Investigation of Phytochemical and Antioxidant Capacity of Fennel (Foeniculum vulgare Mill.) Against Gout

Yeganeh Teimoori-Boghsani, Mohammad Bagher Bagherieh-Najjar* and Manijeh Mianabadi

Department of Biology, Faculty of Science, University of Golestan, Shahid Beheshti Ave., Gorgan, Iran

Article History: Received: 22 January 2017 /Accepted in revised form: 11 January 2018
© 2013 Iranian Society of Medicinal Plants. All rights reserve

Abstract

Fennel, Foeniculum vulgare Mill. (Apiaceae) is an aromatic plant, with medicinal and culinary applications, widely naturalized worldwide, especially near the sea coasts and riversides. Fennel has long been used in Iran as a traditional remedy against gout. The objective of our study was to uncover the scientific basis of this traditional gout therapy. Different plant parts were extracted by methanol and used in phytochemical assessment and examined for possible inhibitory effects on xanthine oxidase -the main enzyme responsible for uric acid accumulation in blood. FRAP and β-carotene bleaching assays, total anthocyanin, carotenoids, soluble sugars, phenolics and flavonoids content were measured spectrophotometrically, while caffeic acid, chlorogenic acid and quercitin contents were measured by HPLC. The experiments were performed using a 3-stage nested statistical design with three biological replications. Results showed that the flower extract exhibited the most xanthine oxidase inhibitory effect (80% of Allopurinol), the highest amounts of total phenol, flavonoid and cafeic acid (53.55, 7.71 and 0.049 mg gdw⁻¹, respectively), as well as significant antioxidant activity in scavenging free radicals. These results suggest that flower extract of fennel is a natural source of valuable compounds against xanthine oxidase activity, with potential therapeutic applications in human gout treatment.

Keywords: Foeniculum vulgare, Xanthine Oxidase, Phenolics, Flavonoids, Gout

Introduction

Elevated levels of uric acid in blood often lead to hyperuricemia related diseases such as gout. In human, gout can be developed by elevated production of uric acid via de novo biosynthesis, or breakdown of nucleic acids, and ablated elimination of uric acid via kidney [1]. In the first two mechanisms, conversion of hypoxanthine to xanthine and subsequently to uric acid is catalyzed by an enzyme named xanthine oxidase [2]. Accordingly, in a therapeutic approach against gout, an inhibitor of xanthine oxidase named Allopurinol, as an approved synthetic drug, is widely prescribed; although it has many side effects [1]. In addition to uric acid, in the above indicated reactions catalyzed by xanthine oxidase, H₂O₂ and O₂° are produced, which in turn can induce several cellular modifications, including DNA damage, protein carbonylation and lipid peroxidation, some of which may lead to inflammatory reactions [3, 4]. Application of antioxidants may help patients suffering from gout to tolerate joint inflammation caused by accumulation of hydrogen peroxide and super-oxide molecules. Due to anticipated side effects of synthetic drugs [5], demand for remedies obtained from natural resources, such as medicinal plants, that prevent accumulation of uric acid and oxidative damage is increasing.

Foeniculum vulgare Mill. commonly known as fennel, belongs to the Apiaceae family with aromatic odor, medicinal and culinary applications, and is widely cultivated throughout the temperate and tropical regions of the world, including Iran (known as Razianeh) [6]. Fennel extracts exhibits carminative, flavoring, antioxidant, antibacterial,
fungicidal, diuretic, lactation, stimulant and mosquito-repellent properties [7,8].

In ancient Greece, some prescriptions against human gout contained fennel extracts [11]. Likewise, in traditional medicine of India [12] and Iran [6] fennel was supposed to mitigate gout and act as a diuretic agent by increasing the flow of urine. Since, joint inflammation observed in gout is mainly caused by accumulation of mono sodium urate crystals leading to inflammatory reactions, we hypothesized that fennel extracts, in addition to anti-inflammatory effects, might exhibit a direct inhibitory activity on production of uric acid. Therefore, Xanthine oxidase-inhibition potential and antioxidant properties of fennel extracts collected from different plant organs during various growth stages were examined.

**Material and Methods**

**Plant Collection and Extraction**

Wild type *Foeniculum vulgare* plants, were cultivated in Khaf, Northeast of Iran (34º North latitude to 60º East longitude). The plant identity was confirmed as *F. vulgare* with the herbarium code of 38425; by the Botanical Research Institute of Mashhad University, Iran. Different parts of the plant, collected during various stages of plant growth were separated and dried (Table 1). For extraction, one gram of each sample was soaked in methanol for 48 h at room temperature and kept at 4 ºC for further analysis.

