TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2021) 45: 723-738
© TÜBİTAK
doi:10.3906/bot-2109-19

Medicinal plants of Pakistan and their xanthine oxidase inhibition activity to treat gout: a systematic review

Kanwal ASHIQ[1,*], Khalid HUSSAIN[1], Muhammad ISLAM[1], Naureen SHEHZADI[1], Ejaz ALI[1], Sana ASHIQ[2]
[1]Punjab University College of Pharmacy (PUCP), University of the Punjab Lahore, Pakistan
[2]Centre for Applied Molecular Biology, University of the Punjab Lahore, Pakistan

Received: 10.09.2021 • **Accepted/Published Online:** 21.11.2021 • **Final Version:** 31.12.2021

Abstract: Gout is the most prevalent form of arthritis that arises due to hyperuricemia. Xanthine oxidase inhibitors (XOI) are usually prescribed to treat gout. Although treatment options are available, these therapies are associated with side effects, and natural remedies may be proven helpful in this regard. This systematic review aims to present consolidated information on the xanthine oxidase inhibition potential of the plants located in Pakistan. An extensive literature review reveals that no such study that systematically summarizes existing data is currently available. The present study is registered with PROSPERO (ID: CRD42021247416) and performed according to PRISMA guidelines. Several databases were searched (from January 2000 to April 2021) for the keywords, including "hyperuricemia", "xanthine oxidase inhibitory activity", "medicinal plants", "gout", "Pakistan", "in vivo", and "in vitro". The SYRCLE's RoB tool was used to assess the risk of bias. Total 22 articles met the inclusion criteria. Following plants had higher XO inhibitory activity (>75%); *Bryophyllum* pinnatum, *Zingiber officinale*, *Laggera aurita*, *Trachyspermum ammi*, *Tribulus terrestris*, *Trianthema triquetra*, and *Croton sparsiflorus*. Numerous phytochemicals were also identified, and the followings had potent activity; acacetin 1 (11.92 ± 0.01 μM), ranuncoside (IC50 = 11.69 μM), chrysin 2 (IC50= 73.74 ± 0.02 μM), methyl-3,4,5-trihydroxybenzoate (IC50= 59.5 μM), costinone A (IC50= 90.3 ± 0.06 μM), and umbelliferone (IC50= 97.12 ± 0.01 μM). This study will provide beneficial insight into medicinal plants and serve as a reference for future studies on gout treatment. A rigorous pharmacological and clinical evaluation of medicinal plants is required to gauge their efficacy and safety.

Key words: Gout, uric acid, xanthine oxidase, medicinal plants, arthritis, hyperuricemia

1. Introduction

Gout is a metabolic disorder and the most prevalent form of arthritis caused by hyperuricemia. First, in 2640BC, Egyptians documented this disorder. The word "gout" originates from the Latin term "gutta" meaning drop. Gout is also renowned as the "disease of kings", as it is more likely to be connected with lifestyle (Ashiq et al., 2021). It is associated with various co-morbidities such as hypertension, diabetes, stroke, obesity, chronic kidney disease (CKD), and cardiovascular disorders (CVDs) (Bardin T and Richette, 2017). Worldwide, the frequency of this disease is quite uneven and affects developed countries more than developing countries (Kuo et al., 2015). It is also known as "men's disease" as affecting men four times than women. The lower incidence in women may be due to the shielding effect of estradiol against the synthesis of urate crystal. However, after menopause, females are also at a higher risk of developing gout due to the loss of protective effect induced by estrogen (Kanwal et al., 2018).

Gout is caused by increased serum uric acid levels (>6.8mg/dL). Uric acid is mainly derived from the catabolism of purine bases. In a first step, hypoxanthine forms due to the breakdown of these nitrogenous bases, and it is then converted into xanthine. Uric acid is produced from xanthine by the action of the xanthine oxidase enzyme (El Ridi and Tallima, 2017). Sustained high serum uric acid levels ultimately lead to the formation of monosodium urate crystals (MSU). MSU crystals start to deposit in and around the joints where these crystals provoke cell necrosis and inflammation. Acute gout usually presents with symptoms of fever, deep pain, and marked inflammation of joints. The term "podagra" describes the condition when the first metatarsal joints are affected by urate crystals deposition during an acute attack of the disease. An acute stage lasts about 5 to 10 days followed by an asymptomatic period of hyperuricemia. Chronic gout persists for a longer time and presents with the formation of tophi. As the disease progresses, multiple tophi can be

* Correspondence: pharmacist.kanwal6@gmail.com

found in any region of the body like bones, cutaneous tissues, and joint spaces. If these tophi are left untreated, permanent disability can be resulted (Hainer et al., 2014).

Over the last ten years, the mortality and morbidity rate of gout has increased significantly, and the disease put more economic burden on the healthcare system. Joint pain, inflammation, restricted mobility, and treatment expenses negatively influence the patient's quality of life (QoL). Lack of awareness, suboptimal treatment, shortage of diagnostic facilities, and lifestyle of the patients are the key challenges that are needed to address to prevent or manage this disorder (Khanna et al., 2017; Ashiq et al., 2017). Currently, many treatment options are available such as NSAIDs (ibuprofen, naproxen, aspirin, and indomethacin), corticosteroids, uricosuric agents (probenecid and colchicine), cyclooxygenase 2 inhibitors (etoricoxib), and xanthine oxidase inhibitors (allopurinol, febuxostat and oxypurinol). However, side effects linked with existing therapies may limit their use in clinical practice, hence more future research is required to identify an effective treatment with minimum side effects and cut down the therapeutic expenses (Soskind et al., 2017; Abu Bakar et al., 2018; Bredemeier et al., 2018).

The use of medicinal plants is rising considerably to develop new pharmaceuticals that will have optimum pharmacological activity with minimum or no side effects. The biological activities of medicinal plants rely on the presence of valuable phytochemicals, for example, flavonoids, tannins, saponins, and alkaloids, etc. (Latif et al., 2019). Pakistan has a wide range of treasured plant flora due to its diverse topographic and climatic conditions. To date, almost 6000 vascular plants have been identified that play an imperative role in the socio-economy of Pakistan. Despite this fact, only a little scientific work has reported on their medicinal aspects, hence it demands more future research. Currently, very little literature is available that summarizes medicinal plants of the region and their potential role in therapeutics (Islam et al., 2021).

1.1. Rationale

Through an extensive literature survey, we have not found a single study that attempts to precisely recapitulate earlier studies conducted on Pakistani medicinal plants and their potential role in xanthine oxidase inhibition to treat gout.

1.2. Objective

The objective of this review is to assess systematically all published articles on medicinal plants located in Pakistan and their potential anti-gout effect. We hope that the present study will provide a considerable evidence-based comprehension of drug discovery and development methods, and it will prove as a reference for coming studies.

2. Methodology

2.1. Study reporting and registration

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA, 2009) statement has been followed for this study (supplementary file no: 1). This systematic review is registered with PROSPERO, NIHR (International Prospective Register of Systematic Reviews, National Institute for Health Research, United Kingdom). The registration number is CRD42021247416 (supplementary file no. 2).

2.2. Search strategy

Google Scholar, PubMed, Scopus, ScienceDirect, MEDLINE and Cochrane Library were searched for the keywords, including medicinal plants, Pakistan, hyperuricemia, xanthine oxidase inhibition, gout, in vivo, and in vitro. The articles published from January 2000 to April 2021 are included in this study.

2.3. Inclusion and exclusion criteria

In vitro and in vivo studies conducted on medicinal plants of Pakistan were considered for this systematic review. Studies that identified active compounds were used for this study as well. Articles published only in the English language were included in this study.

Narrative reviews, comments, systematic reviews, books, meta-analyses, duplicate articles, posters, case reports, doctoral thesis, and presentations were excluded from the current study.

2.4. Data extraction and statistical analysis

Six independent investigators were involved in extracting and examining the data. For this review, the data were collected according to a pre-designed form. The following information was included in the form: the plant name, family, part used, solvent for extraction, data on the bioassay/model used, inhibitory concentration (IC50), phytochemicals present in the extracts, major outcomes, author name, and year of publication. The data were entered and saved in an Excel sheet, checked for any duplication, and removed. Microsoft Office Excel Worksheet (2010) was used for constructing graphs. Due to methodological heterogeneity, meta-analysis was not performed.

2.5. Quality assessment of studies

A careful review of the retrieved reports was done independently by five researchers. The assessments included were as follows: carrying out a literature search, sequentially screening their titles, evaluating abstracts, and examining relevant studies. In case of any discrepancy, the sixth researcher was consulted to resolve a disagreement. For the experimental design of in vivo studies, the risk of bias was tested by using SYRCLE's RoB tool for animal studies (Hooijmans et al., 2014).

