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In-vitro Screening of Extracts for their Xanthine Oxidase Inhibitory Potential of Some Indian Medicinal Plants and Active Fraction of Selected Plants

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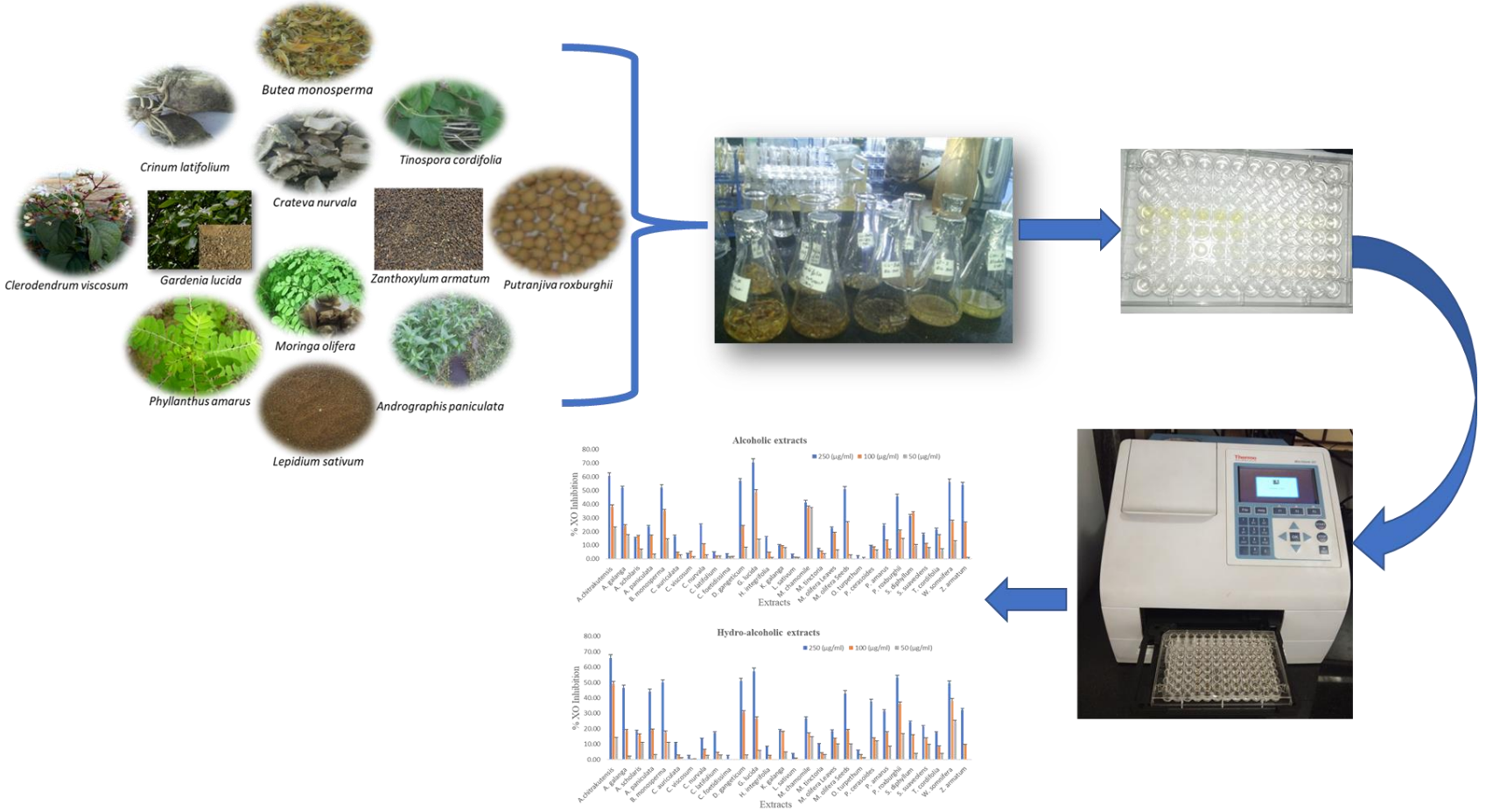
Graphical Abstract



Abstract

Background: The activity of xanthine oxidase (XO) enzyme plays main role in the induction of hyperuricemia, gout and raising superoxide radical level in blood through oxidation of xanthine and hypoxanthine into uric acid. Thus, inhibition of xanthine oxidase activity is regarded as an effective treatment of hyperuricemia, gout, inflammation and other XO associated diseases.

