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Screening of methanol extracts of sixty plants from Kerman for their potential xanthine oxidase inhibitory activity

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ABSTRACT

Introduction: Gout is a chronic metabolic disease in which xanthine oxidase plays a crucial role. Many natural compounds such as various flavonoids have been reported to have inhibitory effect on xanthine oxidase. In this study we aimed to screen hydromethanol extracts of various plants for their anti-xanthine oxidase activity to find safer and cheaper medicines in prevention and control of related diseases.
Methods: The xanthine oxidase activity was measured by spectrophotometric method at 290 nm. Kinetic study of the enzyme was performed in presence and absence of the extracts.
Results: Among sixty hydromathanolic (70% methanol) extracts, *Quercus infectoria* and *Mentha longifolia* showed more than 70% inhibitory effect on xanthine oxidase. *M. longifolia* showed competitive inhibition and *Q. infectoria* showed non-competitive inhibition by double-reciprocal Lineweaver-Burk plot analysis. The K_m value of xanthine for xanthine oxidase was 1.81 mM and V_{max} value was 2.01 mM min^{-1}.
Conclusion: The data suggest that these plants might be good candidates for treatment of gout disease.

Implication for health policy/practice/research/medical education:
The results of this study scientifically validate the use of *Quercus infectoria* and *Mentha longifolia* in treatment of gout and related diseases. However, further studies are needed to prove this hypothesis.
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Introduction

Uric acid elevation is seen in some chronic metabolic and inflammatory diseases. Urate crystals are deposited in the joints and kidneys, causing swollen and painful joints and the kidneys are being injured (1). Xanthine oxidase plays a crucial role in physiopathology of this process. The enzyme converts hypoxanthine and xanthine into uric acid (2). The activity of the enzyme consumes oxygen, and produces large amounts of free radicals and superoxide. Damage caused by free radicals in cells results in the occurrence and progression of many diseases such as cancer, gout, aging, diabetes and cardiovascular disease (2). Free radical production by this enzyme is so great that it is in the design of free radical generating systems used for research (3).

The most common anti-gout drug is Allopurinol. In this regard, research on allopurinol and oxypurinol showed that inhibiting xanthine oxidase was effective in reducing brain damage caused by ischemia (4). The consumption of allopurinol as a drug leads to nausea, vomiting, liver and kidney damage (5).

Plants are important sources of drugs and their medicinal use has a long history (6). Many studies on the inhibition of xanthine oxidase have been done in India (7), Vietnam (8), North America (9), Chile (10) and Australia (11). Among these natural inhibitors are flavanols (12), steroid alkaloids (13), coumarins (14), pterin (15) and anthocyanidins (16). Many natural compounds classified as inhibitors of xanthine oxidase have been suggested as the most important flavonoids (12). It is reported that some plant extracts have strong xanthine oxidase inhibitory activity (17). Xanthine oxidase inhibitory activity was reported for the species *Artemisia vulgaris* (8), *Artemisia asiatic*a (18), *Artemisia scoparia* (19), *Artemisia princeps* (20) and *A. minor* (21).

In this study we aimed to screen hydromethanol extracts

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of various plants for their anti-xanthine oxidase activity to find safer and cheaper medicines in prevention and control of related diseases.

Materials and Methods

Plants

Sixty plants were collected during spring-summer 2015 from various states of Iran or purchased from the medicinal herbal markets in Kerman city and all of them were botanically identified. A voucher specimen was deposited at the herbarium of the Herbal Medicines Research Center, Faculty of Pharmacy, Kerman University of Medical Sciences, Iran (Table 1). *Quercus Infectoria* was purchased from Kerman medicinal markets, therefore, the codes are not known to the researchers. *Mentha longifolia'*s code is KF1353. Each plant was air dried and grounded into fine powder. 20 g of each plant powder was suspended in 200 mL of methanol (70%), evenly soaked and stirred for one hour using the shaker, then placed at room temperature in dark conditions for 24 hours. The suspensions were filtered and air-dried at room temperature. The resulting powders were stored in dark vials at -20°C until use (22).