3. Results and discussion

3.1. Selection of the literature and study characteristics

A total of 338 records were identified based on the search strategy. We found 22 articles eligible for the current study based on inclusion criteria. Eleven articles including studies performed on synthetic compounds (n=6), in silico studies (n=2), and the plant material imported from other countries (n=3) were disqualified based on specific reasons. The literature selection process is illustrated in Figure 1.

The characteristics of the included studies are described in Table 1, encompassing the author's name, year of publication, study area, and the plant used. Also, the number of papers published per year was computed, and the results are displayed in Figure 2. The present study results have revealed that for the first time in 2006, research was published on Pakistani medicinal plants to test their potential inhibitory effect on xanthine oxidase. Before the year 2006, not a single scientific work was discovered by an exhaustive literature survey. Among 22 studies, only two studies have documented in vivo activity of the medicinal plants against gout. The first in vivo study was reported in 2008, followed by the second investigation documented in 2019.

3.2. In vitro studies

The xanthine oxidase inhibition activity has been assessed in 22 medicinal plants located in Pakistan. The present study outcomes have shown that plants selected for investigation belong to a diverse range of 22 families. Merely in four families named Apiaceae, Leguminosae,

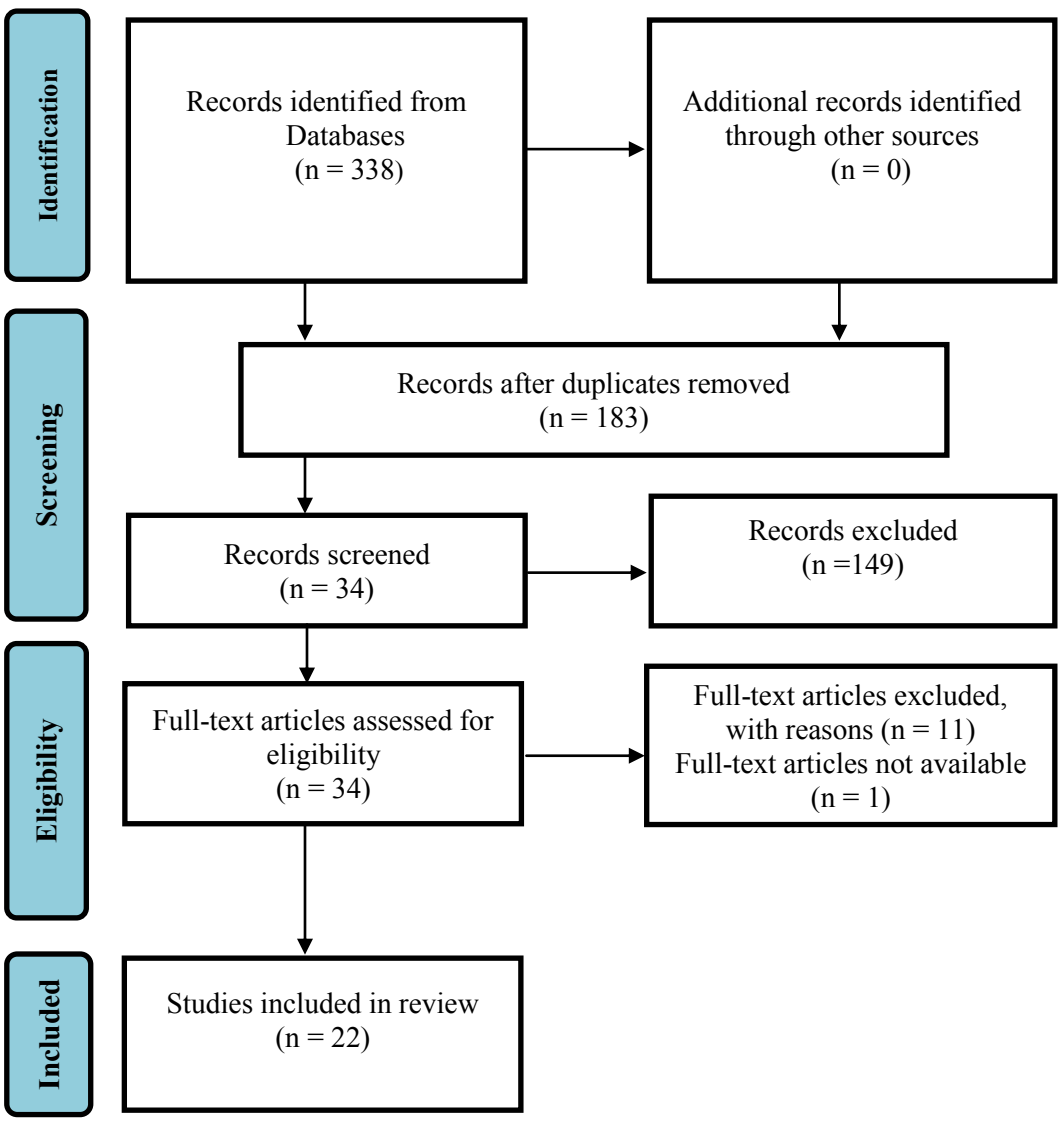

Figure 1. Flow diagram of study selection.

Table 1. Study characteristics.

Reference	Area of the plant collection in Pakistan	Plant name
Ahmad et al., 2008	Murree Hills	*Pistacia integerrima*
Ahmad et al., 2010	Kala Shah Kaku, Muridke	*Croton sparsiflorus*
Ahmad et al., 2010	North-West Frontier Province (N.W.F.P.) Swat	*Isatis costata*
Ahmad et al., 2017	Pattoki, District Kasur, Punjab	*Lagenaria siceraria*
Ahmad et al., 2019	Rawalpindi	*Cassia absus*
Ahmad et al., 2020	Karachi	*Apium graveolens, Colchicum autumnale, Berberis vulgeris, Trachyspermum ammi*
Akram et al., 2014	Karachi	*Apium graveolens, Colchicum autumnale, Tribulus terrestris, Withania somnifera, Zingiber officinale*
Alam et al., 2021	Tandyani (Hazara) KPK	*Viburnum grandiflorum*
Arshad et al., 2020	Cholistan Desert near Bahawalpur, Punjab,	*Indigofera argentea*
Bilal et al., 2019	Bahawalpur	*Trachyspermum ammi*
Gul et al., 2021	Karak, KPK	*Ricinus communis*
Karim et al., 2009	Karachi	*Barleria acanthoides*
Khan et al., 2006	Karachi	*Amberboa ramose*
Khan et al., 2008	Nathia Gali, Hazara Hills, N.W.F.P.	*Ranunculus repens*
Khurshid et al., 2019	Cholistan desert, district Bahawalpur	*Trianthema triquetra*
Latif et al., 2020	Botanical garden of University of the Punjab, Lahore	*Bryophyllum pinnatum*
Rauf et al., 2016	Gilgit	*Potentilla evestita*
Raziq et al., 2017	Peshawar	*Ranunculus muricatus*
Saeed and Ahmed, 2015	Collected from the hills near Abbottabad	*Carissa opaca*
Saeed and Shahwar, 2015	Lahore	*Syzygium aromaticum*
Shaheen et al., 2020	Multan	*Mimosa himalayana*
Shahwar et al.,2012	Lahore	*Laggera aurita*

Euphorbiaceae, and Ranunculaceae, a maximum of two species have been investigated. In the remaining families, only one plant species was chosen for the evaluation of xanthine oxidase activity. The data on the plant parts used in all studies is exhibited in Figure 3. The plant parts are used as whole plants (39%), roots (18%), seeds (11%), and leaves (7%). Fresh flowering shoots, aerial parts, tubers, bark, mesocarp of fresh fruit, and stem each constituted 4% individually. Pakistan has a rich diversity of botanical flora and a vast hub of many important medicinal plants, and, according to estimation, about 700 out of 6000 plant species have therapeutic value. Pakistani population greatly depends on medicinal plants for the treatment of various ailments. However, in Pakistan, the exploration of plants for medicinal purposes is still ignored. To date, only twenty-two plant species were evaluated for their potential anti-gout effect, and most of these researches were conducted in vitro. More concentration on exploratory studies of medicinal plants is desired in the future to address scientific gaps and for novel drug discovery and development (Jan et al., 2019).

