

XANTHINE OXIDASE INHIBITION BY SOME MEDICINAL PLANTS**¹Khalil Ahmed Ansari, ²M.Akram, ¹H. M. Asif, ¹M.Riazur Rehman, ¹S. M. Ali Shah, ²Khan Usmanghani. ¹Naveed Akhtar, ²E. Mohiuddin**¹College of Conventional Medicine, Islamia University, Bahawalpur²Shifa ul Mulk Memorial Hospital, Hamdard University, Karachi, Pakistan

ABSTRACT : Xanthine oxidase inhibitory activity has been reported from different plants *such as Cinnamomum cassia, Chrysanthemum indicum, Lycopodium europaeus, Polygonum cuspidatum, Acacia confuse, Coccinia grandis, Datura metel, Strychnos nux-vomica, Vitex negundo, Coccinia grandis, Vitex negundo, Fraxinus angustifolia, Pistacia lentiscus, Hyptis obtusiflora, H. lantanaefolia, Artemisia vulgaris, Caesalpinia sappan, Blumea balsamifera, Chrysanthemum sinense, Tetracera scandens, C. sinense, Allium Cepa, Pistacia integerrima, Caesalpinia sappan and Caesalpinia sappan.* This review very clearly specify that plants could be utilized for the inhibition of xanthine oxidase and out of them *Clerodendrum floribundum, Eremophila maculata, Stemodia grossa Benth, Eucalyptus deglupta, Syzygium malaccense and Larix laricina* exhibited 84%, 61%, 57%, 51%, 64%, 86 % xanthine oxidase inhibition at concentration of 50 µg/ml, 50 µg/ml, 50 µg/ml, 44.5 µg/ml, 51 µg/ml, 6.26mg/dl respectively.

Key Words: Xanthine oxidase, inhibitory effects, medicinal plants

INTRODUCTION

Dietary and endogenous purines are metabolized into hypoxanthine and xanthine, which are further catalyzed by xanthine oxidase (XO) into uric acid, the end product of purine catabolism in hominoid species (Hershfield, 2000). During this reaction of xanthine and XO molecular oxygen acts as electron acceptor, producing super oxide radicals and hydrogen peroxide (Cimanga et al., 2001)

Hyperuricemia due to increased catabolism or decreased excretion may precipitate urate crystals causing nephrolithiasis and gouty arthritis, activating lipoxygenases and cyclooxygenases that result in further liberation of reactive oxygen radicals (Middleton et al., 2000). Presently anti-inflammatory drugs are used to relieve the symptoms of gout and xanthine oxidase inhibitor is involved to block the production of uric acid from purines (Kong et al., 2002). Allopurinol is the only xanthine oxidase inhibitor available for hyperuricemic patients

The xanthine oxidase inhibitory activity can be measured using a spectrometric procedure Xanthine oxidase is the enzyme that acts as a catalytic agent in the conversion of xanthine to uric acid. Inhibition of this enzyme will decrease the blood levels of uric acid and result in antihyperuricemic effect. Uric acid is normally dissolved in the blood. When the concentration rises, uric acid forms crystals in the joint. The crystals set up the inflammation process called acute gout.

Disorders of Purine Salvage

Lesch-Nyhan syndrome: This is a rare, X-linked, recessive disorder caused by deficiency of hypoxanthine-guanine phosphoribosyl transferase (HPRT); degree of deficiency creating manifestations vary with the specific mutation. HPRT deficiency results in failure of the salvage pathway for hypoxanthine and guanine. These purines are instead degraded to uric acid. Additionally, a decrease in inositol monophosphate and guanosyl 1 monophosphate leads to an increase in conversion of 5-phosphoribosyl-1-pyrophosphate (PRPP) to 5-phosphoribosylamine, resulting in uric acid overproduction. Hyperuricemia predisposes to gout and its complications. Patients also have a number of cognitive and behavioral dysfunctions, etiology of which is unclear; they do not seem related to uric acid.