**Biochemical Measurements**

Xanthine oxidase activity was assayed according to the procedure of Noro et al. [13] as previously described [14]. Antioxidant activity of the extracts was assessed by FRAP, using the Benzene and Strain method [15] as well as by β-carotene bleaching assay [14]. Total anthocyanin and carotenoid contents were determined according to Mita et al. [16] and Lichtenthaler [17], respectively. Soluble sugar content was measured by phenol-sulfuric acid method, as described by Kochert. [18]. Total phenolic content was measure by the Folin–Ciocalteu method [18] and expressed as gallic acid equivalents. Total flavonoids were quantified using aluminum chloride reagent (AlCl₃) according to Chang et al. [20] and expressed as quercetin equivalent. Caffeic acid, chlorogenic acid, quercetin and gallic acid contents were measured by High-Performance Liquid Chromatography (HPLC), as described by Owen et al. [21]. The HPLC was performed by a Hitachi model, UV/VIS detector, C18 column (Lichroshper 100 reversed phase, 250×4 mm; 5 μm). Mobile phases included water, methanol and acetic acid with an 80:18:2 ratio for caffeic acid, chlorogenic acid and gallic acid; and a 60:39:1 ratio for quercetin with a 1 ml/min flow rate. The UV detector was operated at 330 nm for caffeic acid and chlorogenic acid, 280 nm for gallic acid and 360 nm for quercetin, respectively. 100 μl of samples were injected into equipment and 20 μl of each were loaded.

**Statistical Analyses**

All experiments were conducted at least in triplicates and the results were reported as mean ± standard error (SE). The experiments were performed according to a 3-stage nested design and statistically significant differences at the %5 confidence level were determined using the LSD method. The correlation index was used to confirm the effect of parameters on Xanthine oxidase inhibition, using SAS (9.2) and Minitab (16.1.1) software.

**Result and Discussion**

**The Inhibitory Effect of Plant Extract on Xanthine Oxidase Activity**

In the traditional Uighur formula known as Karapxa Decoction [22] as well as Iranian traditional medicine, *F. vulgare* was used to treat gouty patients [6]. In order to study the scientific basis for these traditional applications, we extracted various organs of the plant at different growth stages (table 1) and measured there in vitro anti-xanthine oxidase activity. Data depicted in Figure 1 suggest that the lowest percentage of xanthine oxidase inhibition was found in stem of fruiting stage while the maximum capacity of inhibition was observed in flowers (82± 2.6%).

**Determination of Antioxidant Capacity of Fennel Extracts**

Since hydrogen peroxide and super-oxide molecules are by-products of xanthine oxidase activity, we reasoned that fennel extracts might reduce the side effects of gout through scavenging reactive oxygen species. Therefore, total antioxidant activities of fennel extracts during
various plant growth stages were measured by FRAP and β-carotene bleaching assays. Data presented in Table 2 indicate that all analyzed samples demonstrated significant antioxidant activity. These data suggest that traditional application of fennel extracts against gout might be related to the plant antioxidant potential.

Biochemical Basis of Fennel Antioxidant Activity

In order to determine potential compounds that might directly or indirectly scavenge hydrogen peroxide and superoxide molecules, produced by xanthine oxidase, the amount of several compounds in various plant extracts were identified and presented in Table 2. Total phenolic content was higher in flowers (33.55 ± 1.41 mg GA·g⁻¹ dw⁻¹) and leaves of the flowering stage (30.37 ± 0.35), as compared to other organs. Phenolic compounds which are commonly found in both edible and non-edible plants have been reported to exhibit multiple biological effects, including antioxidant activity [23-25].

Among the samples, flowers (7.71±0.14 mg Q·g⁻¹ dw⁻¹) and leaves of the vegetative state (5.55± 0.26) showed the highest amounts of flavonoids (Table 2). Many of the pharmacological effects of flavonoids are linked to their known biological functions as antioxidants, due to free-radical scavenging and metal chelating [24]. Total anthocyanin content represented a dynamic change in different stages of plant growth and development. The highest amount of anthocyanin was detected in leaves of the flowering stage (7.56± 0.25 mg/gdw) while the lowest amount was detected in stem and root of the vegetative stage. It has been shown that anthocyanins are strong antioxidants with free radical scavenging properties, attributed to the phenolic hydroxyl groups present in their structures, while different hydroxylation and glycosylation may modulate their antioxidative properties [26].