The in vitro studies data encompassing author's name, year, plant name, family, extraction, xanthine oxidase inhibition (XOI) method followed, XOI %, IC 50 (ug/mL), phytochemicals, and outcomes of the assay are summarized in Table 2. The XOI% data was not available for the following plants: *Amberboa ramose*, *Barleria acanthoides*, *Isatis costata*, *Pistacia* integerrima, *Ranunculus repens*, *Ranunculus muricatus*, *Viburnum grandiflorum*, and *Potentilla evestita* (Khan et al., 2006; Ahmad et al., 2008; Karim et al., 2009; Ahmad et al., 2010; Rauf et al., 2016; Raziq et al., 2017; Alam et al., 2021). IC50 information was not recorded for these plants *Lagenaria siceraria*, *Cassia absus*, *Indigofera argentea*, and *Bryophyllum pinnatum* (Ahmed et al., 2017; Ahmad et al., 2019; Arshad et al., 2020; Latif et al., 2020). In seven medicinal plant species included, *Amberboa ramose*, *Barleria acanthoides*, *Isatis costata*, *Potentilla evestita*, *Ranunculus muricatus*,

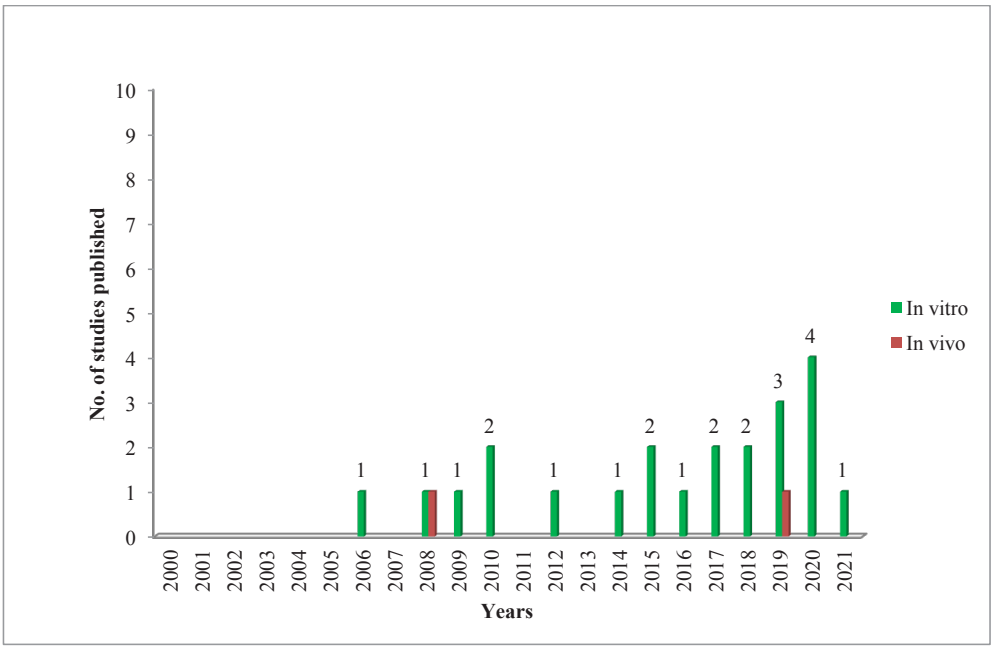

Figure 2. Number of papers published per year.

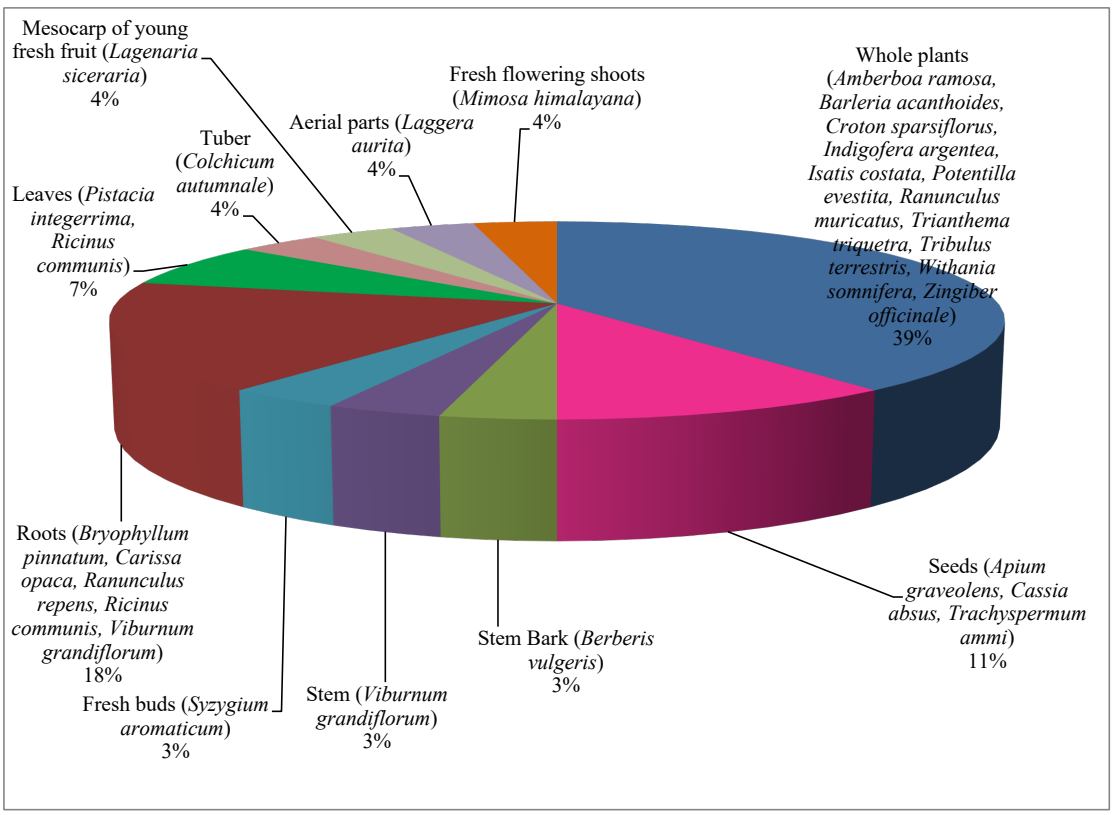

Figure 3. Parts of medicinal plants used in different studies.

Ranunculus repens, and *Syzygium aromaticum*, several phytochemicals were isolated and elucidated for potential XOI activity (Khan et al., 2008; Karim et al., 2009; Ahmad et al., 2010; Saeed and Shahwar, 2015; Rauf et al., 2016; Raziq et al., 2017). IC50 is a concentration at which 50% of the xanthine oxidase activity was inhibited. Khan et al.

Table 2. In vitro studies investigated to evaluate medicinal plants inhibitory effect on xanthine oxidase.

Reference	The plant scientific name	Family	Common/local name	Part used	Extraction	XOI (%)	IC50 (for the extracts and isolated compounds units are µg/mL and µM, respectively)	Active compound(s)	Outcomes of the assay
Ahmad et al., 2008	*Pistacia integerrima*	Anacardiaceae	Villana, Thoak, kakarsinghi, habb-e-suranjan (traditional dosage form)	Fresh leaves	Aqueous extract and solvent fractions	NA	Aqueous extract = 85 Ethanol:water (4:1) extract = 59 Ethanol:water (1:1) extract = 60 Chloroform extract = 44 Ethyl acetate extract = 20 n-butanol extract = 19	Flavonoids	Significant inhibitory activity against the enzyme xanthine oxidase
Ahmad et al., 2010	*Croton sparsiflorus*	Euphorbiaceae	Ban Tulasi, Raan Tulas	Whole plant	Extraction was done in nine different solvents in sequential manner (n-hexane, dichloromethane, chloroform, ethyl acetate, acetone, methanol, ethanol, n-butanol and water)	n-hexane extract = 7.1 ± 0.1 at 100 µg/mL Dichloromethane extract = 73.2 ± 0.3 at 100 µg/mL Chloroform extract = 75.1 ± 0.5 at 100 µg/mL Ethyl acetate extract = 74.2 ± 0.2 at 100 µg/mL Acetone extract = 70.4 ± 0.3 at 100 µg/mL Methanolic extract = 45.3 ± 0.8 at 100 µg/mL Ethanolic extract = 61.1 ± 1.0 at 100 µg/mL n-butanol extract = 55.8 ± 0.2 at 100 µg/mL Water extract = 8.5 ± 0.5 at 100 µg/Ml	n-hexane extract = -- Dichloromethane extract = 23.82 Chloroform extract = 17.34 Ethyl acetate extract = 9.31 Acetone extract = 11.35 Methanolic extract = -- Ethanolic extract = 43.25 n-butanol extract = 87.51 Water extract = --	NA	Moderate to significant inhibition of the xanthine oxidase enzyme
Ahmad et al., 2010	*Isatis costata*	Brassicaseae	NA	Whole plant	Ethanolic extract and solvent fractions	NA	Compound 1 Costinone A = 90.3±0.06 Compound 2 Costinone B =: 101.7±0.02 Compound 3 Isatinone A = 117.5±0.03 Compound 4 Isatinone B = 130.6±0.05 Compound 5 Indirubin = 170.5±0.01 Compound 6 Trisindoline = 179.6±0.04	Costinone A, Costinone B, Isatinone A, Isatinone B, Indirubin, Trisindoline	Compounds 1-6 showed a significant to moderate inhibition of the xanthine oxidase enzyme activity

Table 2. (Continued).

