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Protective potency of *Meristotropis xanthioides* against nephrotoxicity in a rat model along with its antioxidant and antibacterial activities

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ABSTRACT

Objective: To investigate nephroprotective potential of *Meristotropis xanthioides* (*M. xanthioides*) extract against ethanol-induced nephrotoxicity in Wistar rats, and also its total phenolics content, antioxidant and antibacterial activities.

Methods: Total phenol and flavonoid amounts of the leaf and stem extracts were determined by Folin–Ciocalteu and aluminum chloride reagents, respectively. Antioxidant and antibacterial activities of the extracts were investigated by 2,2-diphenyl-1-picrylhydrazyl radical scavenging and disc diffusion methods, respectively. In addition, protective potential of the leaf extract against ethanol-induced nephrotoxicity was studied by histological and biochemical analyses.

Results: Obtained results indicated high total phenol [(10.26 ± 0.46) mg GAE/g of dry extract] and flavonoid [(3.63 ± 0.62) mg QE/g of dry extract] amounts in the leaf extract. The leaf and stem extracts possessed stronger antioxidant activity [IC_{50}: (0.119 ± 0.006) mg/mL and IC_{50}: (0.133 ± 0.009 mg/mL)] than that of ascorbic acid [IC_{50}: (0.142 ± 0.002) mg/mL]. Also, the extracts showed good antibacterial activity against the most of bacteria taken in this research, especially Gram-positive ones. Histological examinations revealed tissue injury in the kidney of rats treated with ethanol. Results from biochemical assays showed reduction in total protein content and also in superoxide dismutase activity. In addition, remarkable increased levels ($P < 0.05$) of H_2O_2 and malondialdehyde were found in ethanol-treated rats in comparison to control group. However, these injuries were significantly improved in rats treated by *M. xanthioides* leaf extract.

Conclusions: Results from present study demonstrates strong pharmaceutical potential of *M. xanthioides* extract to apply as a new drug supplement.

1. Introduction

Polyphenols particularly flavonoids, are a large group of organic compounds in plants that showed important pharmaceutical activities such as antiradical properties [1–3]. In organisms, free radicals such as reactive oxygen are formed normally during processes such as cell respiration and have important roles in cell signaling [4]. Also, free radicals may be formed through different exogenous agents such as UV radiation, smoking and both acute and chronic alcohol exposures [5]. High amounts of the free radicals are harmful and cause complete degradation of important molecules including lipids in cell membrane, protein such as enzymes and nucleic acids that play important roles in induction and development of different diseases [6,7]. Ethanol consumption has adverse effects on tissues of kidney, testis, nervous system and cardiovascular system [8]. Ethanol causes reactive oxygen production such as H_2O_2, resulting in structural and morphological changes and finally creation of injury in many tissues [9,10]. Antioxidant supplementation specially with a natural source may use to cure these injuries. Phytochemical compounds, particularly flavonoids in foodstuffs and other natural compounds act as scavenger, reducing, quencher agents and/or activators of cellular antioxidant enzymes to prevent free radical damages in biological systems [11–14]. In recent years, rapid increase in antimicrobial resistance to antibiotics is a serious concern in medical sciences [15]. Also, there is an increasing interest about use of plant extracts as

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source of new antimicrobial agents against antibiotic-resistant human pathogens [16].

The genus *Glycyrrhiza* L. belonging to the family Fabaceae is native to most regions of the world especially Asia [17]. The genus includes about 20 species and has the highest distribution in the northeast and west of Iran. *Merstotropis* Fisch. etMey. (Fabaceae) with the only one species of *Meristotropis xanthioides* (*M. xanthioides*) Vassilcz. is very close to the genus *Glycyrrhiza*. Plants of both genera are widely utilized in pharmacy, metallurgy, for food and other industrial branches and in agriculture. They contain various phytochemical compounds including essential oils, phenolic acids and terpenes such as saponinglycoside [18,19]. The aim of present research was to investigate total phenolic contents, antioxidant capacity, antibacterial activity and protective role against nephrotoxicity of *M. xanthioides* extract.

2. Material and methods

2.1. Plant materials

Fresh plant materials of *M. xanthioides* were collected randomly from Khorasan Province, NE Iran in July 2016. The taxonomic identification of the studied species was confirmed by Dr. Negaresh and the voucher specimen was deposited at the Bu-Ali Sina University Herbarium, Hamedan, Iran.