The highest amounts of carotenoids were observed in leaves. Beta-carotene and other carotenoids are believed to provide antioxidant protection to lipid rich tissues, similar to vitamin E [27]. Carotenoids have also been linked with improvement of human immune system and decreased risk of degenerative diseases, such as cancer, cardiovascular disease, age-related macular degeneration and cataract formation [28].

The highest amount of soluble sugars was observed in the leaves of flowering stage and roots of fruiting stage and the lowest amount of that was in stems of vegetative stage. Many beneficial health properties of agaro-oligosaccharides have been attributed to their anti-oxidative activities in scavenging free hydroxyl and superoxide anion radicals, and inhibiting lipid peroxidation [29].

The Content of Caffeic Acid, Chlorogenic acid, Quercetin and Gallic Acid

To determine which one of the phenolic compounds is effective in antioxidant activity and xanthine oxidase inhibition, caffeic acid, chlorogenic acid and gallic acid contents and quercetin, as an important flavonoid were assayed by HPLC (Table 3). The results showed that the caffeic acid was the highest in flowers (0.05 mg g⁻¹ dw⁻¹) followed by leaves of flowering stage (0.02 mg g⁻¹ dw⁻¹). A similar trend was observed in total phenolic compounds (Table 2). The chlorogenic acid was the highest in leaves of vegetative stage (5.98 mg g⁻¹ dw⁻¹) and flowers (2.85 mg g⁻¹ dw⁻¹) with any specific order. The quercetin content was very low and negligible.

A correlation analysis showed a significant linear correlation between xanthine oxidase inhibition and phenolic/flavonoid contents of the extracts (Table 4). Among the phenolic compounds, caffeic acid which was higher in flowers was correlated with xanthine oxidase inhibition (Table 4). The HPLC results showed that pure caffeic acid had a low concentration in the extracts (Table 4) suggesting that most caffeic acid molecules were converted to its derivatives. Accordingly, Faudale et al. [30] reported that in fennel the amount of caffeic acid derivatives are high. Several experimental models showed that flavonoids and other phenolic compounds are considered as antioxidants not only because of their ability as free radical scavengers, but also because of their ability to directly inhibit Xanthine oxidase [31]. It is possible that the inhibitory effect of many phenolic compounds on xanthine oxidase activity is caused by their similar structures or by non-competitive inhibition.

Studies have shown that xanthine oxidase inhibitors might also be useful for successful treatment of hepatic disease, which is caused by the generation of uric acid and superoxide anion radicals [32]. Xanthine oxidase-derived superoxide anion has been linked to post-ischaemic tissue injury and edema [33]. Therefore, control of hydrogen peroxide and superoxide anions is very important.
Table 1 Plant growth stages and date of their collections.

<table>
<thead>
<tr>
<th>Sampling stage</th>
<th>Phenological stage</th>
<th>Date of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>sowing</td>
<td>24/3/2012</td>
</tr>
<tr>
<td>2</td>
<td>Vegetative stage</td>
<td>22/5/2012</td>
</tr>
<tr>
<td>3</td>
<td>Flowering stage</td>
<td>24/6/2012</td>
</tr>
<tr>
<td>4</td>
<td>Fruiting stage</td>
<td>10/8/2012</td>
</tr>
</tbody>
</table>

Table 2 Total phenol, flavonoid, anthocyanin, carotenoid and soluble sugar content of Foeniculum vulgare Mill. and antioxidant activity in the presence of plant extract from different stages and several organs.

<table>
<thead>
<tr>
<th>Vegetative stage</th>
<th>Flowering stage</th>
<th>Fruiting stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>Stem</td>
<td>Root</td>
</tr>
<tr>
<td>Phenol content</td>
<td>26.59±0.19</td>
<td>3.60±0.06</td>
</tr>
<tr>
<td>Flavonoid content</td>
<td>5.55±0.26</td>
<td>0.89±0.09</td>
</tr>
<tr>
<td>Anthocyanin content</td>
<td>2.65±0.44</td>
<td>1.52±0.19</td>
</tr>
<tr>
<td>Carotenoid content</td>
<td>19.5±0.12</td>
<td>8.96±0.31</td>
</tr>
<tr>
<td>Soluble Sugar content</td>
<td>0.03±0.00</td>
<td>0.02±0.00</td>
</tr>
<tr>
<td>Antioxidant activity (FRAP)</td>
<td>69.39±0.16</td>
<td>8.99±1.69</td>
</tr>
</tbody>
</table>