Ahmad et al., 2017	*Lagenaria siceraria*	Cucurbitaceae	Ghia or Ghia kaddu	Mesocarp of young fresh fruit	Methanolic extract of the fruit and its fractions	Aqueous extract = 0 at 100 µg/mL n-hexane = 20 at 100 µg/mL Methanol = 34 at 100 µg/mL Ethyl acetate = 35 at 100 µg/mL n-butanol = 52 at 100 µg/mL	NA	Revealed the presence of a variety of chemical compounds, including 4-(methoxymethyl) phenol, 2,2′-methylenebis, phenol, methyl 2-hydroxy-3-phenylpropanoate, oxacyclododecane-2,8-dione, N-(2-(4-hydroxyphenyl)ethyl] acetamide, 2,3-Epoxycarane, 4-(propan-2-yl) benzaldehyde, octadec-1-ene, 4amethyl-1-methylidene-1,2,3,4,4a,9,10,10a-octahydrophenanthrene, 2,6-di(propan-2-yl) naphthalene, methyl 3-(4-hydroxy-3,5-dimethylphenyl) propanoate, 4,8a-dimethyl-6-(prop-1-en-2-yl)-3,5,6,7,8,8a hexahydronaphthalen-2(1H)-one 1,3-dihydro-2-benzofuran, methyl N-hydroxybenzene carboximidoate, linoleic acid, and palmitic acid	Significant activity shown by n-butanol extract while no activity was reported in aqueous extract
Ahmad et al., 2019	*Cassia absus*	Leguminosae	Chaksu	Seeds	Ethanolic extract and its fractions	Ethanolic extract = 10.1±0.02 at 5 mg/mL n-butanol = 29.2±0.02 at 5 mg/mL Chloroform = 26.8±0.03 at 5 mg/mL n-hexane = 35.5±0.02 at 5 mg/mL Aqueous = 23.7±0.04 at 5 mg/mL	NA	NA	Weak to moderate inhibition of xanthine oxidase
Ahmad et al., 2020 Akram et al., 2014	*Apium graveolens*	Apiaceae	Tukhm-e-karafs	Seeds	Ethanolic extract prepared by soaking plant powder in solvent	54 at 100 µg/mL	Ethanolic extract = 180	Phenolic compounds, Flavonoids	Concentration-dependent XO inhibitory effect
Ahmad et al., 2020	*Berberis vulgeris*	Berberidaceae	Barbbery	Stem bark	Ethanolic extract prepared by soaking plant powder in solvent	52 at 100 µg/mL	Ethanolic extract = 121	Phenolic compounds, Flavonoids	Concentration-dependent XO inhibitory effect

Table 2. (Continued).

Ahmad et al., 2020 Akram et al., 2014	*Colchicum autumnale*	Colchicaceae	Suranjan shirin	Tuber	Ethanolic extract prepared by soaking plant powder in solvent	61 at 100 µg/mL	Ethanolic extract = 42	Phenolic compounds, Flavonoids	Concentration-dependent XO inhibitory effect
Ahmad et al., 2020	*Trachyspermum ammi*	Apiaceae	Ajwain	Seeds	Ethanolic extract prepared by soaking plant powder in solvent	78 at 100 µg/mL	Ethanolic extract = 19.8	Phenolic compounds, Flavonoids	Concentration-dependent XO inhibitory effect
Akram et al., 2014	*Tribulus terrestris*	Zygophyllaceae	Gokshur or Gokharu	Whole plant	Ethanolic extract prepared by soaking plant powder in solvent	78 at 100 µg/mL	Ethanolic extract = 19.8	NA	Concentration-dependent XO inhibitory effect
Akram et al., 2014	*Withania somnifera*	Solanaceae	Ashwagandha, Indian ginseng	Whole plant	Ethanolic extract prepared by soaking plant powder in solvent	69 at 100 µg/mL	Ethanolic extract = 95	NA	Concentration-dependent XO inhibitory effect
Akram et al., 2014	*Zingiber officinale*	Zingiberaceae	Adrak, Ginger	Whole plant	Ethanolic extract prepared by soaking plant powder in solvent	80 at 100 µg/mL	Ethanolic extract = 23.6	NA	Significant inhibitory activity against the enzyme xanthine oxidase
Alam et al., 2021	*Viburnum grandiflorum*	Caprifoliaceae	Guch	Stem and roots	Ethanol extract and its chloroform soluble fraction	NA	Compound 1 Benzofuran = 49.52 + 0.03	2, 3-dihydro-2-(4′-hydroxy, 3′-methoxyphenyl)-3-hydroxymethyl-5-(2-formylvinyl) 7-hydroxybenzofuran (benzofuran)	Significant concentration dependent inhibition of the enzyme
Arshad et al., 2020	*Indigofera argentea*	Fabaceae	Neel, Jantari, Hathio	Whole plant	Methanol, n-hexane, chloroform, and n-butanol extracts/ fractions	n-hexane extract = 47.3 ± 0.5 at 5 mg/mL Chloroform extract = 50 ± 0.7 at 5 mg/mL n-butanol extract = 52.5 ± 0.9 at 5 mg/mL Methanolic extract = 67.85 ± 0.4 at 5 mg/mL	NA	Flavonoids, Lithocholic acid, terpenes	Moderate to significant inhibition of the xanthine oxidase enzyme
Bilal et al., 2019					Ethanolic extract prepared by maceration method	72±0.5 at 0.5 mg/mL	Ethanolic extract = 68.6±0.2	Phenolic compounds, Flavonoids	Significant inhibitory activity against the enzyme xanthine oxidase
Gul et al., 2021	*Ricinus communis*	Euphorbiaceae	Castor Bean	Roots and leaves	Methanolic extract and its green synthesis of sliver nanoparticles	Root extract = 23 at 100 µg/mL Leaves extract = 24 at 100 µg/mL Root silver nanoparticles extract = 83.6 at 100 µg/mL Leaves silver nanoparticles extract = 83 at 100 µg/mL	Root silver nanoparticles extract = 3.60 ± 0.04 Leaves silver nanoparticles extract = 3.8 ± 0.03	NA	Significant inhibitory activity against the enzyme xanthine oxidase
Karim et al., 2009	*Barleria acanthoides*	Acanthaceae	Thath	Whole plant	Methanolic extract and solvent fractions	NA	Compound 1 Barlerisides A = 419.0±3.167 Compound 2 Barlerisides B = 426.7±1.87	Two new glycosides called Barlerisides A and B, acteoside, p-hydroxycinnamic acid	Comparable but weak inhibitory activity against the enzyme xanthine oxidase

Table 2. (Continued).

Khan et al., 2006	*Amberboa ramose*	Compositae	Jafri	Whole plant	Methanolic extract and solvent fractions	NA	Compound 1 5,7,4′-trihydroxy-3,8-dimethoxylflavone 5-O-β-D-gluco-pyranoside = 408.5 Compound 2 6,4′-dihydroxy-3,5,7-trimethoxyflavone = 139.2 Compound 3 5,7-dihydroxy-4′-methoxyflavone = 177.8	New flavonol glycoside reported; 5,7,4′-trihydroxy-3,8-dimethoxylflavone 5-O-β-D-gluco-pyranoside, 6,4′-dihydroxy-3,5,7-trimethoxyflavone, 5,7-dihydroxy-4′-methoxyflavone, (23R)-5α-cycloart-24-ene-3β,21,23-triol	Moderate inhibition of xanthine oxidase enzyme by compound 2 and compound 3
Khan et al., 2008	*Ranunculus repens*	Ranunculaceae	Buttercup	Roots	Methanolic extract and solvent fractions	NA	Compound 1 R(+)-dalbergiphenol = 217.8 Compound 2 R(+)-4-methoxydalbergione = 359.2 Compound 3 methyl-3,4,5-trihydroxybenzoate = 59.5	R(+)-dalberg phenol, R(+)-4-methoxydalbergione, methyl-3,4,5-trihydroxybenzoate	Significant inhibitory activity against the enzyme xanthine oxidase
Khurshid et al., 2019	*Trianthema triquetra*	Aizoaceae	Red spinach, Choti Ulwaiti	Whole plant	Methanolic extract and its fractions	Methanolic extract = 26.32±0.9 at 5 mg/mL n-hexane = 77.7±0.7 at 5 mg/mL Ethyl acetate = 51.9±0.5 at 5 mg/mL n-butanol = 19.1±0.4 at 5 mg/mL Aqueous = 45.7±1 at 5 mg/mL	Methanolic extract = -- n-hexane = 0.9±0.5 Ethyl acetate = 4.7±0.8 n-butanol = -- Aqueous = --	9,12-octadecadienoic acid (Z,Z)-, methyl ester, hexadecanoic acid, methyl ester, 9-octadecenoic acid (Z)-, methyl ester, 9,12-octadecadienoic acid, ethyl ester, hexadecanoic acid, ethyl ester, ethyl oleate, 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester, vitamin E	Significant inhibitory activity against the enzyme xanthine oxidase
Latif et al., 2020	*Bryophyllum pinnatum/ Kalanchoe pinnata*	Crassulaceae	Pathar chat	Roots	Sequential extraction was carried out by using solvents; n-hexane, chloroform, methanol and water	n-Hexane extract = 7.34 + 0.002 at 2mg/mL Chloroform extract = 31.54 + 0.005 at 2mg/mL Methanolic extract = 88.30 + 0.004 at 2mg/mL Water extract = 44.22 + 0.04 at 2mg/mL	NA	Polyphenols, Flavonoids, Glycosaponin, Polysaccharides	Significant inhibitory activity against the enzyme xanthine oxidase
Rauf et al., 2016	*Potentilla evestita*	Rosaceae	NA	Whole plant	Methanolic extract and solvent fractions	NA	Compound 1 Acacetin 1 = 11.92±0.01 Compound 2 Chrysin 2 = 73.74±0.02 Compound 3 Umbelliferone 3 = 97.12±0.01	Acacetin 1, Chrysin 2, Umbelliferone 3	Significant inhibitory activity against the enzyme xanthine oxidase
Raziq et al., 2017	*Ranunculus muricatus*	Ranunculaceae	Chambel, Crowfoot, Buttercup	Whole plant	Methanolic extract and solvent fractions	NA	Compound 1 Ranuncoside = 43.3 ± 0.22	Ranuncoside	Significant inhibitory activity against the enzyme xanthine oxidase

Table 2. (Continued).

Saeed and Ahmad 2015	*Carissa opaca*	Apocynaceae	Granda	Roots	Methanolic extract prepared by cold maceration and its fractions	Methanolic extract = 45 at 100 µg/mL Ethyl acetate = 43 at 100 µg/mL Chloroform = 43 at 100 µg/mL n-butanol = 18 at 100 µg/mL n-hexane = --	Methanolic extract = 156 Ethyl acetate = 129 Chloroform = 154.2	Polyphenols, flavonoids, Limonene, vanillin, lupeol, rutin, quercetin, β-sitosterol, Vitamin E, 2-hydroxyacetophenone, naphthalenone, 2,3,3-trimethyl-2-(3-methylbuta-1,3-dienyl)-6-methylenecyclohexanone, and 2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester.	Significant inhibitory activity against the enzyme xanthine oxidase
Saeed and Shahwar, 2015	*Syzygium aromaticum*	Myrtaceae	Clove	Fresh buds	Extract prepared by steam distillation using Dean Stark apparatus	Compound 1 Essential oil = 85.1±2.2 at 150 µg/mL Compound 2 Eugenol = 81.2±3.0 at 150 µg/mL Compound 3 Hyoscine = --	Compound 1 Essential oil = 61.9±2.1 Compound 2 Eugenol = 43.5±2.6 Compound 3 Hyoscine = --	Essential oil, Eugenol, Hyoscine	Significant inhibitory activity against the enzyme xanthine oxidase
Shaheen et al., 2020	*Mimosa himalayana*	Leguminosae/ Mimosaceae		Fresh flowering shoots	Ethanolic extract prepared by maceration	66.1 at 100 µg/mL	Ethanolic extract = 83.5	NA	Significant inhibitory activity against the enzyme xanthine oxidase
Shahwar et al., 2012	*Laggera aurita*	Asteraceae	NA	Aerial parts	Extraction was done by steam distillation using Dean Stark apparatus.	79.05 ± 7.39 at 100 µg/mL	Extract = 45 ± 0.009	Fatty acids, sesquiterpene, hydrocarbon, Monoterpene, phenolic derivatives	Significant inhibitory activity against the enzyme xanthine oxidase

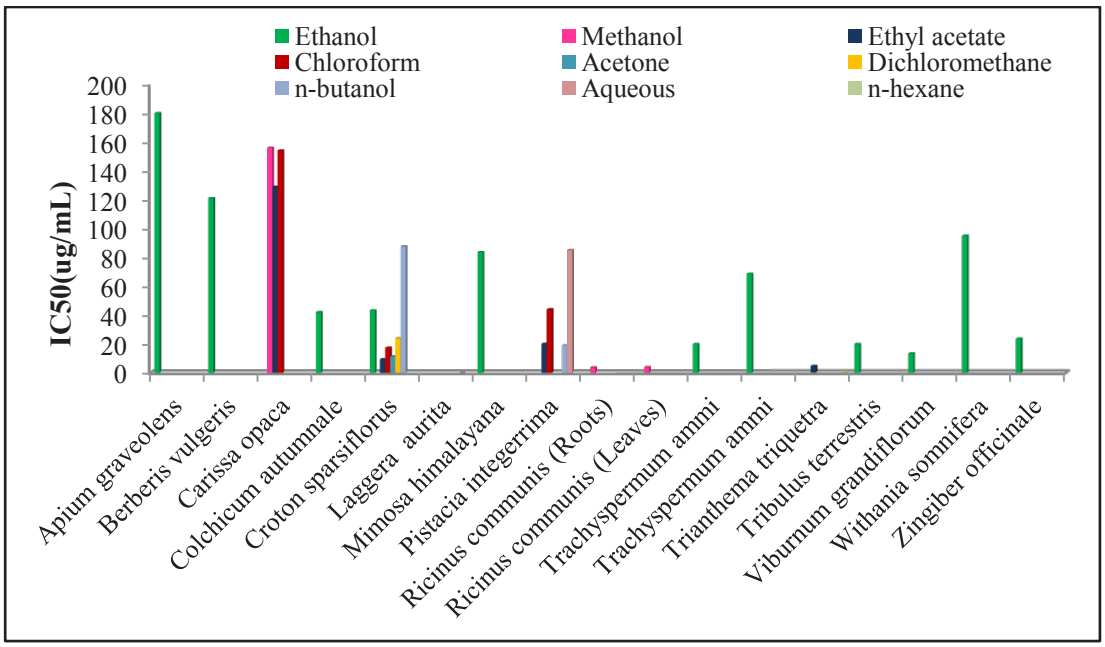

Figure 4. IC50 comparison of the plants in various extracts.

(2006) investigated *Amberboa ramosa* and isolated three flavonoids (5,7,4′-trihydroxy-3,8-dimethoxylflavone 5-O-β-D-gluco-pyranoside (IC50= 408.5 µM), 5,7-dihydroxy-4′-methoxyflavone (IC50= 177.8 µM), and 6,4′-dihydroxy-3,5,7-trimethoxyflavone (IC50= 139.2 µM) that possessed weak to moderate xanthine oxidase inhibitory activity (Khan et al., 2006). In proceeding studies, the presence of many chemicals (cinnamic acid, stigmasterol, marrusidin A, and marrusidin B) has been confirmed in *Amberboa ramose;* however, limited data are available on its pharmacological activities (Ibrahim et al., 2012). *Barleria acanthoides* contains two potent phenolic glycosides called barlerisides A (IC50= 419.0 ± 3.167 µM) and barlerisides B (IC50= 426.7 ± 1.87 µM), but they possessed weak activity against xanthine (Karim *et al.,* 2009). Six oxindole alkaloids (costinone A: IC50= 90.3 ± 0.06µM, costinone B: IC50= 101.7±0.02 µM, isatinone A: IC50= 117.5 ± 0.03 µM, isatinone B: IC50= 130.6 ± 0.05 µM, indirubin: IC50= 170.5 ± 0.01 µM, and trisindoline: IC50= 179.6 ± 0.04 µM) were isolated from *Isatis costata,* and these entire chemicals showed moderate to substantial xanthine oxidase inhibition activity (Ahmad et al., 2010). The assessment of other plants revealed the occurrence of many valuable phytochemicals *Potentilla evestita*; acacetin 1 (IC50= 11.92 ± 0.01 µM), chrysin 2 (IC50= 73.74 ± 0.02 µM), and umbelliferone (IC50= 97.12 ± 0.01 µM), *Ranunculus repens*; 3R(+)-dalbergiphenol (IC50= 217.8 µM), R(+)-4-methoxydalbergione (IC50= 359.2 µM), and methyl-3,4,5-trihydroxybenzoate (IC50= 59.5 µM), *Ranunculus muricatus*; ranuncoside (IC50= 11.69 µM), and *Syzygium aromaticum*; essential oil (IC50= 61.9 ± 2.1 µM) and eugenol (IC50= 43.5±2.6 µM) have displayed a significant inhibitory action on the xanthine oxidase enzyme. Until now, these phytochemicals were not further examined or developed into a formulation to treat gout (Khan et al., 2008; Saeed and Shahwar, 2015; Raziq et al., 2017; Rauf et al., 2018).

In fifteen plants, xanthine oxidase inhibition activity was measured in different extracts (Table 2, Figure 4). Ethanol was frequently used as a solvent for the extraction followed by methanol and water. The selection of solvent has a considerable influence on the isolation of chemical compounds, pharmacological activity, and toxicity (Abu Bakar et al., 2018). *Apium graveolens, Berberis vulgeris, Colchicum autumnale, Trachyspermum ammi, Tribulus terrestris, Withania somnifera,* and *Viburnum grandiflorum* exhibited concentration-dependent inhibition of xanthine oxidase. A significant activity against xanthine oxidase inhibition was also shown by *Pistacia integerrima*, *Lagenaria siceraria*, *Ricinus communis*, *Trianthema triquetra*, *Bryophyllum pinnatum*, *Carissa opaca*, *Mimosa himalayana*, and *Laggera aurita*. *Croton sparsiflorus and Indigofera argentea* exhibited mild to significant activity, while *Cassia absus* had weak to moderate activity against the xanthine oxidase.

The maximum XOI% of plants according to the available information is found as: *Bryophyllum pinnatum* 88.30% (methanolic extract) at 2 mg/mL, *Zingiber officinale* 80% (ethanolic extract) at 100 µg/mL, *Laggera aurita* 79.05% (ethanolic extract) at 100 µg/mL, *Trachyspermum ammi*

Table 3. In vivo studies investigated to evaluate medicinal plants inhibitory effect on xanthine oxidase.

Author (Year)	Scientific Name	Family	Common/ local name	Part used	Extraction	Experimental animal model	Dose	Active compound(s)	Toxicity	Outcomes of the assay
Ahmad et al., 2008	*Pistacia integerrima*	Anacardiaceae	Thoak, kakarsinghi, marketed in a traditional dosage form 'habb-e-suranjan' by Hamdard Laboratories (WAQF) Pakistan	Fresh leaves	Aqueous extract and solvent fractions	Uric acid lowering effect in fructose- induced hyperuricemic mice model as described by Newman and Price (1996)	10 mg/kg to 160 mg/kg extract dose given to mice. Blood samples were collected after every 1 to 4 hours to measure serum uric acid	Flavonoids	ED 20 = 28 mg/kg	Dose-dependent inhibitory activity against xanthine oxidase enzyme
Bilal et al., 2019	*Trachyspermum ammi*	Apiaceae	Ajwain	Seeds	Ethanolic extract prepared by maceration method	Hyperuricemia was induced in rats by administration of potassium oxonate as described by Zaho et al., (2006)	250 mg/kg and 500 Mg/kg for 1, 3, and 7 day s, respectively.	Phenolic compounds, Flavonoids	No data on toxicity was reported. However, previous dose fixation studies showed non-toxic nature of *T.ammi* seeds extract from the dose range of 1 and 5 g/kg body weight in normal rats.	Inhibit xanthine oxidase enzyme activity in time-dependent manner

78% (ethanolic extract) at 100 µg/mL, *Tribulus terrestris* 78% (ethanolic extract) at 100 µg/mL, *Trianthema triquetra* 77.7% (n-hexane extract) at 5 mg/mL, *Croton sparsiflorus* 75.1% (chloroform extract) at 100 µg/mL, *Indigofera argentea* 67.8% (methanolic extract) and 72% (ethanolic extract) at 5 mg/mL, *Withania somnifera* 69% (ethanolic extract) at 100 µg/mL, *Indigofera argentea* 67.8% (methanolic extract), *Mimosa himalayana* 66.1% (ethanolic extract) at 100 µg/mL, *Lagenaria siceraria* 52% (n-butanol extract) at 100 µg/mL, *Apium graveolens* 54% (ethanolic extract) at 100 µg/mL, *Berberis vulgeris* 52% (ethanolic extract) at 100 µg/mL, *Colchicum autumnale* 61% (ethanolic extract) at 100 µg/mL, and *Cassia absus* 29.2% (n-butanol extract) at 5 mg/mL. Gul et al., (2021) conducted a study on *Ricinus communis* roots and its leaves methanolic extract, and also prepared their silver nanoparticles. This research suggested that XOI% of the methanolic extract of silver nanoparticles (roots 83.6% and leaves 83%) showed more effectiveness in comparison to simple methanolic extract (roots 23%, and leaves 24%).

The phytochemical investigation was not performed on *Croton sparsiflorus*, *Zingiber officinale*, *Withania somnifera*, *Tribulus terrestris*, *Ricinus communis*, and *Mimosa himalayana* hence there is a need for more in-depth study. Other than these plants, the phytochemical analysis demonstrated the presence of many important compounds, i.e. flavonoids, polyphenols, alkaloids, and glycosides. It is proven by previous studies that flavonoids, alkaloids, glycosides, and phenolic compounds can inhibit or lessen the production of uric acid. These constituents tend to reduce inflammation by their uricosuric effect. It is proposed that those plants that owned anti-oxidant potential could be used as a potent inhibitor of the xanthine oxidase. In light of the above-mentioned facts, it is suggested that further research should be conducted to spot the bioactive compounds of the medicinal plants and to comprehend their mechanism of action so that the wisdom behind their anti-gout effect can be justified. It will also extend the usage of natural therapies with minimum side effects and maximum efficacy, as there is hardly a known natural remedy that is available to treat gout (Ling and Bochu, 2014; Ojha et al., 2017). Medicinal plants indigenous to Pakistan have shown a significant anti-gout effect. These plants have important bioactive compounds (i.e. polyphenols, flavonoids, tannins, steroids, etc.) that can be used to treat gout particularly in alternative or complementary medicines. The regional difference could have an impact on the synthesis and activity of bioactive compounds. The current information can be used to correlate the findings of the local region to the other regions of the world (El Dahiyat et al., 2020). For example, a study conducted in Pakistan on *Zingiber officinale* has shown similar xanthine oxidase inhibitory activity (80%) in relation to the study conducted in Malaysia (81.56%) (Abu Bakar et al., 2018). *Apium graveolens* seeds have exhibited higher (54 at 100 µg/mL) xanthine oxidase activity in a Pakistan study as compared to a study conducted in Czech on aerial parts of the plant (1.4 at 200 µg/mL) (Havlik et al., 2010).

3.3. In vivo studies

The results obtained from the in vitro studies provide valuable knowledge and guidance for further screening in animals. The data relating to in vivo studies are summarized in Table 3. So far, in vivo studies were conducted only about in two plants, named *Pistacia integerrima* (Ahmad et al., 2008) and *Trachyspermum ammi* (Bilal et al., 2019). Both plants exhibited significant in vitro activity as well. Ahmad et al. (2008) used a fructose-induced hyperuricemia model. Fructose solution in large doses amplifies the production of AMP and ADP and causes the up-regulation of AMP deaminase. The over-activity of xanthine oxidase (XO) enzyme and substrate will boost the production of uric acid and reactive oxygen species (ROS) (Nakagawa et al., 2019). *Pistacia integerrima* fresh leaves were extracted in an aqueous solvent, and its various fractions were made in different solvents. Ethyl acetate fraction showed promising results and had a dose-dependent (varying from 8% at 20 mg/kg to 57.52% 20 mg/kg) effect on serum uric acid level. The study outcomes revealed that *Pistacia integerrima* showed efficacy almost equal to that of quercetin (45.6% at 25 mg/kg) but less than allopurinol (77.4% at 10 mg/kg). The phytochemical analysis demonstrated the presence of flavonoids in the plant (Ahmad et al., 2008).

Trachyspermum ammi seeds were extracted in ethanol, and the potassium oxonate hyperuricemia model was applied to measure the anti-gout effect. Potassium oxonate induces hyperuricemia by blocking uricase as this enzyme is important for the excretion of uric acid. At the first day of treatment, allopurinol considerably decreases the serum uric acid level (2.8 mg/dL at a dose of 10 mg/kg), while *Trachyspermum ammi* exhibited moderate response (4.1 mg/dL at 250 mg/kg and 3.8 mg/dL at 500 mg/kg). At the seventh day, *Trachyspermum ammi* demonstrated comparable response (2.5 mg/dL at 250 mg/kg and 2.1 mg/dL at 500 mg/kg) with allopurinol (2.51 mg/dL at 10 mg/kg). It is evident from the findings that *Trachyspermum ammi* displayed a time-dependent response and also enhanced the renal excretion of uric acid. The chemical analysis of the plant indicated that it contains flavonoids and polyphenols. The toxicity studies of the plant ware not performed (Bilal et al., 2019).

