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The Evaluation of Antioxidant Compounds of Some Medicinal Plants and Their Effects on Controlling Gout Disease

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Abstract

Gout is a chronic metabolic disease leads raise uric acid levels in the blood. The most effective enzyme in creating Gout is xantine oxidase. Many plants have been introduced to treat Gout in traditional medicine around the world, especially in Iran. The purpose of this study is to investigate the antioxidant compounds of some medicinal plants and their effect on Xanthine Oxidase (XO) enzyme. Sample plants such as Berberis vulgaris, Cichorium intybus, Urtica dioica, Apium graveolens, and Equisetum arvense were collected from the Tonekabon and their scientific names were specified according to herbarium plant. The antioxidant activity of extracts was measured by Ferric Reducing Ability of Plasma (FRAP) method. Also, the content of total phenol, flavonoid, anthocyanin, carotenoid, luteolin, quercetin and rutin was determined and their inhibitory effect on XO enzyme was measured. The results shown that the highest inhibitory effect on XO observed by 0.3 m^{-1}ml of B. Vulgaris extract was equivalent to 28.06%. Total phenol, flavonoid, rutin, anthocyanin, and carotenoid contents in B. vulgaris were significantly greater than the other samples. The most quercetin content was identified in U. dioica (0.021 ± 0.00044 g^{-1} dw) and the highest amount of luteolin was also observed in A. graveolens. The results suggested that the extract of B. vulgaris is a valuable source of antioxidant compounds and it has a high inhibitory effect on XO activity which can be suggested for treatment of the diseases that arise due to the oxidants such as Gout.

Keywords: Flavonoid, Ferric reducing ability of plasma, Methanolic extract, Xanthine oxidase.

1 | Introduction

The Reactive Oxygen Species (ROS) damage to cells leading to the development and the progression of many diseases including cancer, Gout, diabetes, and cardiovascular disease [1], [2]. Gout is a chronic metabolic disease that high levels of uric acid and inflammation are its symptoms and the enzyme Xanthine Oxidase (XO) is a key enzyme in Gout through producing uric acid [3]. During of purine catabolism, enzyme XO

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causes hypoxanthine and xanthine to be converted into uric acid [4]. Moreover, XO generates free radicals and super oxide anion though the consumption of oxygen and that makes XO as an excellent source of such products [5], [6]. Nowadays, Allopurinol are employed as XO inhibitor for the therapy of Gout, but this drug has some adverse impacts such as nausea and vomiting, bone and brain damage, liver and kidney damage [7]. Also, in the case of Gout, people tend to use medicinal plants to treat due to the nature of chronic illness, long-term consumption of drugs and their side effects. Because of these, to find natural XO inhibitors, wide investigations have been done in countries such as India [8], Vietnam [9], Australia [10], America [11], and Chile [12] until now. In addition, there are many natural compounds such as pantagaloil glucose [13], flavonoids as quercetin [14], polyphenols [15], flavonols [16], pterins [17] and anthocyanidins [18] known as XO inhibitors and among these, flavonoids display more potent inhibitory activates. Many plants have been represented to treat Gout in traditional medicine around the world and especially in Iran. However, there are not many reports concerning anti-Gout effect of these plants. Therefore, the purpose of this research is to evaluate the inhibitor impacts of metanolic crude extract on XO in some several of plants from Iran which on basis of some sources have anti-Gout effects and to reach this purpose, plants such as Berberis vulgaris, Cichorium intybus, Urtica dioica, Apium graveolens, and Equisetum arvense were used for the current research.

2 | Materials and Methods

2.1 | Providing of Extracts

Quercetin, gallic acid, 2, 4, 6-tri (2-pyridyl)-s-triazin (TPTZ), XO, xanthine, and High-performance Liquid Chromatography (HPLC) standards were provided by Sigma. Methanol, ethanol, and hydrochloric acid were from Fluka. Other reagents were prepared with the best quality. As a first step, a list of plants using for the treatment of Gout was made and among them five plants including Berberis vulgaris, Cichorium intybus, Urtica dioica, Apium graveolens, and Equisetum arvense were also selected and then they were collected from Tonekabon city and their names were confirmed by herbarium. The samples were dried in the shade and ground. A mount 1 g of ground specimens were extracted using 50 ml of 80% methanol for 48 hours, followed by filtration of the extracts by Whatman filter paper, and then, the residues were re-extracted with 80% methanol for 24 hours. Then, methanol of extracts became vapor by a rotary device at 40°C. The crude extract was kept at 4°C (up to 10 days) [19]. It should be mentioned that in case of Cichorium intybus, Urtica dioica and Equisetum arvense shoot was used and in case of Berberis vulgaris, and Apium graveolens fruit and seed were respectively employed.