Objective: Twenty- six plants extracts were assayed for the novel XO inhibitors to develop a potent lead for the management of hyperuricemia gout, and other XO metabolism related diseases / disorders.

Materials and methods: Traditional knowledge based prioritized plant extracts were screened for XO inhibitory potential through *in-vitro* assay. Microtiter plate based high through spectrophotometric method was employed to determine the inhibition by measuring the uric acid at 295 nm.

Results: The *in-vitro* assay of extracts was found as eight alcoholic and six hydroalcoholic extract found to have highest inhibition more than 50% at a concentration of 250 µg/ml in the assay mixture. While thirteen plant extracts found to have moderate inhibition 20-30% at 250 µg/ml. Plants *Alectra chitrakutensis, Butea monosperma, Dasmodium gangeticum, Gardenia lucida*, *Zanthoxylum armatum* investigated their IC_{50} values below 250 µg/ml.

Conclusion: On the basis of detail phytochemical exploration both from data mining and 3qualitative evaluation, we found that phenolic rich plants particularly *Z. armatum* and *G. lucida* showed highest XOI potential. Present study correlates with the traditional usages of these plants and provides basis for further investigation for lead of natural XOI for treatment of gout and other XO-related disorders.

Keywords: Xanthine oxidase inhibitors; hyperuricemia; Gout; Allopurinol; Uric acid

Introduction

- Plants have been widely used for healing diverse diseases, since ancient times. It is an important sources of effective and minimal side effects natural products which are vary vastly in chemical nature, mechanism of actions and biological activity. Plants used in traditional system of medicines and screenings of their extract for biological studies may provide an idea to identify newer medicaments for the treatment or prevention of various ailments.

- Gouty arthritis is described by the accumulation of monosodium urate crystals in kidneys, joints and surrounding tissues. Hyperuricemia is also occur due to increase of serum uric acid level, thus there are two strategies to reduce serum uric acid level. First, by inhibiting the uric acid production, and second is to accelerate the uric acid excretion though urine.

- Uric acid is the end product of purine metabolism, Xanthine oxidase (XO) an enzyme which catalyzes the purine catabolism. XO catalyzes the oxidation of hypoxanthine to xanthine and then to uric acid, simultaneous increases level of superoxide free radicals (Figure. 1).

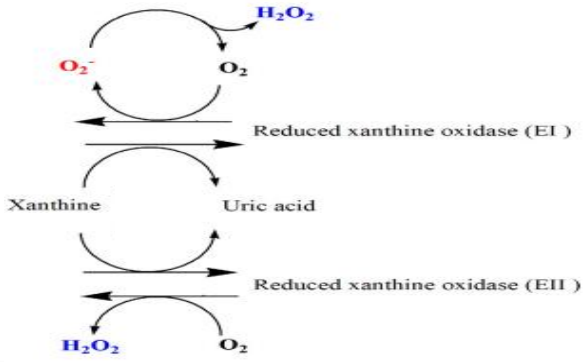


Figure. 1: Proposed mechanism of the O_2^- or H_2O_2 generation and uric acid formation

