

Inhibition Effects of *Teucrium Polium* Extract on GoutElnaz Saghafi,<sup>1</sup> Manijeh Mianabadi,\*<sup>1</sup> Gholamreza Hadadchi<sup>1</sup>

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Article information	Abstract
<p>Article history: Received: 19 May 2012 Accepted: 18 Aug 2012 Available online: 24 Nov 2012 ZJRMS 2013; 15(11): 24-28</p> <p>Keywords: Teucrium polium Xanthine Oxidase Inhibition Antioxidant Gout</p> <p>*Corresponding author at: Department of Biology, Faculty of Science, Golestan University, Gorgan, Iran. E-mail: <a href="mailto:m.mianabadi@gu.ac.ir">m.mianabadi@gu.ac.ir</a></p>	<p><b>Background:</b> <i>Teucrium polium</i> (Lamiaceae) is a natural source containing numerous antioxidant compounds and <i>T. polium</i> had advised for treatment of wide range of diseases including gout, inflammation and diabetes. In this Study, inhibitory effect of extracts, the most important antioxidant contents of <i>T. polium</i> and correlation between them had determined.</p> <p><b>Materials and Methods:</b> Flowering branches of <i>T. polium</i> were collected from nine regions in north-eastern provinces of Iran and dried and powdered afterwards. The inhibitory effects of the plant extract on xanthine oxidase were assayed <i>in vitro</i>. Total Phenol and anthocyanin and soluble sugar content of each extract were matured. Then, their correlations with the inhibitory effects on xanthine oxidase were also determined. All these measurements were repeated three times and variance analysis was used for comparing means.</p> <p><b>Results:</b> All extract in different habitat exhibited a good inhibition effects on xanthine oxidase activity, the concentration of 0.3 mg/ml of samples were inhibited the enzyme from 11.44 to 91.45%. The highest inhibitory effect on xanthine oxidase was found in Ramian by 91.45%. Anthocyanin content in Golestan's samples (Tilabad) was remarkably more than other samples, 4.26 mg/g DW. Razari khorasan's samples (Garmab) had the highest of total phenol and soluble sugar contents, 28.11 and 6.84 mg/g DW respectively.</p> <p><b>Conclusion:</b> These results suggest that <i>T. polium</i> extract in different regions is a rich source of antioxidant and has inhibitors effect on xanthine oxidase. Golestan samples had the highest inhibitory effect on the xanthine oxidase activity and are recommended for pharmacological studies.</p> <p>Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.</p>

## Introduction

Although the study about the plant effect on treatment of diseases has been traditionally considered, the need for further study is still felt. Damages to cells caused by free radicals lead to many diseases such as a cancer, goat, aging, diabetes and cardiovascular diseases [1]. Goat is a chronic metabolic disease with symptoms such as increased uric acid and tissue inflammation [2]. Xanthine oxidase generating uric acid and oxidant is a key enzyme in tissue damage. This enzyme converts hypoxanthine and xanthine to uric acid [3]. Xanthine oxidase using oxygen generates a high amount of free radicals and super oxide and so is a main source of generating super oxide ion and free radicals in body [4]. Allopurinol is one of the most important anti-gout which acts through the inhibition of xanthine oxidase. Using this drug causes nausea and vomiting, bone marrow suppression, kidney and liver damages<sup>5</sup>. Herbal plants are one of the natural sources of antioxidants available and effective in treating gout [5].

*T. polium* (Lamiaceae) is a perennial herbaceous, woody, fragrant plant with white cottony appearance. This plant grows in different geographic areas. Studies suggest that the presence of different compounds with different concentrations in this plant depend on its geographic area [6-8] despite this, a systematic study of the actual assessment of the plant effect on treating gout has not yet

been conducted. So, the inhibitory effect of *T. polium* methanolic extract in different areas on xanthine oxidase activity, the most important factor in developing gout, was examined.