It is believed that the presence of flavonoids and phenolic compounds in the plant makes them a favorable choice to treat a triad of disorders like gouty arthritis, ROS, and hyperuricemia due to their potential antiinflammatory, antioxidant, and xanthine oxidase

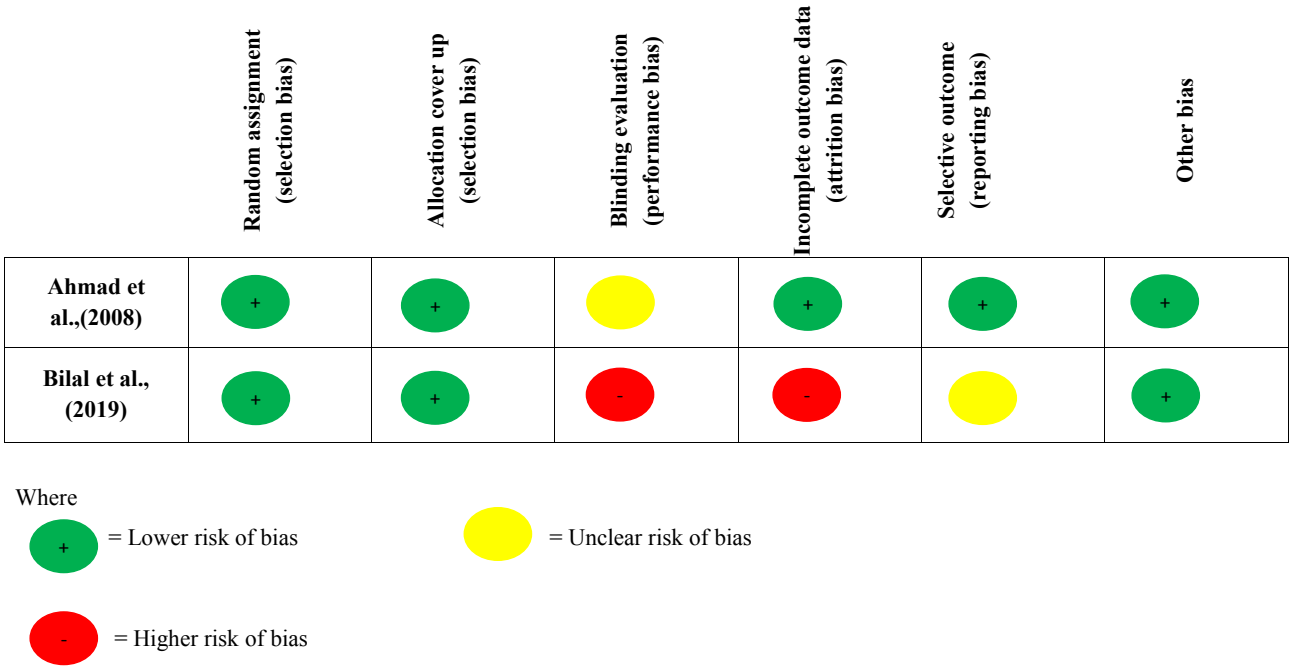

Figure 5. Categories of different risk of bias in two in vivo studies

inhibitory effect (Tungmunnithum et al., 2019). Advance research is recommended in order to evaluate medicinal plants in terms of their potential efficacy, bioavailability, and possible toxicity. Furthermore, the clinical assessment of the formulations based on medicinal plants must be performed (Abu Bakar et al., 2018).

3.4. Study methodological quality and risk of bias

The risk of bias (RoB) was checked according to SYRCLE's RoB tool to augment the transparency and applicability of the animal studies. The results are illustrated in Figure 5. The SYRCLE's RoB tool is a tailored version of the Cochrane RoB tool, which is implemented to critically evaluate animal interventions. These papers were characterized based on the following parameters: random allocation of the animals, detailed procedure of animal confinement, guidelines followed for animal handling, blinding details, control and reference groups, experimental design, and reporting of the data. Only in one study, information on blinding evaluation and estimation of the plant toxicity was not reported. The animal studies lacking information on the randomization and blinding process are considered less significant scientifically while applying animal models (Hooijmans et al., 2014; Gandhi et al., 2016).

3.5. Limitations

For the current review, articles only published in the English language were considered. This review only focused on medicinal plants that are available in Pakistan, and the results obtained from other regions of the world must be summarized to have a comprehensive document. The standardization of plant extract is essential to validate therapeutic potency and safety. As many extracts are used without standardization, care must be taken when generalizing the findings. Only preliminary phytochemical investigation of the plants was done in animal studies therefore, exhaustive analysis is a prerequisite to identify the chief component responsible for pharmacological activity. In addition, toxicological studies of the extracts must be performed so that possible side effects will be elucidated before the clinical trials.

4. Conclusion

This systematic review assembled scattered data on xanthine oxidase activity of medicinal plants which are sited in Pakistan. Many isolated phytochemicals prove to be beneficial in the reduction of hyperuricemia. So far, little scientific work is found, and a majority of the studies are conducted in vitro. Ethnic and cultural knowledge may help in identifying medicinal plants that have desired curative effects. More inclusive pharmacological and clinical studies are warranted to ensure the safety, efficacy, and toxicity of the medicinal plants, and their translational use in human therapeutics.

Competing interests

The authors declare no conflict of interest among them.

References

Abu Bakar FI, Abu Bakar MF, Rahmat A, Abdullah N, Sabran SF et al. (2018). Anti-gout potential of Malaysian medicinal plants. Frontiers in Pharmacology 9: 261-275. doi: 10.3389/fphar.2018.00261

Ahmad I, Ijaz F, Fatima I, Ahmad N, Chen S et al. (2010). Xanthine oxidase/tyrosinase inhibiting, antioxidant, and antifungal oxindole alkaloids from *Isatis costata*. Pharmaceutical Biology 48 (6): 716-721. doi: 10.3109/13880200903271298

Ahmad N, Shahwar D, Khan MA, Mughal MS (2010). Antioxidant potential and xanthine oxidase inhibitory activity of *Croton sparsiflorus*. Asian Journal of Chemistry 22 (1): 555.

Ahmad NS, Farman M, Najmi MH, Mian KB, Hasan A (2008). Pharmacological basis for use of *Pistacia integerrima* leaves in hyperuricemia and gout. Journal of Ethnopharmacology 117 (3): 478-482. doi: 10.1016/j.jep.2008.02.031

Ahmad S, Hassan A, Rehman T, Basit A, Tahir A et al. (2019). In vitro bioactivity of extracts from seeds of *Cassia absus* L. growing in Pakistan. Journal of Herbal Medicine 6:100258. doi: 10.1016/j.hermed.2019.100258

Ahmad S, Mohiuddin E, Shah SM, Akram M, Amjad M et al. (2020). Therapeutic efficacy of urinile against gouty arthritis. Dose-Response 18 (4): 1559325820946934. doi: 10.1177%2F1559325820946934

Ahmed D, Dar P, Chaudhery R, Masih R (2017). Chemical constituents of *Lagenaria siceraria* mesocarp and its xanthine oxidase and alpha-amylase inhibitory activities. International Journal of Fruit Science 17 (3): 310-322. doi: 10.1080/15538362.2017.1315628

Akram M, Usmanghani K, Ahmed I, Azhar I, Hamid A (2014). In vitro xanthine oxidase inhibitory and in vivo hypouricemic activity of herbal coded formulation (Gouticin). Pakistan Journal of Pharmaceutical Sciences 27 (3): 541-550.

Alam M, Uddin G, Rashid U, Rauf A, Raza M et al. (2021). In vitro and in silico xanthine oxidase inhibitory potential of benzofuran isolated from *Viburnum grandiflorum* Wall. Ex DC. South African Journal of Botany (in press) doi: 10.1016/j.sajb.2021.01.010

Arshad A, Rehman T, Saleem H, Khan S, Saleem M et al. (2020). In vitro enzyme inhibition, antibacterial, UHPLC-MS chemical profiling and in silico studies of *Indigofera argentea* Burm. f. for potential biopharmaceutical application. South African Journal of Botany (in press). doi: 10.1016/j.sajb.2020.12.001

Ashiq K, Bajwa MA, Tanveer S, Qayyum M, Ashiq S et al. (2021). A comprehensive review on gout: the epidemiological trends, pathophysiology, clinical presentation, diagnosis and treatment. Journal of Pakistan Medical Association 71 (4): 1234-1237. doi: 10.47391/JPMA.313

Ashiq, K, Rehman K, Ashiq S, Sundus A (2017). Influence of osteoporosis on quality of life and current strategies for its management and treatment. GSC Biological and Pharmaceutical Sciences 1 (2): 34-40. doi: 10.30574/gscbps.2017.1.2.0051

Bardin T, Richette P (2017). Impact of comorbidities on gout and hyperuricaemia: an update on prevalence and treatment options. BMC Medicine 15 (1): 1-10. doi: 10.1186/s12916-017-0890-9

Bilal M, Ahmad S, Rehman T, Abbasi WM, Ghauri AO et al. (2019). Effects of *Trachyspermum ammi* L.(Apiaceae) on serum, urine and hepatic uric acid levels in oxonate-induced rats and in vitro xanthine oxidase inhibition assay. Indian Journal of Traditional Knowledge 18 (1): 52-57.