2.2 | Measurements of Total Flavonoid and Phenol

The Folin reagent measured the total phenolic content [20]. To 100µ of each sample, 2.8 distill water, 100 µ of Folin reagents and 2 ml of sodium carbonate 2% was put in and incubated for 30 minutes. The samples' absorbance was read at 720 nm. The data were reported as milligram galic acid equivalents per gram of dry weight (mg GAE g^{-1} dw^{-1}). Total flavonoid of samples was assayed by the Chang approach [21]. The calibration curve was created by preparing quercetin solutions at various levels in methanol. The data was reported as milligram quercetin equivalents for each gram of dry weight (QE g^{-1} dw^{-1}).

2.3 | Measurements of Total Anthocyanin

Total anthocyanin contents of extracts were estimated by the Mita method [22]. 0.02 g from dray sample was extracted with 4 ml methanol containing 1% hydrochloric acid. The obtained extract was kept at 4 °C for 24 h, followed by centrifugation at 13000 g for 10 minutes in ambient temperature. The absorption samples were read against a blank at 530 and 657 nm, and anthocyanin content was obtained by the formula below:

$$A = A_{530} - (0.25 \times A_{657}).$$

2.4 | Measurements of Total Carotenoid

The 0.05 g of fresh plant was extracted through 5ml of acetone and then added to 1g anhydrous sodium sulfate and after that filtered. Then, the volume of filtered extract with acetone has increased to 10 ml and next centrifuged at 2600 g for 10 minutes. The absorption of supernatant was measured against a blank at 662, 645, and 470 nm. The total carotenoid of extracts was obtained by the formula below [23]:

$C_a=11.24A_{662} - 2.04A_{645}$.

$C_b =20.13A_{645}-4.19\ A_{662}$.

$C_t=1000A_{470}-1.9A_{Ca}- 63.14\ A_{Cb}/214$.

In these formulas C_a: the amount of chlorophyll a, C_b: the amount of chlorophyll b, and C_t: the amount of total carotenoid.

2.5 | Reducing Power (Ferric Reducing Ability of Plasma)

By Benzie and Strain method, the reducing power was measured [24]. In summary, the Ferric Reducing Ability of Plasma (FRAP) regent consisted of 1 ml of 20 Mm $FeCl_3$, 1 ml of 10 mM TPTZ solution in 40 Mm pure HCL, and 10 ml of 0.3 M sodium acetate buffer (PH 3.6) was freshly prepared. In order to measure such feature, the 0.1 g of dried plant tissue with 5 ml of distilled water was extracted in a mortar and filtered through filer paper. Then, the solution (100 ml) was blended using 3 ml FRAP reagent. The mixture was vortexed and kept at 30°C for 4 minutes. The absorption of samples was read against blank at 593 nm, and the standard curve was created on the basis of absorbed concentration of $FeSO_4$ in the range of 100 to 1000 µM. Therefore, the reducing power was expressed as equivalent mmol Fe^{+2} g^{-1} dw^{-1}.

2.6 | Measurement of Quercetin, Luteolin, and Rutin Contents

Quercetin, luteolin, and rutin contents were measured using HPLC [25]. In this study, HPLC of all extracts was performed through using Hitachi module with a UV-Vis detector RP-C_{18} column (250 ×4mm, 5Mm) with the mobile phase of methanol-acetonitrile-acetic acid-phosphoric acid-H_2O (70:50:1:1:138) at 0.6 ml/min. The detecting wave length was regulated in 352 nm. Also, the peak area of each extract obtained, and their standard calibration curves were also determined, too.

