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Xanthine Oxidase Inhibitory Activity on Selective Medicinal Plants

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Abstract: Gout is a form of inflammatory arthritis that develops in some people who have high levels of uric acid in blood. The acid can form needle like crystals in a joint and cause sudden, severe episodes of pain, tenderness, redness, warmth and swelling. Gout is a metabolic disease associate with over productions of uric acid. Uric acid is found in a more soluble from as sodium urate. In severe hyperuricemia, crystals of sodium urate get deposited in the soft tissues, particularly in the joints. Such deposits are known as tophi. This causes inflammation in the joints resulting painful gouty arthritis.

Gout is a common disorder with a worldwide distribution. Hyperuricemia, associated with gout, is present in 5-30% of the general population. Hyperurecemia results from the over production or under the excretion of uric acid and is greatly influenced by the high dietary intake of food rich in nucleic acids, such as meats, leguminous seeds and some types of seafood. During the last step of purine metabolism, XO catalyses the oxidation of Xanthine and hypoxanthine into uric acid.

Medicinal plants were evaluated for their Xanthine Oxidase (XO) inhibitory potential. Their aqueous extracts prepared from used parts were tested in vitro at 100µg/ml concentration for their inhibition potencies expressed as percentage (%) inhibition of Xanthine oxidase activity. Totally 12 plants were found inhibition (%) activity of Xanthine oxidase namely *Achyranthes aspera* (leaves), *Aegle marmelos* (pulp), *Aerua lanata* (leaves), *Amaranthus viridis* (leaves), *Beta vulgaris* (vegetable), *Boerhavia diffusa* (leaves), *Canthium parviflorum* (leaves), *Cissus quadrangularis* (fruit), *Coriandrum sativum* (leaves), *Decalepis hamiltonii* (root), *Moringa oleifera* (leaves), and *Momordica charantia* (pulp).

The above plants tested for XO inhibition activity. Allopurinol it is used as a positive control. The aqueous, methanolic and alcoholic extractions of these plants were used for experiments of the 12 plant extracts assays, 10 extracts demonstrated xanthine oxidase activity at 100 µg/ml, among 8 extracts showed an inhibition greater than 50% inhibition and IC_{50} values below 100 µg/ml. The methanol extracts of *Canthium parviflorum*, *Boerhavia diffusa*, *Cissus quadrangularis*, *Beta vulgaris*, showed more than 50% inhibition. The most active was the methanol extract of the pulp of *Momordica charantia* (*Cucurbitaeae*) (IC_{50} , 40 µg/ml), the IC_{50} value of allopurinol used as a positive control. The study demonstrated that the effect of medicinal plants used for the gout treatment was based at least in part on the XO inhibitory action.

Keywords: Gout, Xanthine Oxidase inhibitor, Medicinal Plants.

Introduction

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

Enzymatic degradation of hypoxanthine and Xanthine leads to the production of uric acid. Elevated concentrations of uric acid in the blood stream of human blood leads to formation of gout, characterized by hyperuricemia and recurrent attacks of arthritis. It generates superoxide (during oxidation of substrate), subsequently plays an important role in various forms of inflammatory diseases, several types of tissue and vascular injuries, and chronic heart failure [5, 6].

The treatment of gout entails the use of therapeutic agents such as Xanthine oxidase inhibitors (XOI). XOI acts by blocking the biosynthesis of uric acid form purine in the body and it is believed that either by increasing the excretion of uric acid or reducing the uric acid production helps to reduce the risk of gout [7, 8].

Relief of pain and disability is the main therapeutic goal for gout. Urate-lowering agents include Xanthine oxidase inhibitors, uricosuric agents and uricase agents. Xanthine oxidase catalyzes the oxidation of hypoxanthine to Xanthine and of Xanthine to uric acid. Allopurinol is the most commonly prescribed Xanthine oxidase inhibitor. It alleviates symptoms in most patients; however, it is poorly tolerated in some and leads to hypersensitivity reactions [9-11].

Allopurinol is one of the many known synthetic XOIs, that is widely used in the therapeutic and clinical management of gout, conditions associated with hyperuricemia and related inflammatory diseases. However, allopurinol generates superoxide and some people develop rash as they are allergic to allopurinol. Severe reactions also occur including liver function abnormalities, a fatal complication known as "allopurinol hypersensitivity syndrome".

The appropriate use of botanical plants to treat various diseases are gaining new interest and the focus on plant research has increased around the world. Malaysia houses more than 8,000 species of flowering plants, including shrubs, herbs, lianas, and epiphytes. Some tropical plants and their photochemical are worth to be explored as potential XOIs as they are already used as food or food supplements for many years and found safe for human bodies [12-16].