The disease usually manifests between 3 to 12 month of age with the appearance of orange sandy precipitate (xanthine) in the urine; it progresses to CNS involvement with intellectual disability, spastic cerebral palsy, involuntary movements, and self-mutilating behavior like biting. Later, chronic hyperuricemia causes symptoms of gout eg, urolithiasis, nephropathy, gouty arthritis, tophi.

Diagnosis is suggested by the combination of dystonia, intellectual disability, and self-mutilation. Serum uric acid levels are usually elevated, but confirmation by HPRT enzyme assay is usually done to validate this effect.

Adenine phosphoribosyltransferase deficiency: This is a rare autosomal recessive disorder that results in the inability to salvage adenine for purine synthesis. Accumulated adenine is oxidized to 2,8-dihydroxyadenine, which precipitates in the urinary tract, causing problems similar to those of uric acid nephropathy resulting in renal colic, frequent infections, and consequently renal failure.

Diagnosis is done by detecting elevated levels of 2,8-dihydroxyadenine, 8-hydroxyadenine, and adenine in urine and confirmed by enzyme assay. Treatment is with dietary purine restriction, high fluid intake, and avoidance of urine alkalinization and Allopurinol is prescribed to circumvent the malaise.

DISORDERS OF PURINE NUCLEOTIDE SYNTHESIS

Phosphoribosylpyrophosphate synthetase superactivity: This X-linked, recessive disorder causes purine overproduction. Excess purine is degraded, resulting in hyperuricemia and gout and neurologic and developmental abnormalities. Diagnosis is carried out by enzyme studies on RBCs and cultured skin fibroblasts and allopurinol is prescribed for symptomatic treatment.

Adenylosuccinase deficiency: This autosomal recessive disorder causes profound intellectual disability, autistic behavior, and seizures. Diagnosis is achieved by identifying elevated levels of succinylaminoimidazole carboxamide riboside and succinyladenosine in CSF and urine. There is no effective treatment for the disorders involving these types of enzymes.

DISORDERS OF PURINE CATABOLISM

Myoadenylate deaminase deficiency (or muscle adenosine monophosphate deaminase deficiency): This enzyme converts AMP to inosine and ammonia. Deficiency may be asymptomatic or it may cause exercise-induced myalgias or cramping; expression seems to be variable because, despite the high frequency of the mutant allele (10 to 14%), the frequency of the muscle phenotype is quite low in patients homozygous for the mutant allele. When symptomatic patients exercise, they do not accumulate ammonia or inosine monophosphate as do normal people.

Xanthine oxidase deficiency:

Xanthine oxidase is the enzyme that catalyzes uric acid production from xanthine and hypoxanthine. Deficiency causes buildup of xanthine, which may precipitate in the urine, causing symptomatic stones with hematuria, urinary colic, and urinary tract infections. Diagnosis is obtained by analyzing low serum uric acid and high urine and plasma hypoxanthine and xanthine. Enzyme determination requires liver or intestinal mucosal biopsy and is rarely indicated. Treatment begins with high fluid intake to minimize likelihood of stone formation and allopurinol is suggested accordingly.

Inhibition of xanthine oxidase by some Chinese medicinal plants used to treat gout

The enzyme xanthine oxidase catalyses the oxidation of hypoxanthine to xanthine and then to uric acid, which plays a crucial role in gout. A total of 122 traditional Chinese medicinal plants, selected according to the clinical efficacy and prescription frequency for the treatment of gout and other hyperuricemia-related disorders, have been evaluated for the enzyme inhibitory activity. Among the 122 methanol extracts derived from these species, 69 were shown to be inhibitory at 100 µg/ml, with 29 having greater than 50% inhibition. As to the equal amount of water extracts, 40 were disclosed to be active at 100 µg/ml, with 13 possessing more than 50% inhibition. At 50 µg/ml, 58 methanol and 24 water extracts exhibited inhibitory activity, with 15 of the former and two of the latter showing greater than 50% inhibition. The most active was the methanol extract of the twig of *Cinnamomum cassia* (Lauraceae) (IC₅₀, 18 µg/ml), which was followed immediately by those of the flower of *Chrysanthemum indicum* (Asteraceae) (IC₅₀, 22 µg/ml) and the leaves of *Lycopus europaeus* (Lamiatae) (IC₅₀, 26 µg/ml). Among the water extracts, the strongest inhibition of the enzyme was observed with that of the rhizome of *Polygonum cuspidatum* (Polygonaceae) (IC₅₀, 38 µg/ml). The IC₅₀ value of allopurinol used as a positive control was 1.06 µg/ml. The result demonstrated that the effects for these medicinal plants used for the gout treatment were based, at least in part, on the xanthine oxidase inhibitory action (Konga et al, 2000).