Bredemeier M, Lopes LM, Eisenreich MA, Hickmann S, Bongiorno GK et al. (2018). Xanthine oxidase inhibitors for prevention of cardiovascular events: a systematic review and meta-analysis of randomized controlled trials. BMC Cardiovascular Disorders 18 (1): 24. doi: 10.1186/s12872-018-0757-9

El Dahiyat F, Rashrash M, Abuhamdah S, Farha RA, Babar ZUD (2020). Herbal medicines: a cross-sectional study to evaluate the prevalence and predictors of use among Jordanian adults. Journal of Pharmaceutical Policy and Practice 13(1):1-9. doi: 10.1186/s40545-019-0200-3

El Ridi R, Tallima H (2017). Physiological functions and pathogenic potential of uric acid: A review. Journal of Advanced Research 8 (5): 487-493. doi: 10.1016/j.jare.2017.03.003

Gandhi GR, Barreto PG, Santos LB, Quintans JD, Souza AA et al. (2016). Medicinal plants and natural molecules with in vitro and in vivo activity against rotavirus: a systematic review. Phytomedicine 23 (14): 1830-1842. doi: 10.1016/j.phymed.2016.11.005

Gul A, Shaheen A, Ahmad I, Khattak B, Ahmad M et al. (2021). Green synthesis, characterization, enzyme inhibition, antimicrobial potential, and cytotoxic activity of plant mediated silver nanoparticle using *Ricinus communis* leaf and root extracts. Biomolecule 1 (2): 206. doi: 10.3390/biom11020206

Hainer BL, Matheson E, Wilkes RT (2014). Diagnosis, treatment, and prevention of gout. American Family Physician 90 (12): 831-836.

Havlik J, Huebra RG, Hejtmankova K, Fernandez J, Simonova J et al. (2010). Xanthine oxidase inhibitory properties of Czech medicinal plants. Journal of Ethnopharmacology 132 (2): 461-465. doi: 10.1016/j.jep.2010.08.044

Hooijmans CR, Rovers MM, Vries RB, Leenaars M, Ritskes-Hoitinga M et al. (2014). SYRCLE's risk of bias tool for animal studies. BMC Medical Research Methodology 14 (1): 1. doi: 10.1186/1471-2288-14-43

Ibrahim M, Imram M, Hussain A, Aslam M, Rehmani F et al. (2012). Phytochemical studies on *Amberboa ramose*. Pakistan Journal of Chemistry 2 (1): 24-28.

Islam M, Ahmad I, Akhtar N, Alam J, Razzaq A et al. (2021). Medicinal plants resources of Western Himalayan Palas Valley, Indus Kohistan, Pakistan: Their uses and degrees of risk of extinction. Saudi Journal of Biological Sciences 28 (5): 3076-3093. doi: 10.1016/j.sjbs.2021.02.051

Jan HA, Jan S, Bussmann RW, Wali S, Sisto F et al. (2019). Complementary and alternative medicine research, prospects and limitations in Pakistan: A literature review. Acta Ecologica Sinica 40 (6): 451-463. doi: 10.1016/j.chnaes.2019.12.005

Kanwal A, Abida L, Sana A, Ahlam S (2018). A systematic review on the prevalence, pathophysiology, diagnosis, management and treatment of gout (2007-2018). GSC Biological and Pharmaceutical Sciences 5 (1): 50–055. doi: 10.30574/gscbps.2018.5.1.0077

Karim A, Noor AT, Malik A, Qadir MI, Choudhary MI (2009). Barlerisides A and B, new potent superoxide scavenging phenolic glycosides from *Barleria acanthoides*. Journal of Enzyme Inhibition and Medicinal Chemistry 4 (6): 1332-1335. doi: 10.3109/14756360902887046

Khan SB, Afza N, Malik A, Azhar UH, Perveen S et al. (2006). Xanthine oxidase inhibiting flavonol glycoside from *Amberboa ramosa*. Natural Product Research 20 (04): 335-339. doi: 10.1080/14786410500182110

Khan WN, Ali I, Gul R, Choudhary MI (2008). Xanthine oxidase inhibiting compounds from *Ranunculus repens*. Chemistry of Natural Compounds 44 (1): 95-7. doi: 10.1007/s10600-008-0027-7

Khanna PP, Nuki G, Bardin T, Tausche AK, Forsythe A et al. (2012). Tophi and frequent gout flares are associated with impairments to quality of life, productivity, and increased healthcare resource use: results from a cross-sectional survey. Health and Quality of Life Outcomes 10 (1): 1. doi: 10.1186/1477-7525-10-117

Khurshid U, Ahmad S, Rehman T, Arshad MA, Pervaiz I et al. (2019). GC-MS analysis, DPPH and enzyme inhibition assays of *Trianthema triquetra* Rottl. and wild. growing in Pakistan. Latin American Journal of Pharmacy 38 (6): 1181-1187.

Kuo CF, Grainge MJ, Zhang W, Doherty M (2015). Global epidemiology of gout: prevalence, incidence and risk factors. Nature Reviews Rheumatology 11 (11): 649. doi: 10.1038/nrrheum.2015.91

Latif A, Ashiq K, Ashiq S, Ali E, Anwer I et al. (2020). Phytochemical analysis and in vitro investigation of anti-inflammatory and xanthine oxidase inhibition potential of root extracts of *Bryophyllum pinnatum*. Journal of Animal and Plant Sciences 30 (1): 219-228. doi: 10.36899/JAPS.2020.1.0025

Latif A, Ashiq K, Qayyum M, Ashiq S, Ali E et al. (2019). Phytochemical and pharmacological profile of the medicinal herb: *Bryophyllum pinnatum*. Journal of Animal and Plant Sciences 9 (6): 1528-1534.

Ling X, Bochu W (2014). A review of phytotherapy of gout: perspective of new pharmacological treatments. Die Pharmazie 69 (4): 243-256. doi: 10.1691/ph.2014.3642

Nakagawa T, Lanaspa MA, Johnson RJ (2019). The effects of fruit consumption in patients with hyperuricaemia or gout. Rheumatology 58 (7): 1133-1141. doi: 10.1093/rheumatology/kez128

Ojha R, Singh J, Ojha A, Singh H, Sharma S et al. (2017). An updated patent review: xanthine oxidase inhibitors for the treatment of hyperuricemia and gout (2011-2015). Expert Opinion on Therapeutic Patents 27 (3): 311-345. doi: 10.1080/13543776.2017.1261111

Rauf A, Khan R, Khan H, Khan I, Akram M (2016). Xanthine oxidase inhibition of bioactive constituents isolated from *Potentilla evestita*. Journal of the Chemical Society of Pakistan 38 (1): 429-432.

Raziq N, Saeed M, Ali MS, Zafar S, Shahid M, et al. (2017). A new glycosidic antioxidant from *Ranunculus muricatus* L.(Ranunculaceae) exhibited lipoxygenasae and xanthine oxidase inhibition properties. Natural Product Research 31 (11): 1251-1257. doi: 10.1080/14786419.2016.1236098

Saeed A, Shahwar D (2015). Evaluation of biological activities of the essential oil and major component of *Syzygium aromaticum*. Journal of Animal and Plant Sciences 25 (4): 1095-1099.

Saeed R, Ahmed D (2015). Bioactive compounds from *Carissa opaca* roots and xanthine oxidase and alpha-amylase inhibitory activities of their methanolic extract and its fractions in different solvents. Pharmacognosy Research 7 (4): 295. doi: 10.4103%2F0974-8490.158440

Shaheen B, Naureen S, Anwar K (2020). Effect of *Mimosa Himalayana* extract on xanthine oxidase activity in vitro. Pakistan Journal of Medical Health Sciences 14 (1): 185-188.

Shahwar D, Ahmad N, Khan MA, Ahmad SUN (2012). Chemical composition and biological activities of the essential oil of *Laggera aurita* Linn (DC.) grown in Pakistan. Turkish Journal of Biochemistry 37 (3). doi: 10.5505/TJB.2012.04706

Soskind R, Abazia DT, Bridgeman MB (2017). Updates on the treatment of gout, including a review of updated treatment guidelines and use of small molecule therapies for difficult-to-treat gout and gout flares. Expert Opinion on Pharmacotherapy 18 (11): 1115-1125. doi: 10.1080/14656566.2017.1349099

Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. Medicines 5 (3): 93-109. doi: 10.3390/medicines5030093