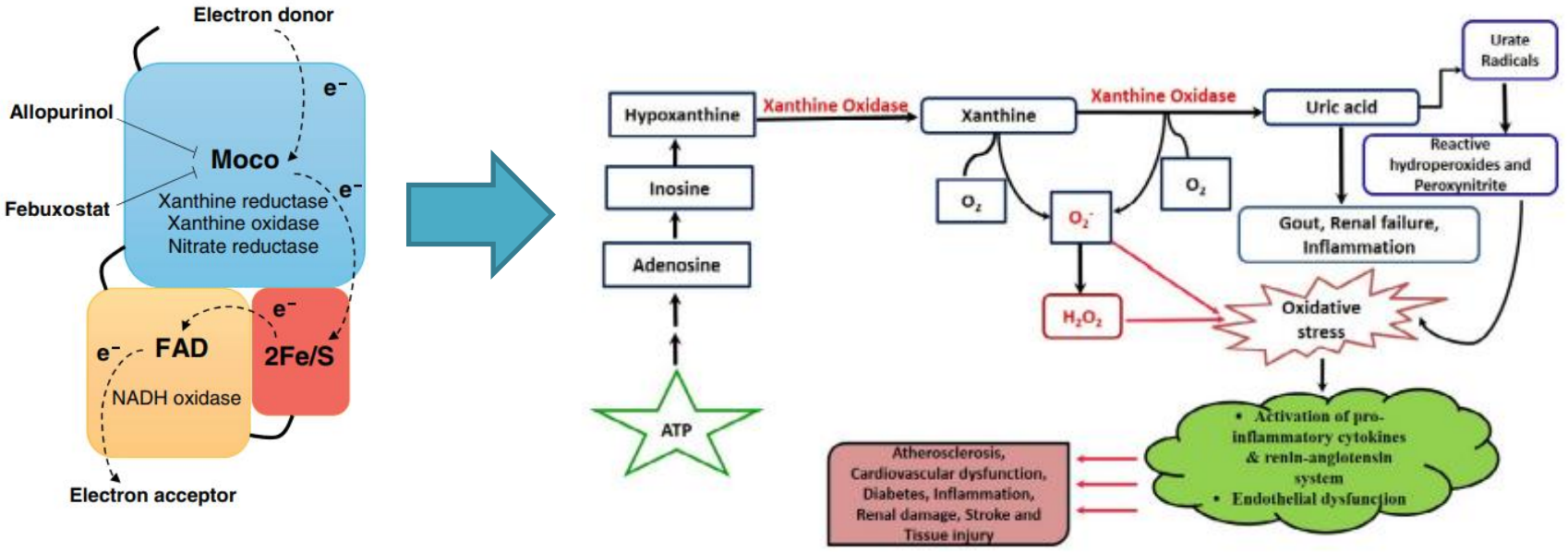


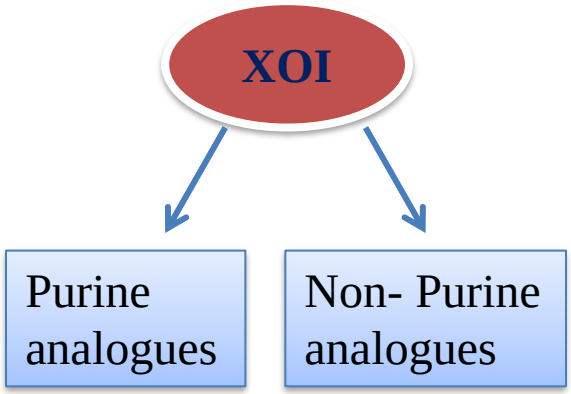
Figure. 2: Structure and enzymic activities and pathophysiology of xanthine oxidase to causing various diseases (Image adopted from M.G. Battelli et al. (2018) 2557–2565 and Ayyappan, P. et al. (2020) 391-416

Introduction

➢The abnormal activity of XO causes a series of pathological condition. Therefor the inhibition of XO activity is regarded as an effective treatment of hyperuricemia, gout, inflammation and other XO associated diseases.

