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HPLC- based bioactivity profiling for investigation of potent Xanthine Oxidase Inhibitor from *Zanthoxylum armatum* fruits

Ranjana[1], Dnyaneshwar U. Bawankule[2,] and Karuna Shanker [1]*

[1]Analytical Chemistry Department, CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow-226 015, India
[2]Molecular Bioprospection Department, CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow-226 015, India

* Corresponding author: kspklko@yahoo.com

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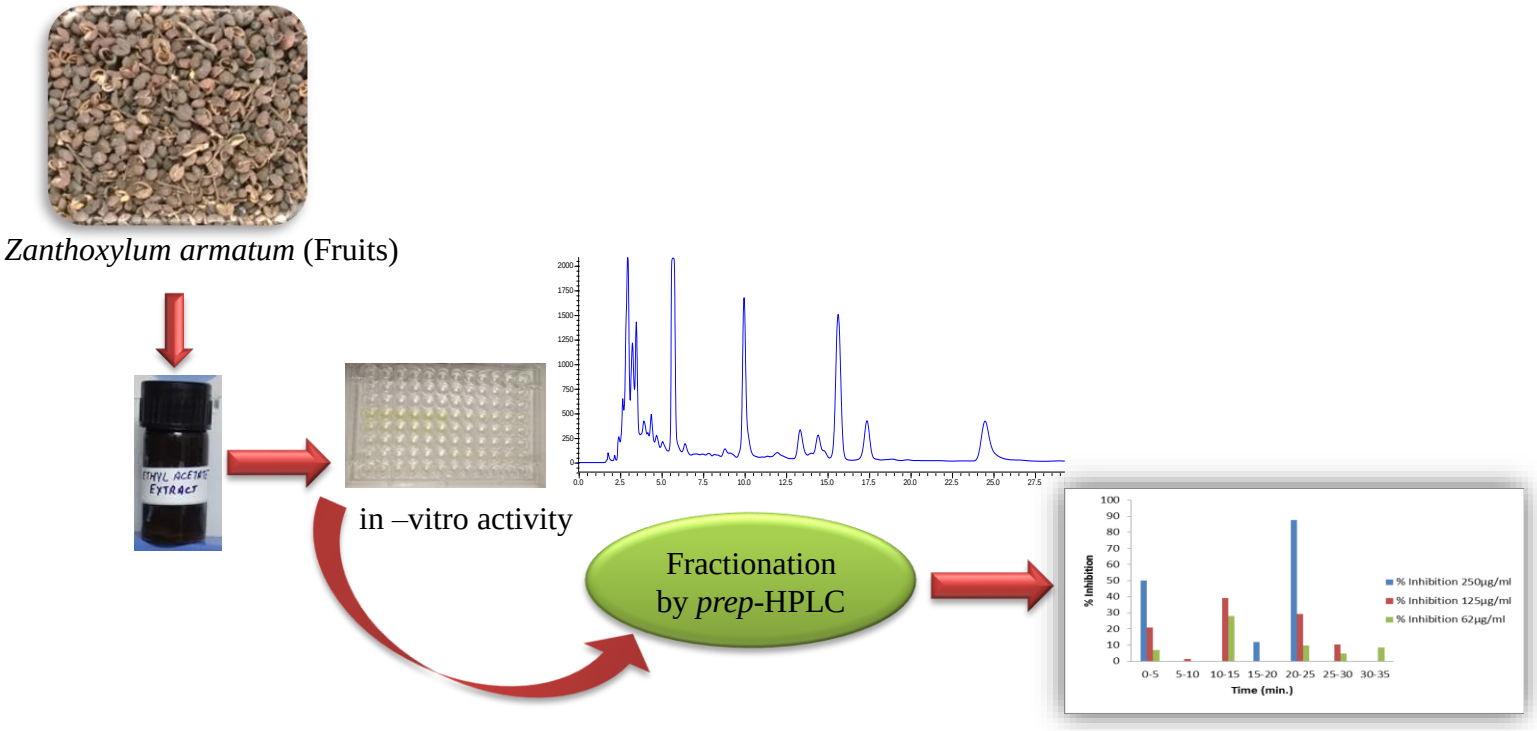
Graphical Abstract