Enzyme assay

Xanthine and xanthine oxidase were purchased from Sigma CO., USA. The xanthine oxidase activity was measured by spectrophotometric method (9). The assay mixtures for these experiments contained xanthine solution (5.2 mg dL^{-1}) and enzyme solution (0.4 unit) in phosphate buffer (1.15M), pH 7.5 (final volume of 2 mL). 5 mg of each extract was dissolved in 1 mL of distillated water and then 20 µL of this solution was added to the test mixture before adding the substrate. The mixture was incubated for 15 minutes at room temperature and the reaction was terminated by addition of 1 mL of HCl solution (1M). The absorbance at 290 nm was determined using spectrophotometer. Blank sample contained whole test mixture and extract without enzyme solution. Allopurinol solution was prepared by dissolving 2.0 mg of allopurinol in 2 mL of 1.15M phosphate buffer pH 7.5 and used as the positive control (11).

The inhibitory activity was calculated using the following formula (9):

Inhibition (%) = $[A_{Control}\text{-}A_{Extract}/A_{Control}]\times100$

Each test was performed 3 times and the mean value was used as inhibitory activity of the plants extract.

Kinetic study

In order to measure the inhibition mode by hydromathanolic extract with high activity, xanthine oxidase activity was assayed with increasing concentrations of xanthine as substrate (1.71×10^{-5}, 3.42×10^{-5}, 6.84×10^{-5}, 13.68×10^{-5}, 20.53×10^{-5} and 27.37×10^{-5} mM) in the absence or presence of one of the extracts at three different extract concentrations (2, 4 and 8 mg mL^{-1}). Optimal doses of the extracts were determined based on the results from inhibitory activity assay as described above. Inhibition type for these extracts was determined by Lineweaver-Burk plot analysis (20).

Results

Plants with high xanthine oxidase inhibitory effect

Among 60 hydromathanolic extracts, *Q. infectoria* and *M. longifolia* showed more than 70% inhibitory effect on xanthine oxidase.

Fumaria parviflora and *Cichorium intybus* extracts showed more than 30% inhibitory effect on xanthine oxidase. The rest of the plant extracts showed less than 30% or no inhibition on the activity of xanthine oxidase (Table 1).

Kinetic analysis of xanthine oxidase inhibition

The inhibition mode of two strong active plants (*Q infectoria* and *M. longifolia*) against xanthine oxidase was analyzed by Lineweaver–Burk plots. The enzyme kinetics demonstrated a non-competitive inhibition by *Q. infectoria.* Enhancing the concentration of this extract showed decrease in the V_{max} and no effect on K_m values (Figure 1). While increased competitive inhibition on xanthine oxidase activity was observed by *M. longifolia;* enhancing the concentration of this extract showed no effect on V_{max} but increased the K_m values (Figure 2). The Ki value of 0.07 mg mL^{-1} for *Q. infectoria* and 0.13 mg mL^{-1} for *M. longifolia* was obtained.

Discussion

Gout is a chronic metabolic disease with a higher prevalence in men older than 30 years and in women older than 50 years of age (23,24). This condition may underlie diseases such as hypertension, diabetes and obesity (2). Gout is commonly treated using allopurinol or 1,5dihyroxu-4H-pyrazolo[3,4-d]pyrimidin-4-one. Allopurinol is non-competitive inhibitor of xanthine oxidase (25). The medicine is associated with adverse side effects such as nausea, vomiting, liver and kidney damage (5). Many studies on the inhibition of xanthine oxidase have been done in China (26), North America (9), Brazil (27) and Australia (11). Aqueous extracts of *Lagerstroemia* showed anti xanthine oxidase activity (28). *Caesalpinia sappan* has shown comparable IC50 values to allopurinol (29). *Stereospermum personatum* was responsible for the xanthine oxidase inhibitory action of this plant (30). *Physalis alkekengi* extracts have high inhibitory effects on xanthine oxidase activity due to collecting hydroxyl radicals (31). In this study, we evaluated the xanthine oxidase inhibitory action of hydromethanol extracts of sixty plants. *Q. infectoria* and *M. longifolia* showed 92% and 75% inhibitory effect on xanthine oxidase. The enzyme kinetics demonstrated a non-competitive inhibition for *Q. infectoria* similar to that of allopurinol while *M. longifolia* showed competitive inhibition on xanthine oxidase (25). *Quercus infectoria* has anti-inflammatory activity (32). The galls of *Q. infectoria* has shown non-competitive inhibitory effect on Alpha mannosidase activity (33). The extract showed antioxidant properties (34). Many natural inhibitors of xanthine oxidase have been classified as flavonoids. Such flavonoids have been shown to be