Inhibition of Xanthine Oxidase by *Acacia confusa* Extracts and Their Phytochemicals

Acacia confusa Merr. (Leguminosae) is traditionally used as a medicinal plant in Taiwan. In the present study, the Xanthine oxidase dehydrogenase (XOD)-inhibitory activity of ethanolic extracts from *A. confusa* heartwood was investigated for the first time. Results demonstrated that the ethanolic extract of *A. confusa* heartwood had a strong XOD-inhibitory activity. Among all fractions derived from heartwood extracts, the EtOAc fraction exhibited the best inhibitory activity. Following column chromatography and reverse-phase high-performance liquid chromatography, eight specific phytochemicals including melanoxetin, 7,8,3',4'-tetrahydroxyflavone, transilitin, okanin, 3,7,8,3'-tetrahydroxy-4'-methoxyflavone, 7,8,3'-trihydroxy-3,4'-dimethoxyflavone, 7,3',4'-trihydroxyflavone, and 7,3',4'-trihydroxy-3-methoxyflavone were isolated and identified from the ethyl acetate fraction. In addition, the IC₅₀ values indicated that okanin showed the strongest XOD-inhibitory effect (IC₅₀ value of 0.076 µM), followed by melanoxetin (0.274 µM) and allopurinol (4.784 µM). The study revealed that okanin and melanoxetin showed excellent inhibition on XOD in noncompetitive and competitive mode, respectively, and their inhibitory activity is better than that of allopurinol (Tang et al, 2010).

Xanthine oxidase inhibitory activity of some Indian medical plants

Xanthine oxidase inhibitory activity was assayed from six species belonging to different families traditionally used for the treatment of gout and related symptoms by indigenous people of India. The aqueous, methanol–water mixture and methanolic extract of these plants were used for the experiment. Of the 18 extracts assayed, 14 extracts demonstrated xanthine oxidase inhibitory activity at 100 µg/ml, among which 10 extracts showed an inhibition greater than 50% and IC₅₀ values below 100 µg/ml. The methanolic extracts of *Coccinia grandis*, *Datura metel*, *Strychnos nux-vomica* and *Vitex negundo* showed more than 50% inhibition, hence, they were screened for their in vivo hypouricaemic activity against potassium oxonate-induced hyperuricaemia in mice. Methanolic extracts of *Coccinia grandis* and *Vitex negundo* showed a significant decrease in the serum urate level (3.90 ± 0.07 mg/dl, *P* < 0.001) and (6.26 ± 0.06 mg/dl, *P* < 0.01), respectively, when compared to hyperuricaemic control (11.42 ± 0.14 mg/dl). This effect observed is almost similar to the serum urate level of allopurinol (3.89 ± 0.07 mg/dl) (Muthuswamy et al, 2007).

Measurement of xanthine oxidase inhibition activity of phenolics and flavonoids with a modified cupric reducing antioxidant capacity (CUPRAC) method.

Various dietary polyphenolics have been found to show an inhibitory effect on xanthine oxidase (XO) which mediates oxidative stress-originated diseases because of its ability to generate reactive oxygen species (ROS), including superoxide anion radical (O₂⁻) and hydrogen peroxide. XO activity has usually been determined by following the rate of uric acid formation from xanthine-xanthine oxidase (X-XO) system using the classical XO activity assay (UV-method) at 295nm. Since some polyphenolics have strong absorption from the UV to visible region, XO-inhibitory activity of polyphenolics was alternatively determined without interference by directly measuring the formation of uric acid and hydrogen peroxide using the modified CUPRAC (cupric reducing antioxidant capacity) spectrophotometric method at 450nm. The CUPRAC absorbance of the incubation solution due to the reduction of Cu(II)-neocuproine reagent by the products of the X-XO system decreased in the presence of polyphenolics, the difference being proportional to the XO inhibition ability of the tested compound. The structure-activity relationship revealed that the flavones and flavonols with a 7-hydroxyl group such as apigenin, luteolin, kaempferol, quercetin, and myricetin inhibited XO-inhibitory activity at low concentrations (IC₅₀ values from 1.46 to 1.90µM). The spectrophotometric method was practical, low-cost, rapid, and could reliably assay uric acid and hydrogen peroxide in the presence of polyphenols (flavonoids, simple phenolic acids and hydroxycinnamic acids), and less open to interferences by UV-absorbing substances (Ozyürek et al, 2009).

Kinetic Study on the Inhibition of Xanthine Oxidase by Extracts from Two Selected Algerian Plants Traditionally Used for the Treatment of Inflammatory Diseases

The inhibitory effect of various extracts from *Fraxinus angustifolia* and *Pistacia lentiscus* was carried out. These plants are used traditionally in Algeria against inflammatory diseases such as rheumatism, arthritis, and gout. The experiments were performed on purified bovine milk xanthine oxidase (XO) activity. The total phenolic contents of the leaves and bark of *F. angustifolia* and the leaves and seeds of *P. lentiscus* were estimated. *P. lentiscus* aqueous fractions from hexane and chloroform extractions and *F. angustifolia* aqueous fraction from ethyl acetate extraction inhibited XO activity by 72.74±2.63% (50% inhibitory concentration [IC₅₀]=27.52 µg/mL), 68.97±3.89% (IC₅₀=42.46 µg/mL) and 53.92±3.17% (IC₅₀=58.84 µg/mL), respectively, at 100 µg/mL, compared to that of reference drug, allopurinol (98.18% [IC₅₀=6.34 µg/mL]). Moreover, at a concentration of 50 µg/mL, both *P. lentiscus* extracts showed inhibition rates higher than 50%. *F. angustifolia* leaf extracts showed only mild inhibition. Lineweaver-Burk analysis showed that the inhibitory activity exerted by *F. angustifolia* bark aqueous extract and *P. lentiscus* aqueous extracts is of mixed type, whereas the leaf extracts from *F. angustifolia* inhibited XO noncompetitively. Positive correlations were established between XO inhibition and total phenols ($r=0.89$) and flavonoids ($r=0.93$) for *P. lentiscus* and with total phenols ($r=0.72$) and tannins ($r=0.54$) for *F. angustifolia*. The therapeutic use of these plants is attributed to xanthine oxidase inhibition, so as to treat inflammatory-related diseases especially gout.

Preparation of ferulic acid derivatives and evaluation of their xanthine oxidase inhibition

Several ferulic acid ethyl esters (3a-h) were synthesized under the Knoevenagel reaction condition and obtained allylic alcohol derivatives (4a-g). Some of them were evaluated for the xanthine oxidase (XO) inhibitory activity. Among them, 3h exhibited a significant inhibitory activity with an IC₅₀ value of 1.35 x 10⁻⁵ M, while the IC₅₀ value of allopurinol used as the positive control was 1.49 x 10⁻⁵ M. The higher acidity of the phenolic OH group in the ferulic acid derivatives infrequently result in improved XO inhibitory activity.

Xanthine oxidase inhibitory activity of some Panamanian plants from Celastraceae and Lamiaceae

Thirty four crude extracts of Panamanian plants, from nine species of Celastraceae and Lamiaceae, were assayed for xanthine oxidase (XO) inhibitory activity. The enzymatic activity was estimated by measuring the increase in absorbance at 290 nm due to uric acid formation. Eighty five percent of the crude extracts were found to possess XO inhibitory activity at 50 µg/ml and all the extracts of the species from Lamiaceae were active even at 1 µg/ml. The ethanol extracts of *Hyptis obtusiflora* Presl ex Benth. (Lamiaceae) and *H. lantanaefolia* Poit. (Lamiaceae) exhibited the highest activity with an inhibition of approximately 40% at 1 µg/ml (Antonio et al, 1995).

Xanthine oxidase inhibitory activity of Vietnamese medicinal plants

Among 288 extracts, prepared from 96 medicinal plants used in Vietnamese traditional medicine to treat gout and related symptoms, 188 demonstrated xanthine oxidase (XO) inhibitory activity at 100 microg/ml, with 46 having greater than 50% inhibition. At 50 microg/ml, 168 of the extracts were active, with 21 possessing more than 50% inhibition. At 25 microg/ml, 146 extracts exhibited inhibitory activity, with 8 showing over 50% inhibition, while 126 extracts presented activity at 10 microg/ml, with 2 having greater than 50% inhibition. The Methanolic extracts of *Artemisia vulgaris*, *Caesalpinia sappan*, *Blumea balsamifera*, *Chrysanthemum sinense* and *Tetracera scandens* exhibited strong XO inhibitory activity with IC(50) values less than 20 microg/ml. The most active extract was the MeOH extract of the flower of *C. sinense* with an IC(50) value of 5.1 microg/ml. Activity-guided fractionation of the MeOH extract led to the isolation of caffeic acid, luteolin, eriodictyol, and 1,5-di-O-caffeoylquinic acid (4). All these compounds showed significant XO inhibitory activity in a concentration-dependent manner, and the activity of some plants was more potent (IC(50) 1.3 microM) than allopurinol (IC(50) 2.5 microM). (Nguyen et al, 2004).

Onion, a Potent Inhibitor of Xantine Oxidase

Onion (*Allium Cepa*) contains high levels of flavonoids. Many studies indicating the inhibitory effects of flavonoids on xanthine oxidase are cited but there is no report on the effect of onion on this enzyme. Therefore, the inhibitory effects of onion on xanthine oxidase are investigated. Fresh filtered juice of onion was prepared and its inhibitory effect on guinea pig liver and bovine milk xanthine oxidase activity was assayed spectrophotometrically using xanthine as substrate. The juice caused more than 80% inhibition on both guinea pig and bovine milk xanthine oxidase. The extract also resulted in a marked inhibition on guinea pig liver (IC₅₀=10 mg/ml) and bovine milk (IC₅₀=13 mg/ml) xanthine oxidase activities. Quercetin exerted its inhibitory effect on bovine milk xanthine oxidase through a linear mixed-type (K_i=0.06±0.04 and K_I=0.22±0.16 mM), whereas, the guinea pig enzyme was inhibited in a competitive manner (K_i=0.11±0.02 mM). In conclusion, it is suggested that consumption of onion as a staple vegetable with a potent inhibitory effect on xanthine oxidase not only could be useful in gout, but infrequently involve in some interactions with those drugs that are metabolized by xanthine oxidase (Jalal et al, 2004).

Pharmacological basis for use of *Pistacia integerrima* leaves in hyperuricemia and gout.

Pistacia integerrima Stew ex. Brandis is an important component of commonly dispensed traditional dosage forms. This study was carried out to determine polyphenolic constituents involved in oxidative stress and have potential to counter hyperuricemia. Radical scavenging activity was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and xanthine oxidase (XO) inhibitory activity assay in vitro. Fructose (FRS) induced hyperuricemic animal model was utilized to assess the serum uric acid (UA) lowering effect by plant products. Ethyl acetate and *n*-BuOH fractions had the highest DPPH radical scavenging activity. Fifty percent inhibitory concentration (IC₅₀) was 6 and 7.6 µg/ml respectively.