Materials and Methods

Materials

1) Plant materials

S/N	Name of the plant	Common Name
1	*Achyranthes aspera*	Chaff flower
2	*Aegle marmelos*	Golden apple, bael
3	*Aerua lanata*	Mountain knot grass
4	*Amaranthus viridis*	Green amaranth
5	*Beta vulgaris*	Beetroot
6	*Boerhavia diffusa*	Punaranava
7	*Canthium parviflorum*	Carray cheddie
8	*Cissus quadraugularis*	Nalleru
9	*Coriandrum sativum*	Coriander
10	*Decalepis hamiltonii*	Swallow root
11	*Moringa oleifera*	Moringa
12	*Momordica charantia*	Bitter melon

2) **Biochemicals:** DMSO, HCL, Absolute ethanol, methanol, KH_2PO_4, K_2HPO_4.

a) Sodium phosphate buffers
b) 0.5 M HCL

c) 70% Methanol
3) Enzymes: a) xanthine; b) xanthine oxidase
4) **Drug: Allopurinol:** Allopurinol is a Xanthine oxidase enzyme inhibitor that is considered to be one of the most effective drugs used to decrease ureate levels and is frequently used in the treatment of chronic gout.

Method

Plant Extraction

The plant were soaked in water, washed to get rid of any adhering dust and impurities, and in shade dried. The dried plants were ground to fine powder using mill and pass through 24 mesh sieve to generate homogeneous powder. The finally powdered plants are kept in a dark place at room temperature until the time of use. About 1gm of the dried powder was soaked with 10ml of extraction solvents [Methanol; Water; Absolute ethanol].

The mixture of the ground sample and solvent were capped with aluminum toil and placed in an incubator shaker. Agitation speed of the incubator shaker was set at 100rpm and run at 16hr at 30^0C. Then these mixture of plant material and extraction solvent was filtrated using What's Man No.1 filter paper. The filtrate [plant extraction] is collected.

Enzyme solution

Freshly prepared enzymes solution in added to the sample solution. The enzyme solution prepared by using Xanthine oxidase. In this 0.2 units of Xanthine oxidase is dissolved in 1ml of phosphate buffer.

Substrate solution

Xanthine is used as a substrate solution 0.5 mm Xanthine is added to the assay mixture.

Solvent extraction

The solvent methanol absolute ethanol water used for extraction of fractionation were single distilled technical grade reagents.

Xanthine Oxidase Inhibitory Activity Assay

The inhibitory effect on XO was measured spectrophotometrically at 250nm. A well-known Xanthine oxidase inhibitor allopurinol (100µg/ml) was used as a positive control for the inhibition test. The reaction mixture consisted of 300µl of 50mM sodium phosphate buffer (pH–7.5).

100µl of sample solution dissolved in distilled water (or) DMSO, 100µl of freshly prepared enzyme solution (0.2 units/ml if Xanthine oxidase in phosphate Buffer) and 100ml of distilled water.

The assay mixture was pre incubated at 37^0C for 15min, and then 200µl of substrate solution [0.15 mM of xanthine] was added in to mixture. The mixture was incubated at 37^0C for 30min. Next, the reaction was stopped with the addition of 200µl of 0.5 N HCL. The absorbance was measured using spectrophotometer at 295nm.

The inhibition percentage of Xanthine oxidase activity was calculated according to the formula.

A control-A sample/ A control x 100%

Results and Discussions

Xanthine Oxidase Inhibitory (XOI) Activity

Xanthine oxidase is the enzyme that catalyzes the metabolism of hypoxanthine to Xanthine and then Xanthine to uric acid in the presence of molecular oxygen to yield super oxide anion and hydrogen peroxide that contribute to oxidative damage of living tissues.

Table 2. Plants and its Xanthine Oxidase inhibition activity

S/N	Plant Name	Local Name	Part of Plant Used	Xanthine Oxidase Inhibition Using Distilled Water		Xanthine Oxidase Inhibition Using Alcohol		Xanthine Oxidase Inhibition Using 70% Methanol	
				% of XO Inhibition (100µg/Ml)	IC50 (µg/ml)	% of XO Inhibition (100µg/Ml)	IC50 (µg/ml)	% of XO Inhibition (100µg/Ml)	IC50 (µg/ml)
1	*Achyranthes aspera*	Chaff flower	Leaves	53.12%	116	41.07%	21	42.70%	29
2	*Aegle marmelos*	Golden apple, bael	Pulp	49.36%	28	36.18%	24	46.70%	33
3	*Aerua lanata*	Mountain knot grass	Leaves	39.79%	23	32.10%	22	37.02%	24
4	*Amaranthus viridis*	Green amaranth	Leaves	29.67%	98	21.36%	90	39.99%	26
5	*Beta vulgaris*	Beetroot	Veget-able	32.70%	98	24.37%	64	28.67%	89
6	*Boerhavia diffusa*	Punaranava	Leaves	31.33%	21	24.13%	96	26.74%	98
7	*Canthium parviflorum*	Carray cheddie	Leaves	43.47%	37	45.37%	31	57.56%	96
8	*Cissus quadrangularis*	Nalleru	Fruit	33.12%	24	44.34%	24	27.34%	86
9	*Coriandrum sativum*	Coriander	Leaves	27.92%	83	42.39%	29	44.37%	22
10	*Decalepis hamiltonii*	Swallow root	Root	29.38%	27	30.33%	20	49.46%	33
11	*Moringa oleifera*	Moringa	Leaves	40.16%	33	31.08%	20	38.51%	26
12	*Momordica charantia*	Bitter melon	Pulp	92.70%	41	94.60%	43	96.50%	40
13	Allopurinol (+ve control)	-	-	68.33%	79	62.39%	81	69.37%	80

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Conflicts of interest: There is no conflict of interest of any kind.

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