In Vitro Xanthine Oxidase Inhibition by Selected Jordanian Medicinal Plants

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ABSTRACT

In the present study, 18 Jordanian medicinal plants were evaluated for their Xanthine Oxidase (XO) inhibitory potential. Their aqueous extracts, prepared from used parts, were tested in vitro, at 200 µg/mL concentration, for their inhibition potencies expressed as % inhibition of XO activity. Five of the tested plants were found most active (% inhibition more than 35%) and their inhibition profiles (dose-dependent) were further evaluated by estimating the IC50 values of their corresponding extracts. These plants were Hyoscyamus reticulatus L. (IC50 = 12.8 µg/mL), Achillea fragrantissima (Forssk.) Sch. Bip. (197.6 µg/mL), Pimpinella anisum L., (300.4 µg/mL), Origanum syriacum L. (317.0 µg/mL), and Origanum vulgare L. (403.9 µg/mL). Moreover, five more plants showed XO inhibitory activity in the range of 14-30%. Namely: Daphne linearifolia L. (29.5% inhibition), Hibiscus sabdariffa L. (19.4%), Aristolochia maurorum L. (15.6%), Citrullus colocynthis (L.) Schr. (14.4%), and Laurus nobilis L. (13.97%). Considering the results of the present screening study, many of the investigated plant species can be used as potential sources of natural XO inhibitors that can be elaborated as successful herbal remedies for gout, arthritis and other XO-related disorders.

Keywords: Xanthine Oxidase, Gout, Medicinal Plants, Jordan, Hyperuricemia, Natural Products.

INTRODUCTION

Natural products are excellent sources of lead compounds in the search for new medications for some kinds of clinical disorders. The renewed interest in natural therapeutic methods and the use of natural product treatments has led to a steadily growing interest in medicinal plants and the classical methods of plant extract preparations 1,2.

However, systematic exploitation of these natural resources for their human health benefits has not been carried out to a significant degree.

Received on 21/3/2010 and accepted for publication on 28/6/2010.
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Jordan, the investigation of the chemical compositions and the biological activities of most Jordanian medicinal plants has not been performed in a greater depth. Therefore, a wide scope exists for the examination of chemistry and bioactivity of these, particularly native plants.

Gout is a common disease with a worldwide distribution. Hyperuricemia, associated with gout, is present in 5-30% of the general population. It seems to be increasing worldwide and is considered an important risk factor in serious disorders like tophaceous gout, gouty nephropathy and nephrolithiasis. Hyperuricemia results from the overproduction or underexcretion of uric acid and is greatly influenced by the high dietary intake of foods rich in nucleic acids, such as meats, leguminous seeds and some types of seafood. During the last step of purine metabolism, XO catalyses the oxidation of xanthine and hypoxanthine into uric acid (Figure 1). Uricosuric drugs which increase the urinary excretion of uric acid, or XO inhibitors which block the terminal step in uric acid biosynthesis, can lower the plasma uric acid concentration, and are generally employed for the treatment of gout. Moreover, XO serves as an important biological source of oxygen-derived free radicals that contribute to oxidative damage of living tissues causing various pathological states such as hepatitis, inflammation, ischemiareperfusion, carcinogenesis, and aging.

Allopurinol is the only clinically used XO inhibitor in the treatment of gout. However, this drug causes many side effects such as hepatitis, nephropathy, and allergic reactions. Thus, the search for novel XO inhibitors with higher therapeutic activity and fewer side effects are desired not only to treat gout but also to combat various other diseases associated with XO activity.

Figure 1: Conversion of hypoxanthine into xanthine then to uric acid by the action of xanthine oxidase
The main aim of the present study was to screen some plant species growing wild (or cultivated) in Jordan with respect to their XO inhibition activity as potential sources of natural XO inhibitors which may be potentially useful for the treatment of gout or other XO-induced diseases.

The extracts of 18 different plants were tested as potential inhibitors of XO enzyme. Some of these plants were selected randomly, while the selection of the others was based mainly on their traditional uses, by some Jordanians, for the treatment of gout or other diseases associated with symptoms such as rheumatism, arthritis and inflammation [25, 26].

**MATERIALS AND METHODS**

**Plant Materials**

Plant materials, of the selected species \( n = 18 \) were collected from different geographical places in Jordan during the flowering periods of these plants. The collected plants were identified taxonomically, by Dr. Khaled Tawaha (Faculty of Pharmacy, Jordan University), and voucher specimens were deposited at the Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan. The plant materials were cleaned of residual soil, air-dried at room temperature, ground to a fine powder using a laboratory mill and finally passed through a 24 mesh sieve to generate a homogeneous powder. The powder materials were stored in a dark place, at room temperature (22–23 °C), until extraction.

**Plant Extraction**

Aqueous extractions were conducted using a 20 gm sample of each ground plant material in 100 mL distilled water at 85°C for 60 min in a shaking water bath. After cooling, the extract was centrifuged at 1500 g for 10 min, and the supernatant was filtered and subsequently freeze-dried. The lyophilized material was collected and stored in a dry condition until analysis.

**Xanthine Oxidase Assay**

The XO inhibitory activity was measured as previously reported [27-31]. The substrate and the enzyme solutions were prepared immediately before use. The reaction mixture contains 80 mM sodium pyrophosphate buffer (pH = 8.5), 0.120 mM xanthine and 0.1 unit of XO. The absorption at 295 nm, indicating the formation of uric acid at 25°C, was monitored and the initial rate was calculated. The aqueous freeze-dried extract, initially dissolved and diluted in the buffer, was incorporated in the enzyme assay to assess its inhibitory activity at a final concentration of 200µg/mL. Moreover, IC\(_{50}\) evaluation was performed for selected plants, which showed enzymatic inhibition more than 35%. In this case, five different concentrations of the freeze-dried extract (50, 100, 200, 300, 400 and 500 µg/mL) were used to determine the concentration that inhibits 50% of the XO enzyme activity (IC\(_{50}\) value). All assays were triplicated; thus inhibition percentages are the mean of 3 observations. A negative control (blank; 0% XO inhibition activity) was prepared containing the assay mixture without the extract. Allopurinol, a known inhibitor of XO, was used as a positive control in the assay mixture. XO inhibitory activity was expressed as the percentage inhibition of XO in the above assay mixture system, calculated as follows:

\[
\% \text{ of inhibition} = \left(1 - \frac{\text{Test Inclination}}{\text{Blank Inclination}}\right) 
\]

Where test inclination is the linear change in the absorbance of test material per minute, and blank inclination is the linear change in the absorbance of blank per minute.

**RESULTS AND DISCUSSION**

Xanthine oxidase is the enzyme that catalyzes the metabolism of hypoxanthine to xanthine and then xanthine to uric acid in the presence of molecular oxygen to yield superoxide anion and hydrogen peroxide that contribute to oxidative damage of living tissues [22]. It has been shown that XO inhibitors may be useful for the treatment of hepatic diseases, gout, post-ischaemic tissue injury and edema, which are caused by the generation of uric acid and superoxide anion radical [21].

Phytochemicals obtained from traditional medicinal plants present an exciting opportunity for the development of newer therapeutics [33]. In the present study, as part of the continuing search for biologically active XO inhibitors from
natural herbal sources, various plants have been screened for their XO inhibitory potential 31. In this study, the extracts of 18 different plants belonging to 16 different families were investigated as potential XO inhibitors. The selected plants and their XO inhibition assay results are summarized in Table 1. The degree of XO inhibition was evaluated for all extracts at concentration of 200 µg/mL. While the IC₅₀ values (concentration of extract that inhibits 50% of the enzymatic activity) were determined only for 5 plants that showed inhibitory activity more than 35% when compared to uninhibited enzymatic reaction. These plants were *Hyoscyamus reticulatus* L. (IC₅₀ = 12.8 µg/mL), *Achillea fragrantissima* (Forssk.) Sch. Bip. (197.6 µg/mL), *Pimpinella anisum* L., (300.4 µg/mL), *Origanum syriacum* L. (317.0 µg/mL), and *Origanum vulgare* L. (403.9 µg/mL). Table 1: The inhibitory effect of aqueous extracts of 18 plant species on Xanthine Oxidase activities.

From the results shown in Table (1), it was obvious that *H. reticulatus* has the most potent XO inhibitory potential. However, in a separate report, this plant was further investigated in terms of its anti-hyperuricemic activity, using *in vivo* animal models, in addition to its antioxidant properties and phenolic contents 31.

Interestingly, *A. fragrantissima*, a plant not previously reported to have anti-XO activity, was shown to possess notable activity. Figure 2, however, shows the inhibitory profile of this plant on enzyme activity. As seen, *A. fragrantissima* inhibits XO in a dose-dependent manner with an IC₅₀ of 197.6 µg/mL.

Table 1: The inhibitory effect of aqueous extracts of 18 plant species on Xanthine Oxidase activities.

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Family</th>
<th>Part used</th>
<th>% of inhibition*</th>
<th>IC₅₀ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Achillea fragrantissima</em> (Forssk.)</td>
<td>Asteraceae</td>
<td>Aerial parts</td>
<td>49.5</td>
<td>197.9</td>
</tr>
<tr>
<td>Sch. Bip.</td>
<td>Rosaceae</td>
<td>Seeds</td>
<td>1.3</td>
<td>–</td>
</tr>
<tr>
<td><em>Amygdalus communis</em> L. var. <em>Dulcis</em></td>
<td>Rosaceae</td>
<td>Aerial parts</td>
<td>15.6</td>
<td>–</td>
</tr>
<tr>
<td><em>Aristolochia maurorum</em> L.</td>
<td>Aristolochiaceae</td>
<td>Aerial parts</td>
<td>14.4</td>
<td>–</td>
</tr>
<tr>
<td><em>Citrus colocynthis</em> (L.) <em>Schr.</em></td>
<td>Cucurbitaceae</td>
<td>Seeds</td>
<td>1.03</td>
<td>–</td>
</tr>
<tr>
<td><em>Colchicum hierosolymitanum</em> Feinbr</td>
<td>Colchicaceae</td>
<td>Daughter corn</td>
<td>2.9</td>
<td>–</td>
</tr>
<tr>
<td><em>Daphne linearifolia</em> L.</td>
<td>Thymelaeaceae</td>
<td>Aerial parts</td>
<td>29.5</td>
<td>–</td>
</tr>
<tr>
<td><em>Fagonia arabica</em> L.</td>
<td>Zygophyllaceae</td>
<td>Aerial parts</td>
<td>-3.5</td>
<td>–</td>
</tr>
<tr>
<td><em>Hibiscus sabdariffa</em> L.</td>
<td>Malvaceae</td>
<td>Calyx</td>
<td>19.4</td>
<td>–</td>
</tr>
<tr>
<td><em>Hyoscyamus reticulatus</em> L.</td>
<td>Solanaceae</td>
<td>Aerial parts</td>
<td>96.8</td>
<td>12.8</td>
</tr>
<tr>
<td><em>Laurus nobilis</em> L.</td>
<td>Lauraceae</td>
<td>Leaves</td>
<td>14.0</td>
<td>–</td>
</tr>
<tr>
<td><em>Linum pubescens</em> Banks &amp;Sol.</td>
<td>Linaceae</td>
<td>Aerial parts</td>
<td>-4.2</td>
<td>–</td>
</tr>
<tr>
<td><em>Malva nicaeensis</em> All.</td>
<td>Malvaceae</td>
<td>Aerial parts</td>
<td>2.5</td>
<td>–</td>
</tr>
<tr>
<td><em>Nigella sativa</em> L.</td>
<td>Ranunculaceae</td>
<td>Seeds</td>
<td>0.9</td>
<td>–</td>
</tr>
<tr>
<td><em>Origanum syriacum</em> L.</td>
<td>Lamiaceae</td>
<td>Aerial Part</td>
<td>45.3</td>
<td>317</td>
</tr>
<tr>
<td><em>Origanum vulgare</em> L.</td>
<td>Lamiaceae</td>
<td>Aerial parts</td>
<td>54.4</td>
<td>403.9</td>
</tr>
<tr>
<td><em>Pimpinella anisum</em> L.</td>
<td>Apiaceae</td>
<td>Fruit</td>
<td>35.6</td>
<td>300.4</td>
</tr>
<tr>
<td><em>Reseda alba</em> L.</td>
<td>Resedaceae</td>
<td>Aerial parts</td>
<td>1.8</td>
<td>–</td>
</tr>
<tr>
<td><em>Silene aegyptiaca</em> (L.) L.f.</td>
<td>Caryophyllaceae</td>
<td>Aerial parts</td>
<td>-1.2</td>
<td>–</td>
</tr>
</tbody>
</table>

* % of inhibition was measured using a 200 µg/mL concentration of the plant extract.
In previous reports, however, this plant was found to be rich in flavonoid, monoterpenes and pyran derivatives\(^{34,35}\). A lot of these constituents, obtained from various sources, were also reported to exert anti-XO activity\(^ {36,37}\), which accordingly may explain the anti-XO activity observed for this plant in the present study.

On the other hand, a dose dependent XO inhibition with IC\(_{50}\)=300.4 µg/mL was also observed for \(P.\) anisum extract (figure 2). The inhibitory activity of this plant was previously reported\(^ {38}\) but without the determination of IC\(_{50}\). The same scenario was also noticed for the two \(Oregano\) species evaluated in this study\(^ {38}\). In our study, however, \(O.\) syriacum and \(O.\) vulgare inhibited the XO activity with IC\(_{50}\) values 317.0 µg/mL and 403.9 µg/mL, respectively. Phytochemical screenings of the latter species revealed that their major constituents are polyphenols, flavonoids and terpenes\(^ {39}\), which might be responsible, at least in part, for the observed XO inhibitory effects.

Of the other studied plants, five showed XO inhibitory activity in the range of 14-30% at a 200 µg/mL concentration of the extract. These plants are \(Daphne\) linearifolia L. (29.5%), \(Hibiscus\) sabdariffa L. (19.4%), \(Aristolochia\) maurorum L. (15.6%), \(Citrullus\) colocynthis (L.) Schr. (14.4%), and \(Laurus\) nobilis L. (13.97%). XO inhibition was previously reported for \(H.\) sabdariffa\(^ {40}\), while, this is the first report for \(A.\) maurorum, \(C.\) colocynthis and \(L.\) nobilis. Moreover, although some XO inhibitory constituents were isolated from \(genkwa\)\(^ {41}\), this the first report about the activity of \(D.\) linearifolia.

It is noteworthy that, most of the studied plants whose extracts showed notable anti-XO activities like \(A.\) fragrantissima, \(P.\) anisum, \(O.\) vulgare, \(H.\) sabdariffa, and \(C.\) colocynthis are herbal medicines indicated in Jordan for treatment of arthritis\(^ {25,26}\). The XO inhibitory effect of these plants, reported here, could be, however, one of their possible mechanisms of action in the management of arthritis.

### CONCLUSION

These \(in\) \(vitro\) results, moreover, suggest that the studied plants can form a good source of effective crude inhibitors for XO which can be used in the treatment of gout and other XO-related disorders.

However, further investigations, using animal models, are necessary to verify the inhibitory activities of these plants under \(in\) \(vivo\) conditions. In future work, these active plants will be further investigated in order to isolate, identify and evaluate the potentially phytoactive compounds responsible for the XO inhibitory activities reported in the present study.

### ACKNOWLEDGMENTS

This project was sponsored by the Deanship of Academic Research at the University of Jordan (grant No.1070). The authors wish to thank the Deanship of Academic Research at the University of Jordan for their generous funds.
REFERENCES

(1) Newman D.J., Cragg G M. Natural products as sources of new drugs over the last 25 years. *J. Nat. Prod.* 2007; 70:461-477


(25) Abu-Irmaileh B E, Afifi F U. Herbal medicine in Jordan with special emphasis on commonly used herbs. *J


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In the study, the activity of xanthine oxidase (Xanthine Oxidase) was investigated in different plant extracts from Jordan.

The IC50 values obtained were: Hyoscyamus reticulatus L. (12.8 µg/mL), Achillea fragrantissima (Forssk.) Sch. Bip. (197.6 µg/mL), Pimpinella anisum L. (300.4 µg/mL), Origanum syriacum L. (317.0 µg/mL), and Origanum vulgare L. (403.9 µg/mL).

The study concluded that these plant extracts could be potential therapeutic agents for the treatment of diseases related to xanthine oxidase, such as gout, arthritis, and inflammation.