➢Among the few therapeutic options available for allopurinol has remained the main inhibitor of XO with clinical use since 1966, although causing undesirable side effects, such as hypersensitivity syndrome, impaired liver function, and renal toxicity, which have motivated the search for new therapeutic options

Commercially available xanthine oxidase inhibitors (XOI)



Synthetic XO inhibitors

Purine analogs	
XO Inhibitors	IC_{50}
Allopurinol	5.9 µM
2-alkylhypoxanthines	20.5 µM
Neoptrin	3.1µM
Lumazine	7.4µM
6-hydoxy lumazine	0.2µM
Non-Purine analogs	
Febuxostat	20nM
Y-700	5.8nM
Thimaltol	104µM
N-hydroxyguanidines	295.7µM

➢To the discovery of potent XO inhibitors from plants, *in-vitro* screening of plants extracts for pharmacological activity may lead to identification of new medicinal entity, for the treatment or prevention of gout, hyperuricemia or various diseases related to overproduction of uric acid.

Materials and method

Chemicals and Regents: Allopurinol and xanthine were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA). xanthine oxidase (buttermilk), Dimethylsulphoxide (DMSO), hydrochloric acid (HCl), Potassium di-hydrogen phosphate and other reagents of analytical grade were obtained from Merck (Darmstadt, FR, Germany).

Instruments: UV–Vis spectrophotometer (Multiskan GO Microplate, Thermo Scientific, USA) was used for the measurements of absorption of samples.

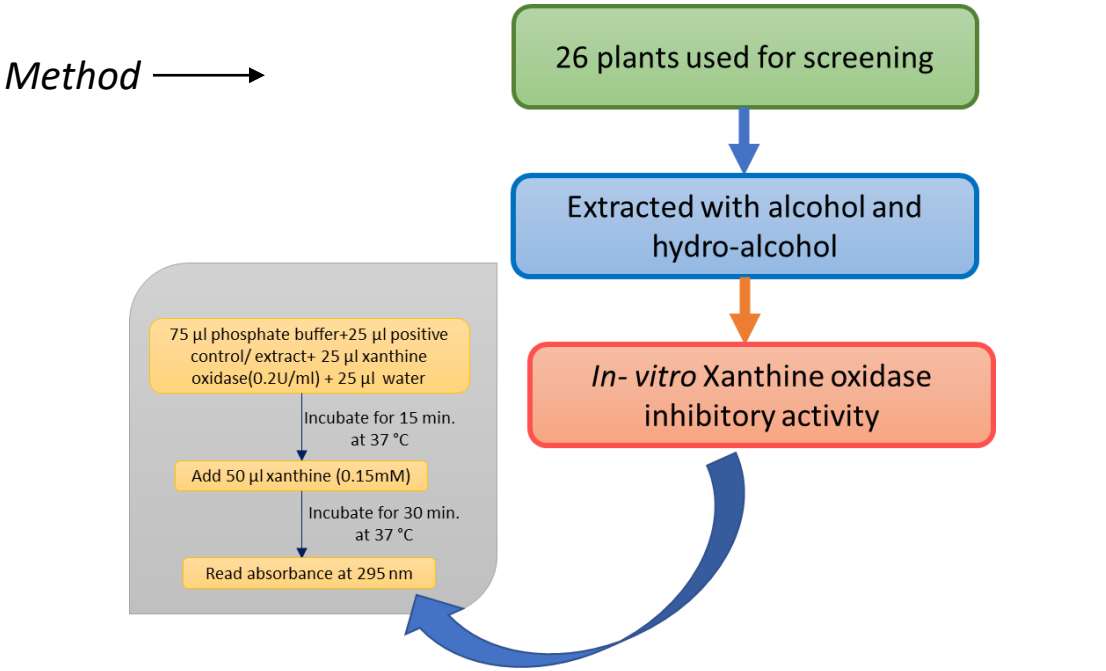


Figure. 3: Schematic diagram for *in vitro* assay of XOI activity methodology

Ref: L . D . Kong et al . / Journal of Ethnopharmacology 73 (2000) 199–207

Results and discussion

The *in-vitro* assay of XOI activity extracts was found as eight alcoholic and six hydroalcoholic extract found to have highest inhibition more than 50% at a concentration of 250 µg/ml. While thirteen plant extracts found to have moderate inhibition 20-30% at 250 µg/ml (Table.1).

