IC_{50} = 0.379 mg/ml) of *Pergularia daemia* showed highest inhibition against xanthine oxidase enzyme while ethanol extract (IC_{50} = 0.410 mg/ml) and water extract (IC_{50} = 0.428 mg/ml) showed moderate enzyme inhibition. The water extract (IC_{50} = 0.530 mg/ml), ethanol extract (IC_{50} = 0.507 mg/ml) and chloroform extract (IC_{50} = 0.498 mg/ml) of *Bauhinia racemose* showed considerable xanthine oxidase inhibition. The chloroform extracts (IC_{50} = 0.540 mg/ml), ethanolic extract ((IC_{50} = 0.568 mg/ml) and water extract of (IC_{50} = 0.672 mg/ml) of *Annona squamosa* showed lowest xanthine oxidase enzyme inhibition. The chloroform extract (IC_{50} = 0.512 mg/ml), ethanolic extract (IC_{50} = 0.539 mg/ml) and water extract (IC_{50} = 0.647 mg/ml) of *Albizia lebbeck* exhibited considerable xanthine oxidase enzyme inhibition. Allopurinol (IC_{50} = 0.102 mg/ml) was used as a standard compound for the inhibition of xanthine oxidase enzyme.

The total phenolic content of the selected medicinal plants was shown in Table 2. The maximum amount of phenolic content was estimated in *Pergularia daemia,* ethanolic extract (83.72 mg/g), chloroform extract (68.30 mg/g), and water extract (54.33 mg/g). The plant *Cissus quadrangularis* ethanolic extract (77.11 mg/g), chloroform extract (59.80 mg/g) and water extract (43.50 mg/g). The other selected plants showed *Annona squamosa* ethanolic extract (53.21 mg/g), water extract (13.08 mg/g), and chloroform extract (22.10 mg/g). The *Albizia lebbeck* plant showed ethanolic extract (46.22 mg/g), water extract (32.40 mg/g) and chloroform extract (47.20 mg/g). The *Bauhinia racemose* ethanolic extract (34.00 mg/g), water extract (7.50 mg/g), and chloroform extract (30.70 mg/g) showed a considerable amount of phenolic content.

Certain research papers claim that the presence of several phytochemicals can cause substantial enzyme inhibitory action (Lin *et al*, 2002). The selected medicinal plants contain a considerable amount of total phenolic content. The plant contains a larger amount of phytoconstituents which exhibits the greater xanthine oxidase inhibitory activity (Nagao *et al*, 1999). The presence of higher amounts of phenolic content in the *Cissus quadrangularis and Pergularia daemia* possess higher xanthine oxidase inhibitory activity.

CONCLUSION:

According to the findings, the chloroform extract of *Cissus quadrangularis* has the highest xanthine oxidase

activity, followed by *Pergularia daemia*, which could be mainly due to the presence of more total phenolic contents that are soluble in organic solvents. More research is needed to identify and purify components from these extracts. This research could be used to replace traditional treatments that are now present in the market.

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Conflict of interest
The author declares that there is no conflict of interest..

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