## Materials and Methods

**Plant collection and extraction:** different parts of *T. polium* including leaves and flowering branches were collected from natural habitat in Iran north-eastern provinces including four regions in Golestan province (Tilabad village in Azad-Shahr city, Shesh-Ab village in Ramian city, Karim-Ishan village in Maraveh-Tape city and Golestan Jungal), two region in Razavi Khorasan province (Jagharqh village in Mashad city, Kameh village in Torbat-e-Heydarieh city), two region in Northern Khorasan (Juzak village in Ashkhaneh city, Garmab in Maneh and Samalqhan city) and one region in Semnan province (Shahroud city) (Table 1). The samples were air dried in the shade and ground afterwards. One g of each sample was soaked in 50 ml of methanol and for 48 hrs was incubated in room temperature. After this time, the extract were filtered, the solution was evaporated by rotary evaporator in -40°C. The rest was kept in the fridge in 4°C to be tested [9].

**Soluble sugar content:** soluble sugar measurement was done using phenol-sulfuric acid methods [10]. 0.05 g of dried powdered plant was poured in test tube; 5 ml ethanol 70% was added and for one week was incubated in the fridge. After a week, the extract was centrifuged for 15 min in 10000 g in room temperature. Supernatant was used for measuring soluble sugar. To measure soluble sugar, 0.5 ml of the extract was poured in test tubes and was increased to 2 ml using distilled water, then concentrated sulfuric acid and phenol 5% (1 and 5 ml respectively) was added to each test tube]. The mixture was well stirred and was kept in room temperature for 30 min. Absorption was measured at 485 nm against the blank. Glucose was used as control for drawing the standard curve. The extract soluble sugar content was reported in terms of mg/dried weight.

**Total phenol content:** For measuring total phenol, 2 ml calcium carbonate 2%, 2.8 ml distilled water and 100  $\mu$ l Folin-ciocalteu's reagent 50% were added to 100  $\mu$ l of the extract. Absorption was measured at 720 nm, after 30 min. Gallic acid was used as standard; the extract total phenol was reported by mg Gallic acid equivalent (mg of GA/g dried weight [11].

**Total anthocyanin measurement:** 0.02 g of dried plant tissue and 4 ml HCL (Methanol 1%) were pulverized in a porcelain mortar. The mixture was kept in a fridge for 24 hr. Then, the solution was centrifuged 10min at 13000 rpm. Upper phase absorption was measured at 530 and 657nm. Hydrochloric acid (contain 1% methanol) was used as control. Anthocyanin level for each extract was calculated using this equation [12]:  $A = A_{530} - (0.25 \times A_{657})$   
A: absorption (subscripts numbers indicate wavelength at which was measured).

**The inhibitory effect of the extract on xanthine oxidase activity:** This measurement was done according to the method of Noro with slightly modified [13]. 405  $\mu$ l potassium phosphate buffer (50 mM) pH=7.5, 20  $\mu$ l the plant extract and 150  $\mu$ l xanthine oxidase 0.25 unit were mixed into a test tube and 425  $\mu$ l potassium phosphate buffer (50 mM) pH=7.5, and 150  $\mu$ l xanthine oxidase 0.25 unit were poured in another test tube. They were incubated in 25°C for 15 min. after this time, 300  $\mu$ l xanthin 300 mM was added to each sample as the enzyme substrate. The sample was again incubated in 25°C for 30 min. After the incubation, 750  $\mu$ l hydrochloric acid 1 N was added to block the enzyme reaction. Absorbance then was measured at 295 nm. Blank contains all component except the enzyme solution. The following equation was then used to determine the percentage of xanthine oxidase inhibition (I %) in each sample.  $I \% = (1 - B/A) \times 100$

Where B and A are the absorbance changes in the presence and absent of the plant extract. Each test was

repeated three times. One factor analysis of variance and rank test was used to verify the results. In case of significant ANOVA, comparison charts and Duncan's test were used for paired comparison. Dennett's test at different stages of sampling is taken for comparison with control group. SPSS-16 software was used to determine the correlation coefficient between the characters.