Table:1 XOI activity of alcoholic and hydro-alcoholic extracts of medicinal plants

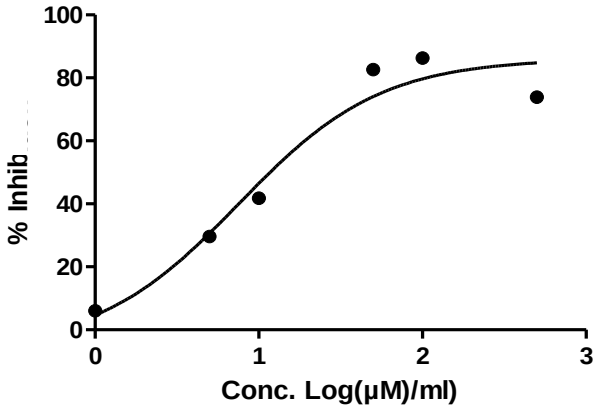
S. no.	Plants/part	Extract	Inhibition (%)			IC_{50}
			250 (µg/ml)	100 (µg/ml)	50 (µg/ml)	
1	*Alectra chitrakutensis* (Rhizomes)	Alcoholic Hydroalcoholic	60.82±2.10 65.80±2.27	38.10±1.33 49.13±1.71	22.29±0.77 13.90±0.45	< 150 µg/ml
2	*Alpinia galanga* (Rhizomes)	Alcoholic Hydroalcoholic	51.94±1.28 46.56±1.74	24.09±0.91 18.85±0.68	16.63±0.6 02.06±0.07	< 250 µg/ml
3	*Alstonia scholaris* (Stem)	Alcoholic Hydroalcoholic	15.25±0.57 18.50±0.68	16.88±0.44 15.90±0.57	6.16±0.16 10.71±0.38	
4	*Andrographis paniculata* (Aerial)	Alcoholic Hydroalcoholic	23.66±0.92 44.10±1.62	16.96±0.64 19.19±0.70	2.67±0.08 3.12±0.11	
5	*Butea monosperma* (Flowers)	Alcoholic Hydroalcoholic	52.14±2.23 50.00±1.65	35.00±1.15 17.85±0.64	13.57±0.42 10.71±0.33	< 250 µg/ml
6	*Cassia angustifolia* (Leaves)	Alcoholic Hydroalcoholic	16.78±0.67 10.78±0.42	4.89±0.28 02.78±0.08	1.9±0.04 1.21±0.04	

7	*Clerodendrum viscosum* (Leaves)	Alcoholic Hydroalcoholic	3.98±0.14 2.65±0.09	5.14±0.24 0.23±0.01	No inhibition	
8	*Crateva nurvala* (Bark)	Alcoholic Hydroalcoholic	24.83±0.67 13.53±0.41	10.86±0.28 6.23±0.51	1.87±0.02 2.68±0.08	
9	*Crinum latifolium* (Bulb)	Alcoholic Hydroalcoholic	5.03±0.21 9.44±0.26	2.07±0.08 4.28±0.54	1.09±0.04 2.78±0.07	
10	*Dasmodium gangeticum* (Root)	Alcoholic Hydroalcoholic	56.99±1.84 51.04±1.68	23.77±0.75 30.76±0.99	7.46±0.23 2.89±0.06	<250 µg/ml
11	*Gardenia lucida* (Gum resin)	Alcoholic Hydroalcoholic	70.56±2.66 57.25±2.28	48.79±1.82 26.61±1.04	13.30±0.49 5.64±0.23	<100 µg/ml
12	*Holoptelea integrifolia* (Fruits)	Alcoholic Hydroalcoholic	15.625±0.61 8.35±0.35	4.89±0.16 2.56±0.11	No inhibition	
13	*Kaempferia galanga* (Rhizome)	Alcoholic Hydroalcoholic	10.08±0.44 18.96±0.61	9.38±0.32 17.71±0.57	7.29±0.26 4.75±0.13	
14	*Lepidium sativum* (Seeds)	Alcoholic Hydroalcoholic	3.42±0.13 3.94±0.13	1.24±0.04 0.93±0.03	No inhibition	

15	*Matricaria chamomile* (Aerial)	Alcoholic Hydroalcoholic	41.3±1.55 26.78±0.93	37.33±1.40 16.78±0.52	36.68±1.36 14.25±0.49	
16	*Morinda tinctoria* (Bark)	Alcoholic Hydroalcoholic	7.25±0.33 10.08±0.34	5.64±0.24 4.43±0.133	2.82±0.09 3.21±0.14	
17	*Moringa olifera* (leaves) *Moringa olifera* (seeds)	Alcoholic Hydroalcoholic Alcoholic Hydroalcoholic	22.48±0.74 18.45±0.82 50.89±1.89 42.8±1.93	18.79±0.61 13.42±0.47 26.34±1.04 18.89±0.73	5.72±0.17 9.73±0.36 2.09±0.09 9.87±0.36	
18	*Operculina turpethum* (Root)	Alcoholic Hydroalcoholic	2.44±0.08 5.95±0.18	3.02±0.13 No inhibition	1.09±0.04 No inhibition	
19	*Prunus cerasoides* (Bark)	Alcoholic Hydroalcoholic	9.64±0.43 7.56±1.47	8.45±0.28 13.78±0.46	5.62±0.24 11.54±0.47	
20	*Phyllanthus amarus* (Aerial)	Alcoholic Hydroalcoholic	24.47±1.06 31.38±1.17	13.28±0.48 17.39±0.63	6.23±0.28 8.26±0.32	
21	*Putranjiva roxburghii* (Seeds)	Alcoholic Hydroalcoholic	45.625±1.71 53.125±1.63	20.3125±0.76 36.25±1.26	13.9375±0.49 16.25±0.48	<150 µg/ml
22	*Solanum diphyllum* (Leaves)	Alcoholic Hydroalcoholic	31.42±1.06 24.15±0.93	33.57±1.02 15.60±0.56	9.52±0.31 3.75±0.18	

23	*Stereospermum suaveolens* (Bark)	Alcoholic Hydroalcoholic	18.10±0.73 21.35±0.75	11.08±0.40 13.48±0.58	7.30±0.32 9.37±0.43	
24	*Tinospora cordifolia* (Rhizome)	Alcoholic Hydroalcoholic	21.42±0.95 17.46±0.72	17.14±0.63 8.51±0.32	6.42±0.18 3.83±0.12	
25	*Withenia somnifera* (Fruits)	Alcoholic Hydroalcoholic	56.29±2.18 49.46±1.59	27.27±1.06 38.23±1.47	12.23±0.39 24.53±0.95	
26	*Zanthoxylum armatum* (Fruits)	Alcoholic Hydroalcoholic	64.08±1.85 32.14±1.16	26.02±1.08 9.69±0.28	04.50±0.18 No inhibition	<100 µg/ml

- Positive control (Alopurinol) IC_{50} =7.5µM



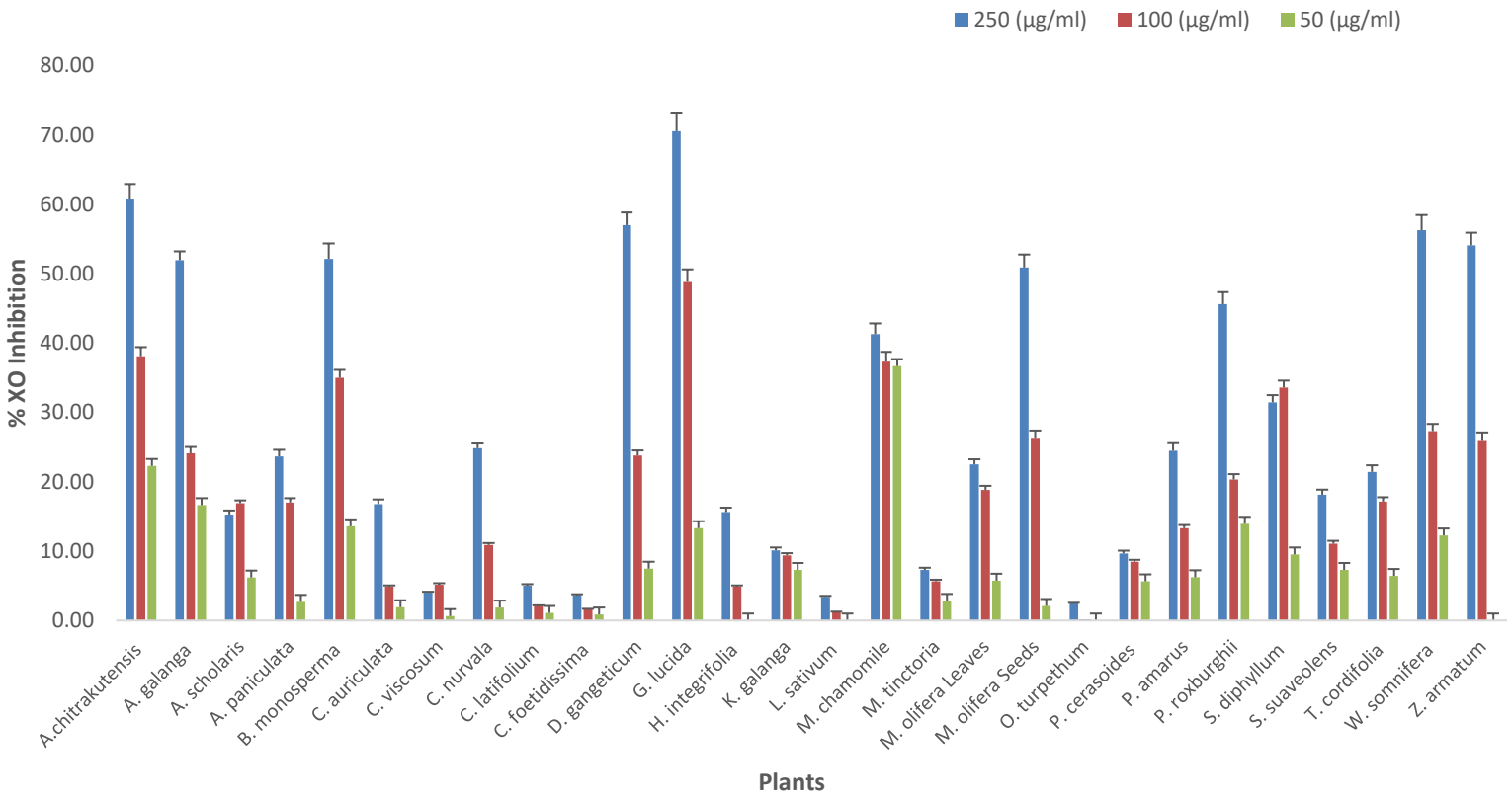


Figure.4: Results are expressed as percent XOI of alcoholic extracts; values expressed as mean ± SD for triplicate experiments