2.7 | Measurement of Xanthine Oxidase Inhibitory Activity

The inhibitory activity of XO was assessed on the basis of the procedure described by Noro with some modification [26]. The measurable mixture included 405 µL of 50 mM phosphate buffer (pH 7.5) 20 µL of test solution and 150 µL enzyme solution (pH 7.5; 0.25 units/ml in 50 mM phosphate buffer), and then incubated at 25°C for 15 minutes. After that, the start of the reaction was by adding 300 µL of substrate solution (300 Mm xanthine in the same buffer). The measurable mixture was again kept at 25 °C for 30 minutes. Further, the reaction was ceased by the addition of 750 µL of 1 N HCl, and the absorbance against blank was read at 290 nm. A control was similarly provided without the enzyme solution. One unit of XO is the amount of enzyme needed to make 1 mmol of uric acid/min at 25°C. XO activity was reported as the XO inhibition rate computed using (1-B/A) × 100 formula where A denotes the alteration in absorbance of the assay without the plant extract (Δabs, with enzyme - Δabs with no enzyme), and B denotes the alteration in assay absorbance with the plant extract (Δabs, with enzyme - Δabs with no enzyme).

2.8 | Statistical Analysis

In the present study, all analyses were done in triplicates, and values were reported as mean ± Standard Deviation (SD). In addition, Data were described by one-way Analysis of Variance (ANOVA) ($p<0.05$), and

the means were separated by Duncan's test by SAS 9.1. Also, Correlation between dates was done by SPSS software.

3 | Results

3.1 | Total Phenol, Flavonoid, Anthocyanin, and Carotenoid Amounts

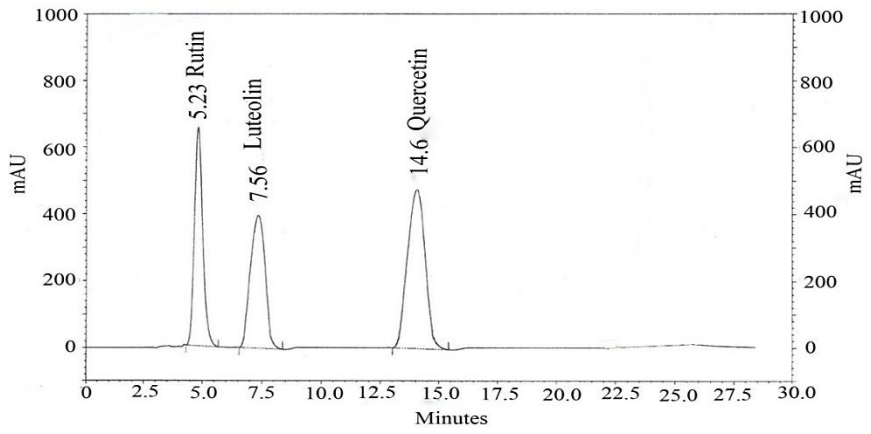
The results of shown that B. vulgaris contained the greatest amount of total phenol and anthocyanin, which were equivalent to 32.16±2.065 mgGAE^{-1}g dw and 8.82±0.114 mg^{-1}g dw respectively (*Table 1*). In addition, the obtained result of total flavonoid content based on aluminum chloride colorimetric procedure has also showed that extracts contained the greatest amounts of total flavonoid content which were equivalent to 1.36±0.037 and 1.472±0.01 mgQE^{-1}g dw respectively (*Table 1*). Regarding total carotenoid content, U. dioica and C. intybus had the highest and lowest amount of total carotenoid, respectively (*Table 1*).

Table 1. The amount of non-enzymetic antioxidant compounds in some medicinal plants.

Plant Samples	Total Phenol (mg GAE^{-1}g dw)	Total Flavonoid (mgQE^{-1}g dw)	Total Anthocyanin (mg^{1}g dw)	Total Carotenoid (mg^{-1}g fw)
Berberis vulgaris	32.16 ±2.065	1.365±0.037	8.829 ±0.114	2.68 ±0.051
Urtica dioica	5.69±0.043	0.747 ±0.042	8.037 ±0.276	2.99 ±0.035
Cichorium intybus	1.92 ±0.435	0.316 ±0.049	2.53 ±0.232	0.462 ±0.039
Apium graveolens	7.871 ±0.195	0.825 ±0.051	3.254 ±0.023	0.801 ±0.018
Equisetum arvense	6.45 ±0.072	1.472 ±0.01	2.62 ±0.365	1.1 ±0.038

3.2 | The Amount of Rutin, Luteolin, and Quercetin Contents

The result reported that U. dioica and B. vulgaris contained the maximum amounts of rutin and quercetin as a sample of flavonoid combines as such a mounts for U. dioica and B. vulgaris were 0.021±0.00004 and 0.783±0.0006 mg^{-1}g dw respectively (*Fig. 1* and *Table 2*). Further, it was found that A. graveolens contained the highest amount of luteolin as a flavonoid combine; while such content was low in *B.* vulgaris (almost zero) (*Fig. 1b*).



a.

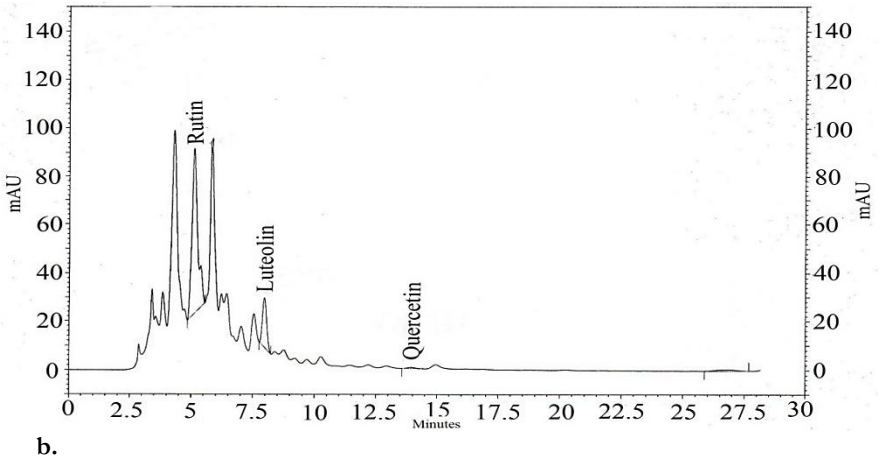


Fig. 1. High-performance liquid chromatography chromatograms; a. Standard flavonoids and b. Extract of Apium graveolens.

Table 2. The amount of rutin, luteolin, and quercetin in some medicinal plants.

Plant Samples	Quercetin (mg^{-1}g dw)	Rutin (mg $^{-1}$g dw)	Luteolin (mg^{-1}g dw)
Berberis vulgaris	0.003 ±0.019	0.006 ±0.783	*
Urtica dioica	0.0004 ±0.021	0.009 ±0.031	0.001 ±0.085
Cichorium intybus	0.0001 ±0.008	0.016 ±0.072	0.016 ±0.107
Apium graveolens	0.0004 ±0.013	0.012 ±0.149	0.013 ±0.62
Equisetum arvense	0.0002 ±0.016	0.056 ±0.282	0.001 ±0.11

*Berberis vulgaris lacks luteoline

3.2|Antioxidant Activity (Ferric Reducing Ability of Plasma)

Extracts reducing power showed that all extracts exhibited antioxidant activity, with B. vulgaris exhibiting maximum antioxidant activity (*Fig. 2*).

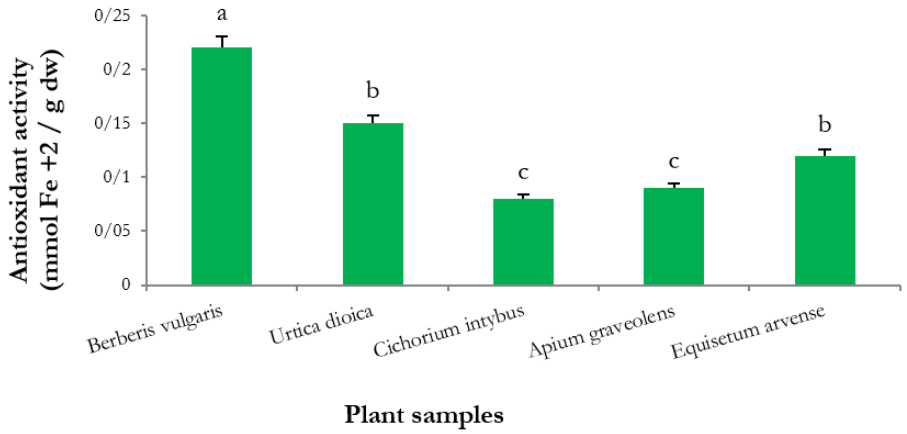
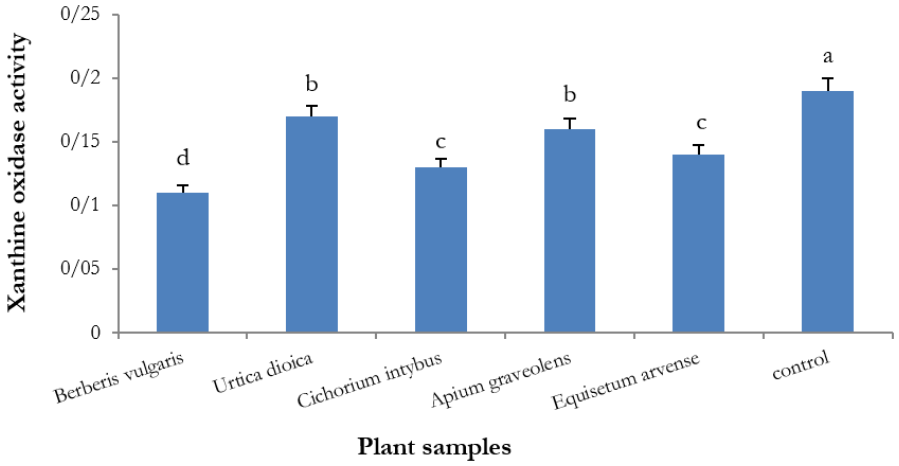


Fig. 2. Antioxidant activity of plant extracts by the ferric reducing ability of plasma method. Data expressed as means±SD. Similar upper-case letters demonstrate no significant differences.

3.3|The Evaluation of the Effect of Inhibition on Xanthine Oxidase Activity

Variance analysis results have shown that lower XO activity in B. vulgaris has resulted from a reduction in enzyme reaction product (Fig. 3a). Also, the calculation of the inhibition rate of XO activity demonstrated that the inhibitory rate of crude extract from B. vulgaris was 28.06% at a concentration of 0.3 mg^{-1}ml which was greater than that of the other plants tested.



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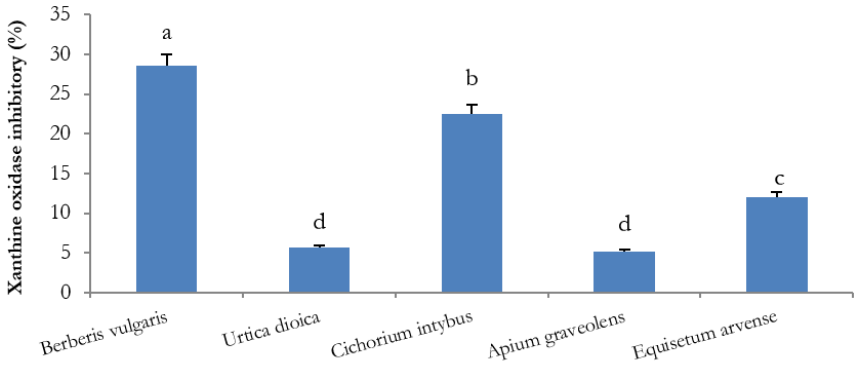

b.

Fig. 3. Comparative inhibitory effects of various plant extracts on xanthine oxidase activity; a. Xanthine oxidase activity, and b. Inhibition percentage of xanthine oxidase. Data expressed as means±S.D. Similar upper-case letters demonstrate no significant differences.

3.4 | Correlation of Xanthine Oxidase Inhibition with Measured Parameters

The analysis of the relationship between antioxidant activity (FRAP) with measured parameters, including flavonoid (5% level), phenol, anthocyanin, rutin, quercetin, carotenoid, and soluble sugar (1% level) contents, showed a positive correlation coefficient. Moreover, XO inhibition activity with total phenol and rutin has also demonstrated a positive correlation coefficient (*Table 3*).

Table 3. Correlation between xanthine oxidase inhibition percentage and measured parameters in Berberis vulgaris, Cichorium intybus, Urtica dioica, Apium graveolens, and Equisetum arvense plants.

	Total Phenol Content	Total Flavonoid Content	Total Anthocyanin Content	Total Carotenoid Content	Quercetin Content	Luteolin Content	Rutin Content	FRAP	XO Inhibition
XO inhibition	0.573*	0.154ns	0.139ns	0.030 ns	0.231ns	0.281ns	0.666 **	0.311ns	1
FRAP	0.767**	0.591*	0.844 **	0.836**	0.708**	-0.581*	0.704 **	1	
Rutin content	0.955**	0.691**	0.499ns	0.383ns	0.355ns	-0.355ns	1		
Luteolin content	0.306ns	-0.209ns	-0.454ns	0.493ns	-0.306ns	1			

Table 3. Continued.

	Total Phenol Content	Total Flavonoid Content	Total Anthocyanin Content	Total Carotenoid Content	Quercetin Content	Luteolin Content	Rutin Content	FRAP	XO Inhibition
Quercetin content	0.434^{ns}	0.542*	0.757**	0.870**	1				
Total carotenoid content	0.549*	0.328^{ns}	0.560*	1					
Total anthocyanin content	0.683**	0.35^{ns}	1						
Total flavonoid content	0.585**	1							
Total phenol content	1								

** Correlation is significant at the 0.01 level.

* Correlation is significant at the 0.05 level.

ns Correlation is not significant.

4 | Discussion

Damage caused by active oxygen to cellular components predisposes to the occurrence or progression of many diseases, such as inflammatory diseases, Gout, diabetes, aging, cancer, and cardiovascular diseases [2]. Protection against these diseases can be provided by antioxidants available in the diet [27]. In all stages of plant growth, as one of the food sources, there is an active antioxidant defense system, including enzymatic and non-enzymatic compounds. The action of these antioxidants is different and varies widely with factors such as maturity stages, weather conditions, and various parts of the plant used, harvesting, and storage conditions [28]. In addition to active oxygen, the increase in uric acid can also lead to various diseases such as Gout, increased blood pressure, increased fats, diabetes, and obesity [29]. Today, Allopurinol is used as one of the main drugs for the therapy of Gout, but this drug has some adverse impacts, such as nausea, vomiting, and liver and kidney injury [7]. The results of this study show that the XO inhibitory effect of methanol extracts of B. vulgaris, C. intybus, U. dioica, A. graveolens, and E. arvense had inhibitory activity at various rates (*Fig. 3b*), and the inhibitory effect had a significant positive correlation with total phenol and rutin (*Table 3*). The highest inhibitory effect of XO enzyme activity was observed in B. vulgaris plants, and the lowest was found in U. dioica and A. graveolens plants. The result also showed that *B.* vulgaris had a significant amount of phenol and flavonoid compounds (*Table 2*). Therefore, there is a strong possibility that the inhibitory effects of the extracts of these plants are related to these compounds. The presence of flavonoid and phenolic compounds increased the absorption changes of the product at the wavelength of 290 nm in B. vulgaris, which is proportional to the increase of the product (uric acid), and the increase of the product is also proportional to the enzyme reaction and the rise in the enzyme activity. Also, flavonoids are considered the most important natural compounds that inhibit this enzyme and the ability of flavonoids to inhibit the enzyme is such that it is used as a positive control (instead of allopurinol) [14]. In addition, flavonoids inhibit XO through competitive inhibition with the active site of the enzyme. With the help of the structural molecular modeling method, it has been shown that among flavonoids, apogenin is the most potent inhibitor of XO, with a stronger interaction with the active site. Among the studied flavonoids, the weakest inhibitory effect on XO is related to isovitexin, which is probably due to the large sugar group attached to it, which prevents the proper interaction of flavonoid with XO by creating spatial hindrance [30], [29]. On the other hand, phenolic and flavonoid compounds cause an increase in the antioxidant activity and further affect the production of ROS by the enzyme.

According to these materials, the role of XO enzyme inhibitors in controlling different diseases such as Gout, arteriosclerosis, and even ischemia is well understood. In Gout, by converting hypoxanthine to uric acid, a large amount of free radicals are produced, which cause tissue damage. In this regard, research conducted on allopurinol and oxypurinol has shown that both are effective in reducing brain damage caused by ischemia [6]. Carefulness in the pathology of Gout reminds us that U. dioica and A. graveolens extracts are not considered as plants lacking anti-Gout properties due to their low inhibitory effect on oxidase enzyme activity, because this plant can improve this disease with its anti-inflammatory properties.

5 | Conclusion

The extracts of Berberis vulgaris and Cichorium intybus had strong inhibitory activity on XO enzyme; therefore, such extracts can be effective in the treatment of many different diseases, such as Gout, caused by the activity of oxidants.

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Data Availability

Data will be made available on request.

Conflicts of Interest

The authors declare no conflicts of interest.

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