Table 1. Plants and their inhibitory effect on xanthine oxidase

Plants name	Family	Inhibition (%)	Used part
Quercus infectoria	*Fagaceae*	92.0	Galls
Mentha longifolia	*Lamiaceae*	75.0	Aerial part
Fumaria parviflora	*Fumariaceae*	35.0	Aerial part
Cichorium intybus	*Asteraceae*	31.0	Roots
Valeriana hispida	*Valerinaceae*	28.5	Rhizomes
Salixalba	*Salicaceae*	28.0	Aerial part
Terminalia chebulla	*Combretaceae*	27.0	Fruits
Scrophularia striata	*Scorophulariace*	26.0	Aerial part
Zataria multiflora	*Lamiaceae*	25.0	Aerial part
Peganum harmala	*Nitrariaceae*	22.0	Aerial part
Trigonella foenum graecum	*Fabaceae*	22.0	Seeds
Zingiber officinale	*Zingiberaceae*	21.0	Rhizomes
Pimpinella anisuml	*Apiaceae*	21.0	Seeds
Rosa damascene	*Rosaceae*	20.5	Floret
Punica granatum	*Lythraceae*	20.3	Fruits
Rosmarinus officinalilis	*Lamiaceae*	20.0	Aerial part
Hyoscyamus senecionis	*Solanaceae*	20.0	Aerial part
Alhagi camelorum	*Fabaceae*	20.0	Aerial part
Papaver orientale	*Papaveraceae*	19.0	Aerial part
Fraxinus excelsior	*Oleaceae*	19.0	Aerial part
Camellia sinensis	*Theaceae*	18.0	Leaves
Teucrium polium	*Lamiaceae*	18.0	Aerial part
Rhuscoriria	*Anacardiaceae*	18.0	Aerial part
Lavandula stoechasl	*Labiatea*	18.0	Aerial part
Otostegia persica	*Lamiaceae*	17.5	Aerial part
Origanum majorana	*Lamiaceae*	16.0	Whole plant
Echium amoenum	*Boraginaceae*	16.0	Flowers
Carthamus oxyacantha	*Asteraceae*	15.0	Whole plant
Equisetum arvense	*Equisetaceae*	15.0	Whole plant
Cordia mixa	*Boraginaceae*	15.0	Fruits
Cardaria draba	*Brassicaceae*	15.0	Aerial part
Vaccinium Arctostaphylus	*Ericaceae*	13.0	Fruits
Bunium persicum	*Apiaceae*	13.0	Seeds
Sanguisorba minor	*Rosaceae*	13.0	Aerial part
Glycyrrhiza glabra	*Fabaceae*	12.0	Aerial part
Hypericum perforayum	*Hypericaceae*	12.0	Aerial part
Matricaria aurea	*Asterraceae*	12.0	Flowers
Hibiscus gossypifolius	*Malvaceae*	12.0	Flowers
Althaea officinalis	*Malvaceae*	12.0	Aerial part
Cuminum cyminum	*Apiaceae*	11.0	Seeds
Ranunculus arvensisl	*Ranunculaceae*	11.0	Aerial part
Rubia tinctorium	*Rubiaceae*	11.0	Roots
Coriandrum sativum	*Coriandera*	10.0	Aerial part
Achillea wilhelmsii	*Asteraceae*	8.5	Aerial part
Urtica dioica	*Urticacea*	7.2	Aerial part
Ocimum basilicum	*Lamiaceae*	6.0	Seeds
Citrus aurantium	*Rutaceae*	5.5	Flowers
Rheum ribes	*Polygonaceae*	0.0	Rhizomes
Mentha piperita	*Lamiaceae*	0.0	Leaves
Sizigium aromaticus	*Caryophyllaceae*	0.0	Leaves
Ziziphus spinachristi	*Rhamnaceae*	0.0	Leaves
Lawsonia inermis	*Lythraceae*	0.0	Leaves
Cinnamomum Zeylanicum	*Lauraceae*	0.0	Dearm
Eucaliptus galbie	*Myrtaceae*	0.0	Leaves
Anacardium occidentale	*Anacardiaceae*	0.0	Leaves
Heracleum persicum	*Apiaceae*	0.0	Fruits
Myrtus communis	*Myrtaceae*	0.0	Leaves
Malvasylvestris	*Malvaceae*	0.0	Flowers
Nigella sativa	*Ranunculaceae*	0.0	Seeds
Crocus sativa	*Iridaceae*	0.0	Leaves

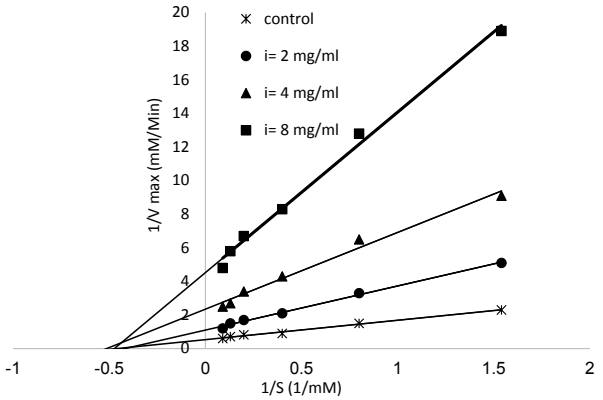

Figure 1. The Lineweaver-Burk plot of kinetic analysis for xanthine oxidase at three different concentrations of *Quercus infectoria* (2, 4 and 8 mg mL^{-1}) in the presence of 6 different xanthine concentrations.

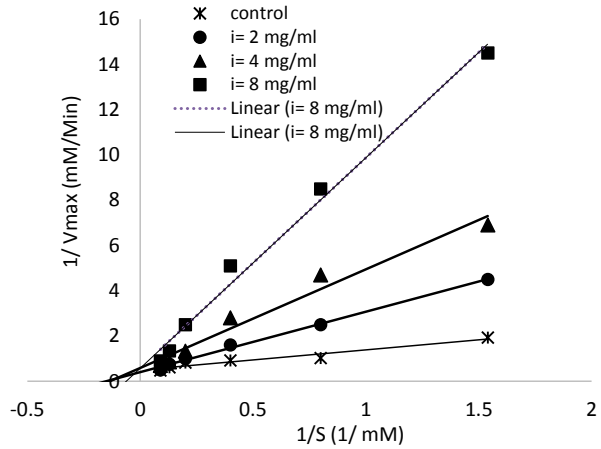

Figure 2. The Lineweaver-Burk plot of kinetic analysis for xanthine oxidase at three different concentrations of *Mentha longifolia* (2, 4 and 8 mg mL^{-1}) in the presence of 6 different xanthine concentrations.

responsible for competitive inhibition of xanthine oxidase (35). *M. longifolia* also has high levels of flavonoids (12). Studies have shown that flavonoid compounds are collectors of hydroxyl radicals and anions dismutase (2). Strong enzyme inhibitor compounds were isolated from *M. longifolia* family plants in this study.

Hydromethanol extracts of *Q. infectoria* and *M. longifolia* showed anti-xanthine oxidase activity. These results scientifically validate the use of them in treatment of gout and related diseases. Further studies are needed to prove this hypothesis.

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Authors' contributions

All authors were participated in the research design,

performance and analysis of the results.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Not applicable.

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