Abstract:

Background: *Zanthoxylum armatum* DC (Family Rutaceae) is traditionally used as carminative, as well as for treatment of inflammation, pain, and gout. Gout and hyperuricemia are caused by accumulation of uric acid, Xanthine oxidase (XO), is an complex enzyme, that catalyzes the oxidative hydroxylation of hypoxanthine and xanthine to uric acid. Xanthine oxidase inhibitors may play a role in treatment of hyperuricemia.

Objective: Investigation of xanthine oxidase (XO) inhibitory activity (*in vitro*) of *Z. armatum* fruits extracts and fractionation of bioactive extracts for separation and isolation of bioactive compounds by HPLC.

Materials and methods: Extract of *Z. armatum were* evaluated for xanthine oxidase inhibitory activity. An efficient method based on bioactivity guided fractionation using preparative-HPLC was used to separate and evaluate the bioactive compounds from ethyl acetate extract. Solvent system methanol: water (65:35, v/v) was used for the isocratic method of *p*-HPLC and seven fractions of 5 mL each were collected. Then the active fractions were evaluated for XOI activity and compounds were isolated by peak-resolved manner.

Results: The ethyl acetate extract shows the most significant effect on XO activity (IC_{50} 115.69µM) and XO inhibitory activity of isolated marker chemical was ranging from 5.62 to 41.21 µM.

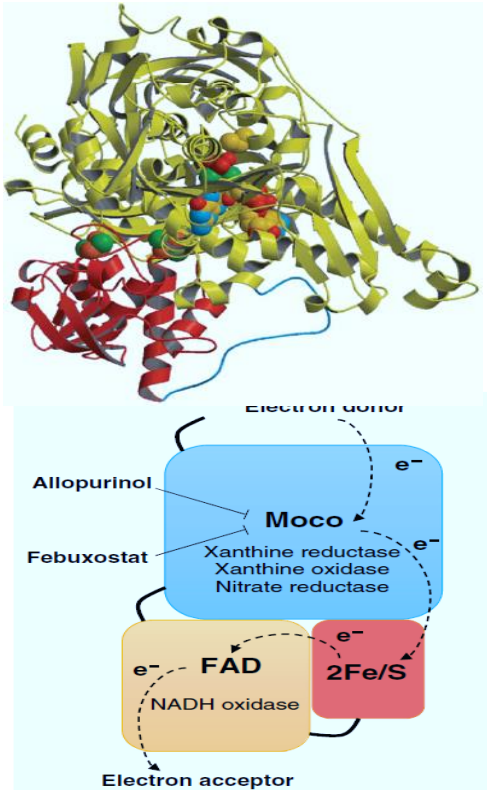
Conclusion: It is concluded that *Z. armatum* possesses a significant inhibitory effect against the xanthine oxidase enzyme. Therefor fruits of *Z. armatum* may lead potential treatment of hyperuricemia.

Keywords: *Zanthoxylum armatum, Xanthine Oxidase Inhibition, HPLC bioactivity profiling.*

Introduction

Xanthine Oxidase (XO)

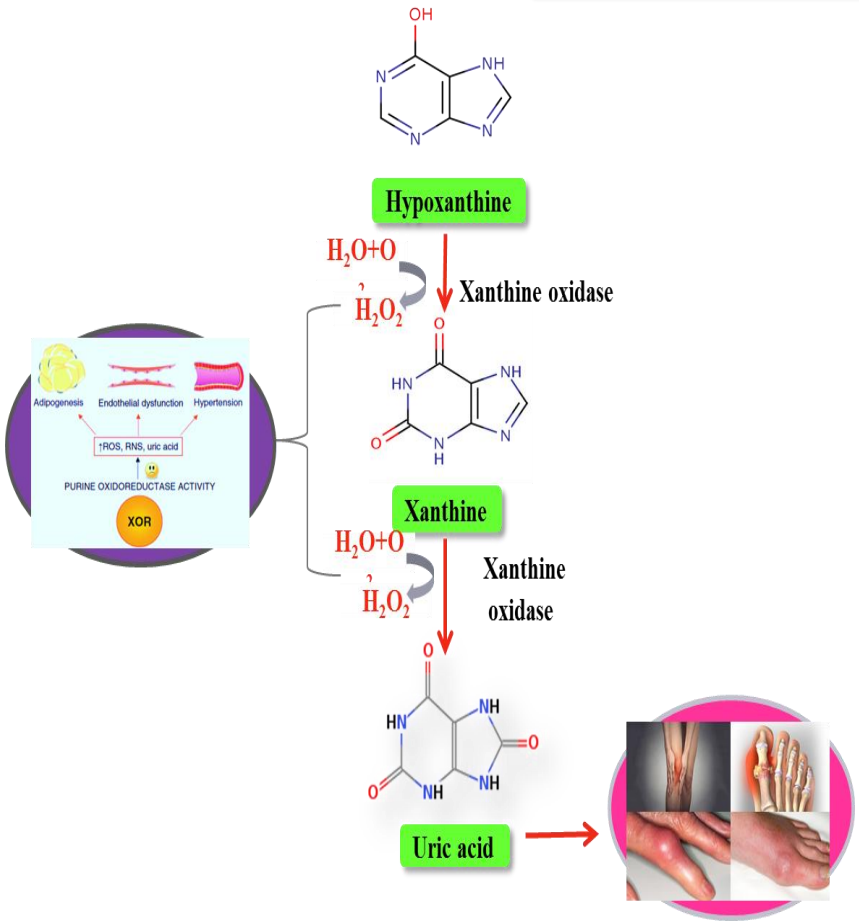
- Xanthine oxidase is a versatile metallo- flavoprotein enzyme
- Its catalyzes the oxidation of hypoxanthine to xanthine and then to uric acid in purine catabolism while simultaneously producing reactive oxygen species
- XO is a homodimer, containing one molybdenum, one of the flavin adenine dinucleotides and two iron-sulfur centers of the ferredoxin type in each of its two independent subunits



Structure and activities of XO

Introduction

Overactivity of XO



- The high expression of XO is associated with overproduction of uric acid that leads to the hyperuricemia
- It is Clinically reported, the key factor uric acid is related to an increased risk of gout
- Increased risk of cardiovascular disorder, nephrolithiasis and diabetes

Introduction

Xanthine Oxidase Inhibitors

- The profusion of uric acid in the blood leads to hyperuricemia, which could be to overproduction of the uric acid. Hyperuricemic condition further prompts another diseased state called gout.

- Gout is marked by a state of inflammation in joints caused by deposition of monosodium urate crystals.

- The selective inhibition of XO result in a broad spectrum therapeutic for gout, cancer, inflammation and oxidative damage.

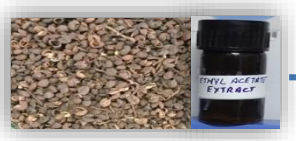
Introduction

- *Zanthoxylum armatum* DC. (Rutaceae) a shrub or small tree, is medicinally significant commonly called Tejovati, Timur or Indian prickly ash.
- It is found throughout India, from Kashmir to Bhutan at altitudes up to 2,500m. It is also widely distributed in Taiwan, Nepal, China, Philippines,Malaysia, Japan, and Pakistan .
- *Zanthoxylum* plant parts have exhibited different biological activities.
- Leaves of the plants are known for its anthelmintic, anti-oxidant, and anti-inflammatory potential and used in arthritis treatment.
- Bark and fruit have hepatoprotective, carminative and piscicide action, respectively.
- The fruits and seeds are also prescribed for the treatment of rheumatism, dysentery, stomach pain, chronic fever, cholera, dyspepsia, toothache, cough, bronchitis and hair diseases.
- Traditionally, it uses to treat asthma, gout, pain, and inflammation. Therefore, *in-vitro* xanthine oxidase (XO) inhibition potential of the extract could be worth to explore prospect in the prevention/treatment of gouty affections of the joints and other diseases.

Introduction

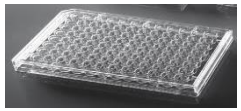
- The prior art shows that the potential of Indian medicinal plants having ***vatasamanam ayurvedic*** **property has not been explored for XO inhibitor. Despite of the fact, Gout and hyperuricemia are caused by the excessive *Vata* as per *Ayurvedic* principles.**

In-vitro XOI activity



Zanthoxylum armatum
(Fruits)

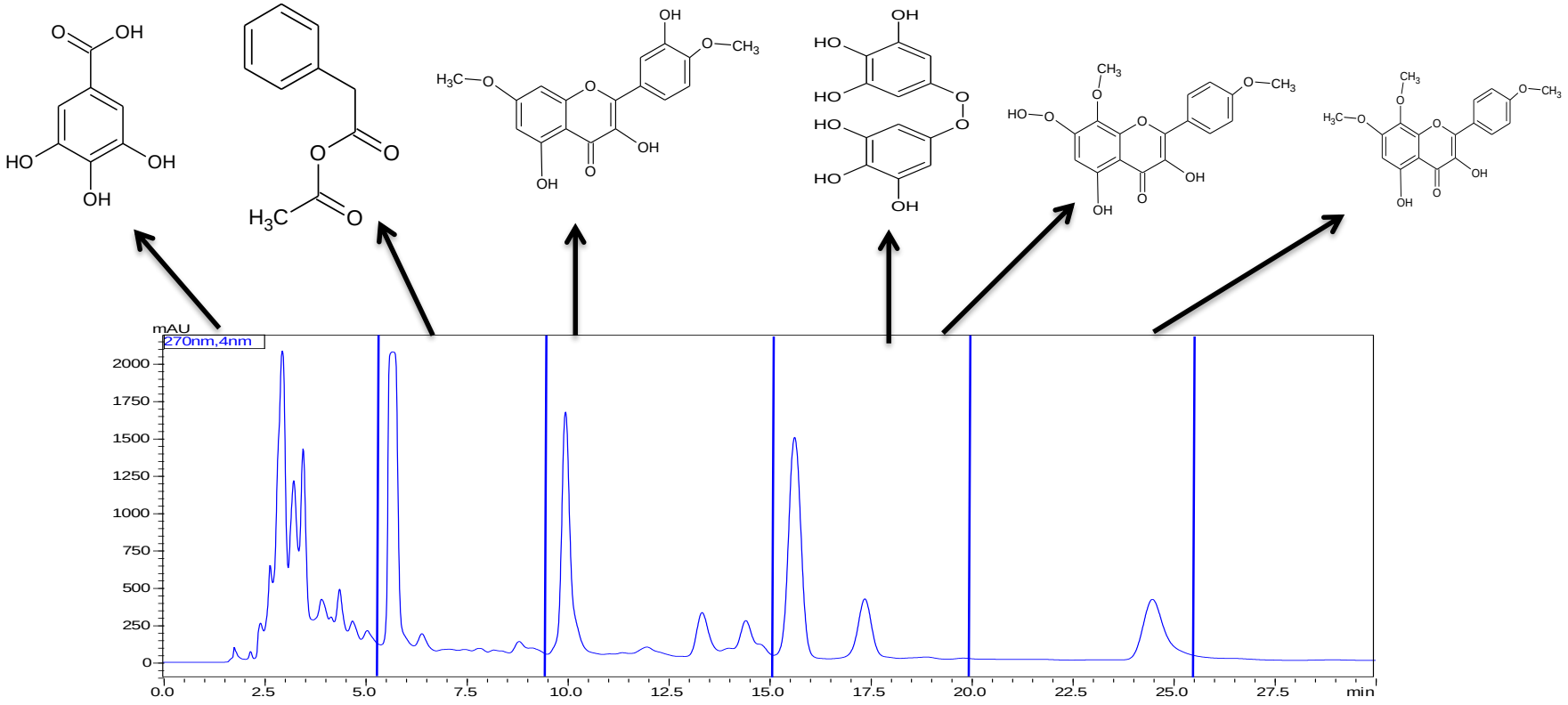
Reaction mixture
Sodium Phosphate Buffer(75µL)
Control/Test (25µL)
0.2U/ml XO (75µL)
Distil. Water (25µL)
0.15mM Xanthine solution (50µL)



Incubate for 30 min.