It was less than quercetin (IC_{50} 0.95 $\mu\text{g/ml}$) and ascorbic acid (IC_{50} 1.76 $\mu\text{g/ml}$). Xanthine oxidase inhibitory activity was comparable between *n*-BuOH and EtOAc (IC_{50} 19 and 20 $\mu\text{g/ml}$) extracts but less than quercetin (IC_{50} 0.65 $\mu\text{g/ml}$) and allopurinol (IC_{50} 0.10 $\mu\text{g/ml}$). The antioxidant activity as well as the inhibitory activity towards the enzyme XO by quercetin-3-*O*- β -D-glucopyranoside, kaempferol-3-*O*- β -D-glucopyranoside, quercetin-3-*O*-(6"-*O*-syringyl)- β -D-glucopyranoside, kaempferol-3-*O*-(4"-*O*-galloyl)- α -L-arabinopyranoside, rutin together with aglycons, quercetin, kaempferol and apigenin was performed to explore in vivo hypouricemic effect. Ethyl acetate extract had dose dependent UA lowering effect in hyperuricemic mice. This effect was comparable with quercetin but less than allopurinol, Therefore, clinical studies in hyperuricemic patients are required to validate the claims. (Naseem et al, 2008).

Neosappanone A, a xanthine oxidase (XO) inhibitory dimeric methanodibenzoxocinone with a new carbon skeleton from *Caesalpinia sappan*.

A dimeric methanodibenzoxocinone, named neosappanone A, has been isolated from the heartwood of *Caesalpinia sappan* L. of Vietnam, and its structure was elucidated on the basis of spectroscopic analysis. Neosappanone A competitively inhibited xanthine oxidase in a concentration-dependent manner (IC_{50} , 29.7 μM ; K_i , 16.3 μM). (Mai et al, 2004)

Table 1. Xanthine oxidase inhibition at different concentrations

S No.	Name of Plant or Ingredient	Concentration
1	Cinnamomum cassia	18 $\mu\text{g/ml}$
2	Lycopus europaeus	26 $\mu\text{g/ml}$
3	Polygonum cuspidatum	38 $\mu\text{g/ml}$
4	Okanin	0.076 $\mu\text{g/ml}$
5	Melanoxetin	0.274 $\mu\text{g/ml}$
6	Coccinia grandis	3.90 mg/dl
7	Vitex negundo	6.26mg/dl
8	flavones and flavonols	1.46 to 1.90 μM
9	<i>P. lentiscus</i> aqueous fractions from hexane extraction	27.52 $\mu\text{g/mL}$
10	<i>P. lentiscus</i> aqueous fractions from hexane and chloroform extractions	42.46 $\mu\text{g/mL}$
11	<i>F. angustifolia</i> aqueous fraction from ethyl acetate extraction	58.84 $\mu\text{g/mL}$
12	Ferrulic acid ethyl ester	1.35 x 10 ⁻⁵ M
13	flower of <i>C. sinense</i>	5.1 microg/ml
14	Artemisia vulgaris, <i>Caesalpinia sappan</i> , <i>Blumea balsamifera</i> , <i>Chrysanthemum sinense</i> . <i>Tetracera scandens</i>	< 20 microg/ml
15	Onion extracts (Inhibition of guinea pig liver xanthine oxidase).	10 mg/ml.
16	Onion extract (Inhibition of bovine milk xanthine oxidase)	13 mg/ml
17	Extracts	20 $\mu\text{g/ml}$
18	Quercetin	0.65 $\mu\text{g/ml}$
19	Neosappanone	16.3 Mm

Table: 2: Xanthine oxidase inhibition %

S. No	Scientific Name	XO inhibition (Percentage)
1	<i>Clerodendrum floribundum</i> R. Br.	84
2	<i>Eremophila maculata</i> extract	61
3	<i>Stemodia grossa</i> Benth.	57
4	<i>Eucalyptus deglupta</i>	51
5	<i>Syzygium malaccense</i>	64
6	<i>Larix laricina</i>	86
7	<i>Swietenia mahagoni</i>	47

Conclusion:

The plants could be utilized for the inhibition of xanthine oxidase and *Clerodendrum floribundum*, *Eremophila maculata*, *Stemodia grossa* Benth, *Eucalyptus deglupta*, *Syzygium malaccense*, *Larix laricina* exhibited 84%, 61%, 57%, 51%, 64%, 86 % xanthine oxidase inhibition at concentration of 50 µg/ml, 50 µg/ml, 50 µg/ml, 44.5 µg/ml, 51 µg/ml, 6.26mg/dl respectively. The gout treatment with plants was due to xanthine oxidase inhibitory effects.

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