2.2. Preparation of plant extracts

The powdered stem and leaf parts of *M. xanthioides* (25 g) were continuously extracted in absolute methanol using a Soxhlet apparatus. The extracts were concentrated to dryness using a rotary evaporator (Lab Tech, Ev 311, Italy) to yield a green gum. In this research, total phenol and flavonoid amounts and also antioxidant and antibacterial activities were assessed in leaf and stem extracts. In addition, for higher total phenol content the leaf extract was used to evaluate protective effect against nephrotoxicity in rats. Data obtained from different assays were the average of triplicate analyses and recorded as means ± standard deviation.

2.3. Determination of total phenol content

Total phenol content was determined by the Folin–Ciocalteu method of Donald *et al* [20]. A volume of 0.5 mL of the extracts (1:10 g/mL diluted with methanol) was mixed with 5 mL Folin–Ciocalteu reagent (1:10 diluted with distilled water) and 4 mL Na_2CO_3 (1 mol/L) solution. After 30 min, the absorbance was determined by a double beam Perkin Elmer UV/visible spectrophotometer (USA) at 760 nm. The amount of total phenol was calculated as mg GAE/g dw.

2.4. Determination of total flavonoid content

$AlCl_3$ colorimetric method was used for total flavonoid determination [21]. Briefly, 0.5 mL of each diluted extract (1:10 g/mL) was separately mixed with 1.5 mL methanol, 0.1 mL 10% $AlCl_3$, 0.1 mL 1 mol/L KCH_3COO and 2.8 mL distilled water. After 30 min; the absorbance was measured at 415 nm by a coulometric method. The amount of total flavonoid was calculated as mg Q/g dw.

2.5. Biological activity

2.5.1. Antiradical capacity

The free radical scavenging potential of the stem and leaf extracts was determined by Mansor *et al* [22]. All the samples (0.2–1.0 mg/mL) were dissolved in absolute methanol mixed with DPPH free radical solution (0.3 mmol/L). In the reaction mixture, remaining DPPH amount was measured at 517 nm after 30 min using a colorimetric method. Ascorbic acid was employed as the control. The antiradical activity (AA%) was calculated by following formula:

$$AA\ (\%) = [1 - (A_s - A_b)/A_c] \times 100.$$

where, A_s is the absorbance 2.5 mL of the extracts in different concentrations (0.2–1.0 mg/mL) and 1 mL DPPH, A_b is the absorbance of the reference (2.5 mL of extracts in different concentrations and 1 mL methanol) and A_c is the absorbance of control sample (1 mL DPPH and 2.5 mL methanol). IC_{50}, which is the concentration of extract to decrease the absorbance of DPPH free radical by 50%, was calculated from antiradical activity percentage against extract concentration.

2.5.2. Animals and experimental design

The adult male Wistar rats (150–200 g) were housed in standard conditions and all experiments were approved by the ethics committee of animal research of Bu-Ali Sina University. Rats were treated by intragastric administration and divided in three different groups containing six animals each: group 1 (the control group) received daily 1 mL water, group 2 (ethanol group) received daily 1 mL 40% ethanol and group 3 (ethanol and extract group) received daily 1 mL extract (500 mg/kg) and ethanol in 4 h intervals. At the end of treatment period, animals were sacrificed by cervical dislocation under mild ether anesthesia and kidneys were harvested, rinsed in saline and stored at −70 °C till further biochemical analysis.

2.5.3. Histological examination

The kidneys fixed in 10% formalin for 24 h. The tissue samples were dehydrated with elevated degree of ethanol and embedded in paraffin for preparing paraffin blocks. Sections from kidney tissue (4–5 μm) were stained with hematoxylin-eosin. The sections were examined and photographed by a BX-51 Olympus microscope (Nagano, Japan) and then evaluated for some parameters including proximal tubule necrosis, per tubular capillary congestion, tubular dilation and mononuclear cells infiltration.

2.5.4. Enzyme extraction

For enzyme extraction, the frozen kidney tissue (0.2 g) was ground in liquid nitrogen to a fine powder and then homogenized in 1.5 mL of extraction buffer including 50 mmol/L PBS (pH 7.8), 0.1 mmol/L EDTA and 0.3% polyvinyl polypyrroidone. After centrifugation (4 °C, 12 000 r/min, 15 min), the supernatant was used for determination of antioxidant enzyme activity.

2.5.5. Determination of total protein content

Briefly, total protein amount was determined according to Bradford's method by bovine serum albumin as standard [23].