## Results

The comparative analysis of the samples collected from different regions on total phenol content in plants was significant (Table 2, 3). Comparison of total phenol in different regions showed that the highest total phenol was observed in Garmab samples, 28.11 mg Gallic acid equivalent/g DW and the lowest total phenol, 19.99 mg Gallic acid equivalent/g DW was found in Ramian samples. The results of measuring total Anthocyanin content in *T. polium* from different regions of sampling suggest that the effects of different sampling sites which represent the different areas the plant growth, is really significant on the amounts of these compounds (Table 2, 3). The highest total Anthocyanin in Tilabad samples (4.26 mg/g DW) and the lowest one was found in Kameh sample, 1.008 mg/g DW. Variance analysis results (Table 2) suggest that samples from different sites had a significant effect on soluble sugar content. Compare the means (Table 3) showed the highest amount of soluble sugar in Garmab region and Shahrood samples and the lowest one was found in Ramian.

**The inhibitory effect of plant extract on xanthine oxidase activity:** Generating excessive uric acid by xanthine oxidase enzyme causes gout. Plants are the natural sources available in effectively treating gout. The variance analysis results (Table 2, 4) suggest that absorbance changes in the presence or absence of xanthine oxidase enzyme treated with the various extracts was significant. The lowest absorbance change, that is a decrease in enzymatic products, was found in Golestan province samples (Table 5). Percent of xanthine oxidase inhibition showed that maximum capacity of inhibition is owned by 0.3 mg/ml Ramian, Jangle Golestan, and Tilabad samples with 91.45, 81.13, 78.12% inhibition respectively, so that these area samples had the highest inhibitory effect on enzyme activity (Table 3).

**Evaluating the correlation between factors measured at different stages of *T. polium* sampling:** evaluating the correlation between the percent of xanthine oxidase inhibition showed a significant negative linear correlation with soluble sugar content (at 1% level), a significant positive correlation with anthocyanin content, and no significant correlation with total phenol (Table 5).

**Table 1.** Geographical location of sampling sites

Sampling Areas	Position	Coordinates	Altitude (meters)
Around the Shahroud	Semnan Province, city of Shahroud	36° 25' 54" 54° 57' 1.5"	1450
Tilabad	Golestan Province, City of Azad Shar	36° 55' 14" 55° 27' 30.4"	1000
Village of Karim Ishan	Golestan Province, Marave Tape County , Village of Karim Ishan	37° 39' 7.3" 55° 43' 32"	305
Around the Garmab	North Khorasan Province, Maneh and Samalqan County, Garmab Village	37° 43' 29.7" 56° 26' 4.2"	600
Around the Juzak Village	North Khorasan Province, City of Ashkhaneh	37° 25' 51.2" 56° 38' 54.1"	1235
Jangal Golestan	Golestan National Park	37° 20' 11" 56° 00' 17"	940
Around the Shesh Ab Village	Golestan Province, City of Ramian,	36° 55' 15" 55° 27' 31"	1370
Around the Kameh Village	Khorasan Razavi Province, City of Torbat-e Heydarieh	35° 29' 09" 59° 12' 17"	1756
Around the Jagharq Village	Khorasan Razavi Province, City of Mashhad	36° 18' 3.66" 59° 19' 6.14"	1500

**Table 2.** Analysis of variance of some *T. polium* antioxidant components and xanthine oxidase activity in the presence of plant extract from different sites

Changing source	Degrees of Freedom (mg/gdw)	Total phenol content (mg/gdw)	Total anthocyanin content (mg/gdw)	Soluble sugar content (mg/gdw)	Xanthine oxidase activity ( $\Delta OD_{295}$ )
Stages of sampling	8	27.9292**	3.1908**	2.9245**	0.00361267**
Error	18	0.1181	0.0566	0.8414	0.00002719

\*\*The level of significance is less than 1% ( $p < 0.01$ )

**Table 3.** Total soluble sugar, phenol, anthocyanin of *T. polium* and xanthine oxidase activity in the presence of plant extract from different sites

Test Sampling areas	Xanthine oxidase inhibition (%)	Xanthine oxidase activity	Soluble Sugar content ( $mg \cdot g^{-1} \cdot dw^{-1}$ )	Total Phenol content ( $mgGA \cdot g^{-1} \cdot dw^{-1}$ )	anthocyanin content ( $mg \cdot g^{-1} \cdot dw^{-1}$ )
Ramian	91.45 ± 6.323 <sup>a</sup>	0.021 ± 0.0081 <sup>e</sup>	3.892 ± 0.124 <sup>e</sup>	19.99 ± 0.376 <sup>f</sup>	2.304 ± 0.295 <sup>d</sup>
Jangal glestan	81.136 ± 3.516 <sup>b</sup>	0.024 ± 0.0038 <sup>f</sup>	5.598 ± 0.198 <sup>e</sup>	27.69 ± 0.374 <sup>a</sup>	3.929 ± 0.251 <sup>a</sup>
Tilabad	78.12 ± 4.522 <sup>b</sup>	0.028 ± 0.0062 <sup>f</sup>	4.848 ± 0.204 <sup>d</sup>	26.78 ± 0.169 <sup>b</sup>	4.26 ± 0.228 <sup>a</sup>
Shahroud	67.88 ± 0.932 <sup>c</sup>	0.041 ± 0.0015 <sup>e</sup>	6.887 ± 0.210 <sup>a</sup>	23.69 ± 0.197 <sup>c</sup>	2.75 ± 0.051 <sup>bc</sup>
Garmab	59.54 ± 2.304 <sup>d</sup>	0.051 ± 0.0036 <sup>d</sup>	6.848 ± 0.155 <sup>a</sup>	28.11 ± 0.321 <sup>a</sup>	1.616 ± 0.176 <sup>c</sup>
Marave tapeh	48.78 ± 3.947 <sup>e</sup>	0.065 ± 0.0045 <sup>c</sup>	5.593 ± 0.379 <sup>e</sup>	22.37 ± 0.340 <sup>d</sup>	2.591 ± 0.176 <sup>cd</sup>
Ashkhaneh	45.21 ± 2.884 <sup>e</sup>	0.07 ± 0.0049 <sup>e</sup>	6.402 ± 0.263 <sup>ab</sup>	26.31 ± 0.310 <sup>b</sup>	3.125 ± 0.223 <sup>b</sup>
Mashhad	20.81 ± 4.396 <sup>f</sup>	0.101 ± 0.0035 <sup>b</sup>	6.461 ± 0.482 <sup>ab</sup>	27.90 ± 0.311 <sup>a</sup>	2.279 ± 0.268 <sup>d</sup>
Kameh	11.44 ± 2.229 <sup>e</sup>	0.113 ± 0.0008 <sup>a</sup>	6.137 ± 0.222 <sup>b</sup>	21.75 ± 0.297 <sup>e</sup>	1.008 ± 0.135 <sup>f</sup>

**Table 4.** Dunnett test for enzyme activity analysis based on the sampling locations

Sampling areas	Tilabad	Ramian	Maraveh Tapeh	Jangal Golestan	Mashhad	Kameh	Shahroud	Ashkhaneh	Garmab
Control	-0.099**	-0.116**	-0.062**	-0.103**	-0.026**	-0.014**	-0.086**	-0.057**	-0.076**

\*\*The level of significance is less than 1% ( $p < 0.01$ )

**Table 5.** Correlation between the percent inhibition of xanthine oxidase and several factors of *T. polium* samples from different regions

	Soluble sugar content (mg/gdw)	Total phenol content (mg/gdw)	Total anthocyanin content (mg/gdw)	Xanthine oxidase inhibition (%)
Xanthine oxidase inhibition (%)	-0.539**	-0.043 <sup>ns</sup>	0.577**	1
Total anthocyanin content (mg/gdw)	-0.295 <sup>ns</sup>	0.377 <sup>ns</sup>	1	
Total phenol content (mg/gdw)	0.448*	1		
Soluble sugar content (mg/gdw)	1			

\*\*The level of significance is less than 1% ( $p < 0.01$ ). \* The level of significance is less than 5% ( $p < 0.05$ ). <sup>ns</sup> No significance correlations between traits

## Discussion

Xanthine oxidase inhibitory effects of *T. polium* methanolic extract suggest that the crude extracts of all sites in a concentration of 0.3 mg/L, inhibited xanthine oxidase activity with varying degrees, from 11.44% to 91.45% (Table 3), which indicate the existence of different types and amounts of compounds within them.

Numerous natural compounds are considered as the xanthine oxidase inhibitors, most notably flavonoids. Among these natural inhibitors are pentagalloylglucose [14], flavonoids such as luteolin [15], polyphenols [16], flavonols [16], steroid alkaloids [17], coumarins [18], ptryns [19] and anthocyanins [20]. These compounds are highly affected by factors such as geographical regions, weather condition different parts of the plant and harvest

and storage conditions [21]. Inhibition percentage of xanthine oxidase had significant negative correlation with soluble sugar and significant positive correlation with anthocyanin content in 1%. But it had no significant correlation with total phenol content (Table 5). Soluble sugars and enzymes associated with their metabolic pathways widely related to oxidative stress and signaling of active oxygen species [22]. So, soluble sugars reduce oxidative stress by refining super oxide and hydroxyl radicals and lipid peroxidation inhibition. Studies suggest that generating free radicals by xanthine oxidase increases in presence of some sugar derivatives of flavonoids. This means that some sugar derivatives particularly amin sugar flavonoids due to space prevention are weaker substrate for xanthine oxidase has less inhibitory effect on oxidase and therefore have less inhibitory effect on the enzyme [23]. Phenolic compound are collecting free radicals and or metal ions chelation, so they have high antioxidant activity [24]. Some flavonoids are competitive inhibitors of xanthine oxidase and some of them have no effect on the enzyme activity [25]. In this plant, phenolic compounds had no significant effect on absorbance increase at 290 nm. Sour cherries are a rich source of anthocyanin with high levels of anthocyanin. This plant extract reduces uric acid levels in rats which is also consistent with inhibition of enzyme activity [26]. Consistent with Results, there is a significant positive correlation between total anthocyanin of *T. polium* and its xanthine oxidase inhibition. Reactive oxygen species cause or are developing disease such as inflammation, gout, diabetes, aging, cancer and cardiovascular diseases by damage to cellular component [27, 28]. Dietary antioxidants can protect the body against these diseases [29]. Increased uric acid levels can also predispose many diseases such as gout, hypertension, hyperlipidemia, diabetes and obesity [30].

Anti gout drug allopurinol, this is now one of the most important works by inhibiting xanthine oxidase. However,

use of this drug may have complications such as nausea and vomiting, bone marrow suppression and liver and kidney damage is [5]. Many studies have been to find herbal xanthine oxidase inhibitors in many countries such as India, Australia and United State [31-33].

Because this enzyme during ischemia, produce large amounts of free radicals that cause tissue damage it is valuable to inhibition [34], this means that Xanthine oxidase activity have crucial role in controlling gout, arteriosclerosis and even Ischemia. In particular, studies suggest that allopurinol and exopurinol are effective on ischemic brain damage by inhibiting xanthine oxidase activity [35]. The results of this study show that *T. polium* methanolic extracts from different habitat had various contents of antioxidant compounds and so inhibitory effect on xanthine oxidase activity. Golestan samples show the highest inhibitory effect on the enzyme activity. Therefore, it is recommended as the proper area for harvested crop and for pharmaceutical studies on animal models. This region is an appropriate region for the plant harvest in surveying animal models and pharmacologic studies.

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### Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

### Conflict of interest

The authors declare no conflict of interest.

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