Table 3 Caffeic acid, chlorogenic acid, quercetin and gallic acid contents of Foeniculum vulgare Mill. from different stages and several organs.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Vegetative stage</th>
<th>Flowering stage</th>
<th>Fruiting stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Stem</td>
<td>Root</td>
</tr>
<tr>
<td>Caffeic acid content (mg. g⁻¹ dw⁻¹)</td>
<td>0.02</td>
<td>0.02</td>
<td>0</td>
</tr>
<tr>
<td>Chlorogenic acid content (mg. g⁻¹ dw⁻¹)</td>
<td>5.98</td>
<td>0.22</td>
<td>0.07</td>
</tr>
<tr>
<td>Quercetin content (mg. g⁻¹ dw⁻¹)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Gallic acid content (mg. g⁻¹ dw⁻¹)</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 4 Correlation between the inhibition percent of xanthine oxidase and several factors of Foeniculum vulgare Mill. samples.

<table>
<thead>
<tr>
<th>Phenol</th>
<th>Flavonoid</th>
<th>Anthocyanin</th>
<th>Carotenoid</th>
<th>Soluble Sugar</th>
<th>Caffeic acid</th>
<th>Chlorogenic acid</th>
<th>Quercetin</th>
<th>FRAP</th>
<th>BCB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.630**</td>
<td>0.659**</td>
<td>0.637**</td>
<td>0.639**</td>
<td>0.150</td>
<td>0.715**</td>
<td>0.369</td>
<td>0.640**</td>
<td></td>
</tr>
<tr>
<td>Antioxidant activity (FRAP)</td>
<td>-0.046</td>
<td>-0.173</td>
<td>0.259</td>
<td>-0.458</td>
<td>-0.137</td>
<td>0.268</td>
<td>-0.517**</td>
<td>-0.355</td>
<td>-</td>
</tr>
<tr>
<td>Antioxidant activity (BCB)</td>
<td>0.826**</td>
<td>0.676**</td>
<td>0.276</td>
<td>0.252</td>
<td>0.300</td>
<td>0.554**</td>
<td>0.312</td>
<td>0.348</td>
<td>0.280</td>
</tr>
</tbody>
</table>

** The level of significance is less than 1% (p < 0.01).
Fig. 1 Xanthine oxidase inhibitory percent of the Foeniculum vulgare Mill. extracts in different stages and several organs.

Fig. 2 Enzymatic conversion of hypoxanthine to xanthine and subsequently to uric acid is catalyzed by xanthine oxidase. Fennel extract may perform anti gout activity via direct inhibition of xanthine oxidase and indirect anti inflammatory effects.

Therefore, we can conclude that fennel extract contains various phenolic and flavonoid compounds, which perform divergent types of antioxidant activities, detected by different methods, some of which can reduce the side effects of xanthine oxidase activity. Altogether, data presented here suggest that fennel extracts may perform anti gout activity via two distinct pathways (Fig. 2). First of all, it may contain compounds with similar structures to xanthine oxidase natural substrate and, thus exhibit competitive inhibitory effects on the enzyme activity leading to reduced uric acid; and secondly, antioxidative compounds present in the plant extract may reduce inflammatory effects of uric acid and $H_2O_2$ accumulations.

Conclusions

In Iranian traditional medicine, fennel (F. vulgare) has been prescribed for treating gout disease. Here, we tried to uncover the scientific basis of this traditional gout therapy. Plant extracts obtained from various organs during different phenological stages were examined on (i) direct inhibition of xanthine oxidase activity and (ii) scavenging of free radicals resulted by xanthine oxidase activity. The results showed that the flower extract of fennel, which exhibited the highest inhibitory effect on xanthine oxidase activity contained the highest amounts of various phenolic/flavonoid compounds. In the phenolic and flavonoid compounds caffeic
acid and quercetin, despite their relatively low contents, showed a good correlation with xanthine oxidase inhibitory effects. On the other hand, the chlorogenic acid content did not show significant correlation with xanthine oxidase inhibition. In addition, we showed that the flower extracts of fennel contain considerable amounts of antioxidants with potential effects on scavenging of free radicals produced by the xanthine oxidase enzyme. Thus, our data justifies traditional application of fennel for gout treatment. Further, in depth in vivo analysis is needed for confirming these data and for suggesting the proper dose of the extract to combat gout in human.

Acknowledgments

This work was supported by a research grant from Golestan University to MBBN and MM. The authors have no conflict of interest to declare.

References

23. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products:


