Xanthine oxidase inhibitory activity of some leafy vegetables collected from Palakkad regions of Kerala

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ABSTRACT

Sauropus androgynus, Aerva lanata & Benincasa hispida have been using as leafy vegetable in kerala. The investigation undertaken was aimed to study xanthine oxidase (XO) inhibitory activity of these three plants and it was demonstrated the usefulness and beneficial effects in the treatment of Gout. Inhibition of XO is an effective therapeutic approach for treating hyperuricemia that causes gout. Allopurinol, a known inhibitor of XO, was used to validate the method and was adopted as positive control in the studies. The degree of inhibition was determined by measuring the increase in absorbance at 295 nm associated with uric acid formation. *Aerva lanata* showed a significant XO inhibitory activity with an IC_{50} value of 62.53µg/ml. *Benincasa hispida* and *Sauropus androgynus* showed a moderate significant XO inhibitory activity with an IC_{50} value of 67.97µg/ml,80.12 µg/ml respectively. The study recommended that leaves possess XO inhibitory activity that might be useful in preventing or slow down the progress of gout.

Keywords: xanthine oxidase, Allopurinol, *Sauropus androgynus, Aerva lanata, Benincasa hispida.*

INTRODUCTION

Gout is an ancient disease. Its name came from the Latin word gutta (drop), points to the belief that a poison falling into the joint drop by drop causing the disease. More recently, it became more prevalent and has increasing complexity which could be related to the development of metabolic syndrome and longevity. We also found that there are more patients refractory to conventional treatment. The disease is more prone to 51 -70 years of age.

It is a chronic inflammatory arthritis presented with excruciating pain, inflammation, erythema and swelling during the acute attack [1]. This chronic metabolic disorder is due to hyperuricaemia[2] leading to deposition of monosodium urate monohydrate crystals in joints or kidneys causing gouty arthritis or uricacid nephrolithiasis[3-4]. Hypeuricaemia is characterised as elevation of serum urate level to more than 7.0 mg/dL for men and more than 5.7 mg/dL for women[5].The treatment for gout is either increasing the excretion of uric acid or reducing the uric acid production. Xanthine oxidase inhibitors (XOIs) are much useful, since they possess lesser side effects compared to uricosuric and anti-inflammatory agents. Allopurinol is the only clinically used XOI, which also suffers from many side effects such as hypersensitivity syndrome, Stevens Johnson syndrome and renal toxicity[6].

Conventional or synthetic drugs used in the treatment of gout sometimes insufficient and can have serious adverse effects. So there is a worldwide trend to go back to traditional medicinal plants. Leafy vegetables which we have been consuming daily, plays an important role in health care. All vegetables enclose several medicinally active compounds .In this research study we have selected the leafy vegetable plants *Sauropus androgynus, Aerva lanata & Benincasa hispida* which are commonly used as food in Kerala. These plants have reported good antioxidants property[7-8] and have been used for the treatment of many diseases [9]. However, there is no scientific claim has been made on the XO inhibitory activity. In view of this, an attempt has been made to investigate the XO inhibitory role of those leafy vegetables.

MATERIALS AND METHODS

Plant material

The fresh leaves of *Sauropus androgynus, Aerva lanata & Benincasa hispida* used for the present studies were collected from Ottapalam, Palakkad, in April 2015 and authenticated by botanist. The collected leaves material were cleaned to remove the adhered dust particles and were then shade dried. The dried plant materials were coarsely powdered, weighed and stored in an air tight container till use.

Preparation of the extract

The coarse powder was packed into Soxhlet column and extracted with water for about 48 h[10]. The solvent was evaporated using electric water bath to get syrupy consistency. Then the dried extract was stored in airtight container in desicator.

Assay of xanthine oxidase inhibitory activity

The XOI activity was assayed spectrophotometrically using xanthine as the substrate. The assay mixture consisted of 1 mL of the fraction (10,20,40,60,80,100 µg/mL), 2.9 mL of phosphate buffer (pH 7.5) and 0.1 mL of xanthine oxidase enzyme (Sigma Aldrich) solution (0.1 units/mL in phosphate buffer, pH 7.5), which was prepared immediately before use. After preincubation at 25 ^{o} for 15 min, the reaction was initiated by the addition of 2 mL of the substrate solution (150 mM xanthine in the same buffer). The assay mixture was incubated at 25^{o} for 30 min. The reaction was then stopped by the addition of 1 mL of 1 N hydrochloric acid and the absorbance was measured at 290 nm using a UV spectrophotometer[11]. Allopurinol (1.0,2.0,4.0,6.0,8.0,10.0 µg /mL), a known inhibitor of XO, was used as the positive control. One unit of XO is defined as the amount of enzyme required to produce 1mmol of uric acid/min at 25^{o}. XOI activity was expressed as the percentage inhibition of XO in the above assay system calculated as percentage Inhibition.

$$\text{Percentage Inhibition (\%)} = (1-[B/A]) \times 100$$

A represents the activity of the enzyme without plant extract and B is the activity of XO in the presence of plant extract. IC_{50} was determined after XO activity. All tests were performed in triplicate.

RESULTS

The Preliminary Phytochemical analysis of the plant extract revealed the presence of flavonoid, alkaloids, Saponins and Glycosides are common in all three selected plants. The observed XO inhibitory activity is attributed to the presence of bioactive compounds in the extracts. The presence of these bioactive compounds in plant extract is known to produce the activity.The percentages of XO inhibitory activity of all crude extracts obtained and comparison was also made among the three plant extracts. Allopurinol (positive control), was used to compare and determine the best plant for XO inhibition.

At a concentration of 100µg/mL, the highest XOI activity was produced by *Aerva lanata* (74.89%), *Benincasa hispida* (69.89%) and *Sauropus androgynus* (59.95%) respectively (Table 2).The *Sauropus androgynus* produce least inhibitory activity at lower concentration (10µg/ml). It was observed that *Aerva lanata* showed a significant XO inhibitory activity with an IC50 value of 62.53µg/ml. *Benincasa hispida* and *Sauropus androgynus* showed a moderate significant XO inhibitory activity with an IC50 value of 67.97µg/ml, 80.12 µg/ml respectively. All the fractions of plant elicited a dose dependent inhibition of xanthine oxidase enzyme activity.

DISCUSSION

The herbal plants and their phytochemicals are important to be explored as potential XO inhibitor as they are already used as food or food supplements for many years and found safe for consume[12]. Plants have mixture of numerous compounds having biological properties such as antioxidant, detoxification enzymes and modulation of metabolic properties. The bioactive compounds that have been derived from plants include flavonoids, terpenoids, phenolic acids, and other categories of phytochemicals, has been outlined for their XO inhibition and antihyperuricemic effect. Hypouricemic agents are commonly employed for the treatment of chronic gouty arthritis, which includes XO inhibitors and uricosuric agents[13].Allopurinol is the drug of choice for the treatment of gout; however it has severe side effects[14]. Thus, new alternatives with improved therapeutic activity and minor side effects are desired. We thus began our study to look for xanthine oxidase inhibitors of phytochemical origin from the various leafy vegetables used in kerala. The selected leafy vegetables *Sauropus androgynus, Aerva lanata & Benincasa hispida* have scientific evidence for the treatment of joint pain and related inflammatory disorders[15,16].

Phytochemical investigation revealed the presence of flavonoid in all three plants. All three plants show XO Inhibitory activity in a concentration-dependent manner. *Aerva lanata* produced better activity compare to other two plants.

CONCLUSION

The study recommended that leaves possess xanthine oxidase inhibitory activity that might be useful in preventing or slow down the development of gout. At higher doses of all fractions, XO would be significantly inhibited. Flavonoids are a group of polyphenolic compounds, which have been reported xanthine oxidase inhibitory activity[17] . It is therefore to speculate that the flavanoids or any other phytoconstituents present in this plant extracts might responsible for the observed XO Inhibitory activity. The findings of the present study concluded that *Sauropus androgynus, Aerva lanata & Benincasa hispida* witnessed a dose dependent significant

activity. Further study should be carried out to identify a potential chemical entity for clinical use in the prevention and treatment of gout and other inflammatory disorders associated with it.

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Table 1. Percent of inhibition and IC_{50} of Allopurinol

No:	Drug	Percentage xanthine oxidase inhibition						IC_{50}
		1.0 µg/ml	2.0 µg/ml	4.0 µg/ml	6.0 µg/ml	8.0 µg/ml	10.0 µg/ml	
1	Allopurinol	13.45 ± 1.03	29.12 ± 1.12	36.32 ± 0.87	48.15 ± 0.98	57.41 ± 0.96	66.57 ± 1.06	6.77

Table 2.Percent of inhibition and IC_{50} of plant extract

No:	Extract	Percentage xanthine oxidase inhibition						IC_{50}
		10 µg/ml	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	
1	*Benincasa hispida*	10.23 ± 1.76	21.73 ± 1.45	33.91 ± 1.76	45.47 ± 2.02	58.09 ± 0.96	69.89 ± 1.76	67.97
2	*Sauropus androgynus*	8.65 ± 0.94	17.93 ± 1.04	28.47 ± 1.23	39.13 ± 0.91	48.29 ± 1.44	59.95 ± 1.25	80.12
3	*Aerva lanata*	12.43 ± 1.45	23.37 ± 2.02	36.33 ± 1.76	51.45 ± 1.76	63.09 ± 0.96	74.89 ± 2.02	62.53
All the data reported are expressed as mean ± SEM.								

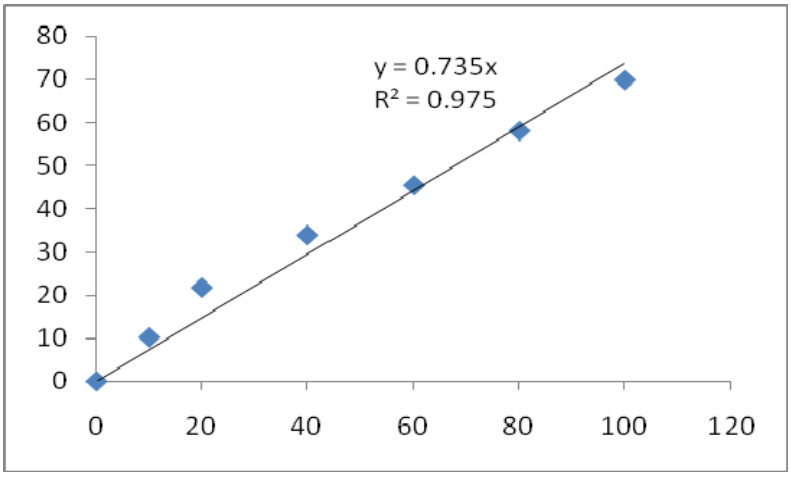

Figure 1. XO inhibitory activity of *Benincasa hispida*

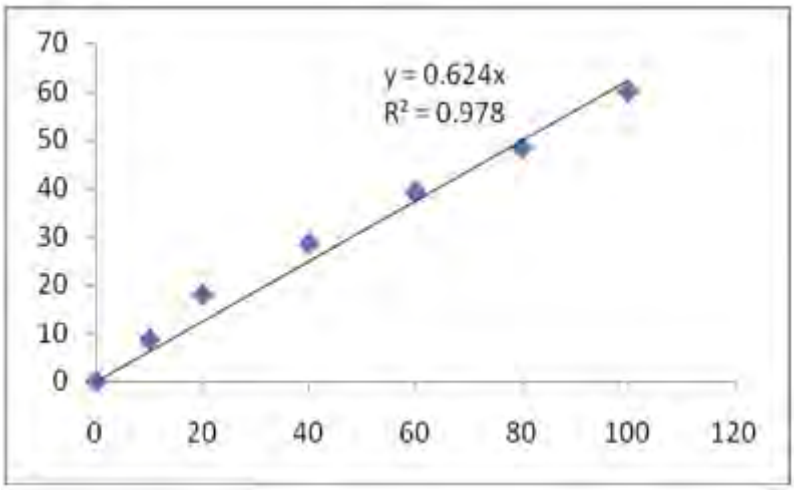

Figure 2. XO inhibitory activity of *Sauropus androgynus*

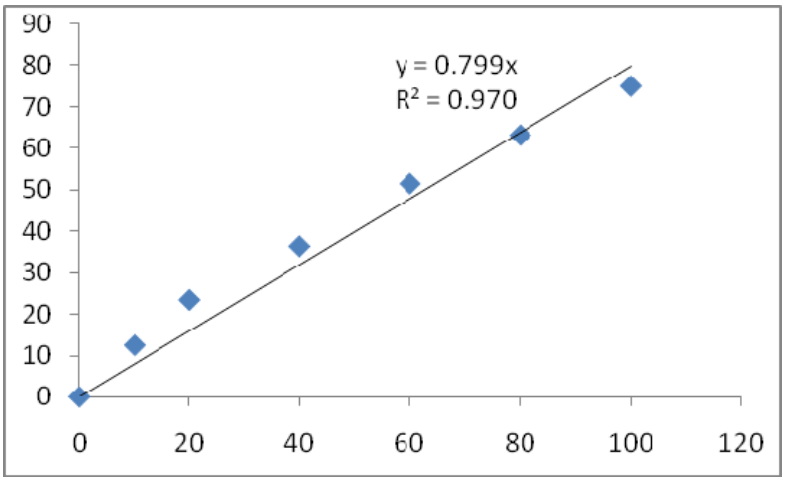

Figure 3. XO inhibitory activity of *Aerva lanata*