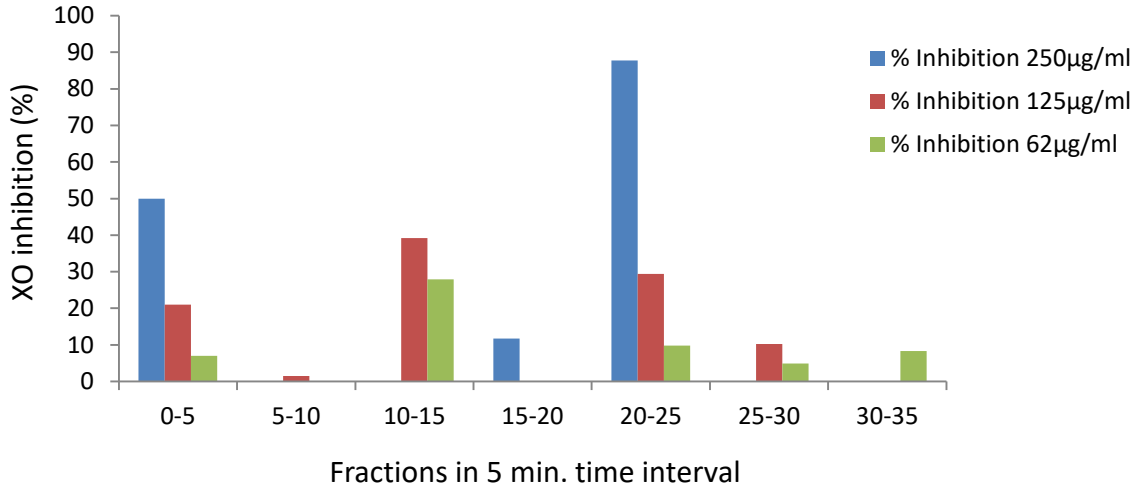
Scan at 290 nm

Results and discussion



HPLC based fractionation of ethyl acetate extract of *Z. armatum*

Results and discussion



Bioactivity Profiling of Fraction collected via *prep*-HPLC

Results and discussion

Isolated compounds which are identified through HRMS and their XOI activity

Compound	m/z	XOI activity IC_{50} (µM)
Gallic acid ($C_7H_6O_5$)	169.0210 $[M-H]^+$	26.15
Acetyl phenyl acetate ($C_{10}H_{11}O_3$)	179 $[M+H]^+$	5.95
3, 5, 3'-Trihydroxy-7, 4'-dimethoxyflavone (Ombuin) $C_{17}H_{14}O_7$	331.0803 $[M+H]^+$	39.63
3, 4, 5, 3 ', 4', 5'-hexahydroxydiphenyl ether ($C_{12}H_{11}O_7$)	267 [M+H]	41.21
3, 5, 7-Trihydroxy-8, 4',-dimethoxyflavone (Prudomestin) $C_{17}H_{14}O_7$	331.0798 $[M+H]^+$	6.73
3, 5-Dihydroxy-7 8, 4'-trimethoxyflavone (Tambulin) $C_{18}H_{16}O_7$	345.0994 $[M+H]^+$	5.62

Conclusions

- This study provides evidence that the Z. armatum has potent XOI activity and may reduce the formation of uric acid.
- Considering the potential benefits may be useful for the treatment of hyperuricaemia and gout, which correlates with the ethno-botanical data on the use of these plants in Indian folklore.

Acknowledgments

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