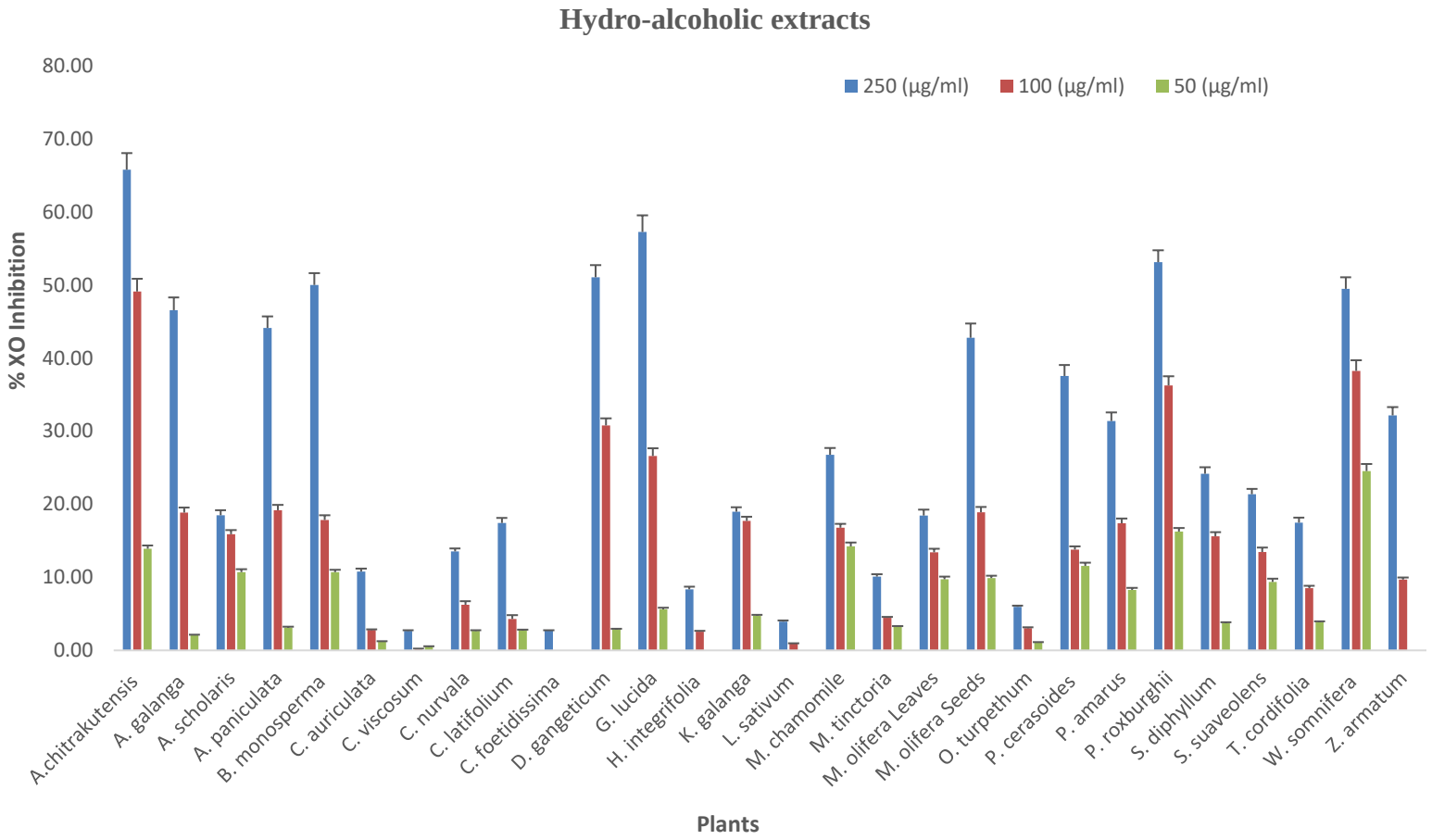


Figure.5: Results are expressed as percent XOI of hydro-alcoholic extracts; values expressed as mean ± SD for triplicate experiments

Conclusions

- Plants *Alectra chitrakutensis, Butea monosperma, Dasmodium gangeticum, Gardenia lucida*, *Zanthoxylum armatum* investigated their IC_{50} values below 250 µg/ml.

- In conclusion, this study indicates two medicinal plants *Z. armatum* and *G. lucida* found highest XOI potential. It may be useful for the treatment of hyperuricemia and gout and other associated diseases. Present study correlates with the traditional usages of these plants and provides basis for further investigation for lead of natural XOI for treatment of gout and other XO-related disorders.

Acknowledgments

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