2.5.6. Determination of malondialdehyde (MDA) content

MDA content of the kidney tissue was determined by trichloroacetic acid/thiobarbituric acid method [24]. Briefly, 0.2 g fresh kidney tissue was finely extracted in 5 mL trichloroacetic acid solution (0.1%, w/v) and then centrifuged (10 000 r/min, 10 min). A total of 4 mL 20% trichloroacetic acid solution containing 0.5% thiobarbituric acid was prepared and added to 1 mL of the tissue extract. Then, the samples were heated in a water bath at 95 °C for 15 min and stopped the reaction immediately by cooling the samples on ice water bath. The samples were centrifuged again (10 000 r/min, 10 min) and the absorbance was read at 532 nm by a double beam Perkin Elmer UV/visible spectrophotometer.

2.5.7. Determination of hydrogen peroxide content

H_2O_2 amount of the kidney tissue was determined according to Velikova *et al* [25]. Tissue extraction was similar to the MDA method and also similar centrifugation (10 000 r/min, 10 min) was used. Then 0.5 mL phosphate buffer (pH 7.0) and 1 mL potassium iodide were added to 0.5 mL of the extract. Its absorbance was recorded at 390 nm by a colorimetric method.

2.5.8. Determination of superoxide dismutase activity

A 1.5 mL reaction mixture containing phosphate buffer saline (50 mmol/L, pH 7.8), EDTA (0.1 mmol/L), nitro blue tetrazolium (75 mmol/L), riboflavin (2 mmol/L), methionine (13 mmol/L) and then enzyme extract were mixed. Then, the samples incubated for 25 min in light condition prepared by a 30-W bulb and the absorbance was recorded at 560 nm by a colorimetric method. Blank contained reaction mixture without light exposure. The superoxide dismutase activity in percent was calculated by following formula:

$$\text{Activity } (\%) = (A_s - A_c)/A_c \times 100.$$

here, A_s is the absorbance of the sample containing reaction mixture reference with enzyme extract (100 μL) and A_c is the absorbance of control sample containing reaction mixture reference without enzyme extract [26].

2.5.9. Antibacterial activity

The potential antibacterial activity of the extracts was assessed against two Gram positive bacteria, namely *Bacillus cereus* (PTCC 1247) and *Staphylococcus aureus* (ATCC 25923) and two Gram negative bacteria, namely *Escherichia coli* (*E. coli*) (ATCC 35218) and *Ralstonia solanacearum* (ATCC 11696) [27]. The extracts were dissolved in DMSO to make different concentrations (1, 0.1 and 0.01 mg/mL) and then sterilized by filtration using a 0.45 μm Millipore. All tests were carried out using 10 mL suspension containing distinct number of bacteria (1.5×10^8 bacteria/mL) spread on Muller-Hinton agar medium. Negative and positive reference standards were prepared using DMSO and the antibiotics gentamicin, penicillin, and streptomycin, respectively.

3. Results

3.1. Total phenol and flavonoid content

Our results showed that the leaf extract represented a higher amount of total phenol [(10.26 ± 0.46) mg GAE/g of dry extract] than that of the stem extract. However, they were not significantly different ($P < 0.05$) in total flavonoid content (Table 1).

3.2. Antiradical capacity

Antiradical capacity of *M. xanthioides* extracts is presented in Table 1. Results showed that the antiradical property of both extracts were concentration-dependent and more than that of vitamin C (72.26%) as a standard (Figure 1). In all methods, a higher biological activity is reflected in a lower IC_{50} value. Here, the DPPH radical scavenging activity ranged in the following descending order: stem extract [IC_{50}: (0.119 ± 0.006) mg/mL] > leaf extract [IC_{50}: (0.133 ± 0.009) mg/mL] > ascorbic acid [IC_{50}: (0.142 ± 0.002) mg/mL] (Table 1).

3.3. Nephrotoxicity assay

3.3.1. Histological examination

Our results indicated that necrosis and atrophy of proximal tubules (yellow arrow in Figure 2B) in only ethanol-treated rats (group 2) remarkably increased in compression to control group (Figure 2A). Also, some necrotic glomeruli were found at renal cortex in rats treated with ethanol (black arrowhead in Figure 2B). Other pathological effects such as tubular dilation

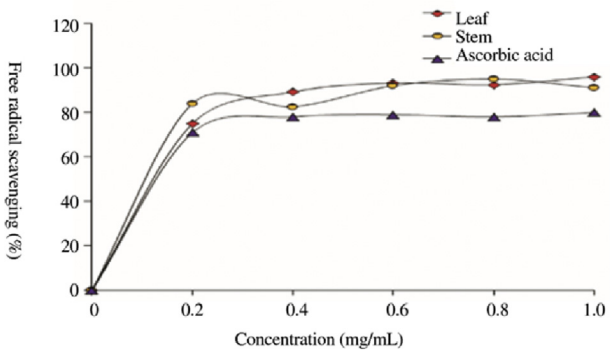

Figure 1. Antiradical property (%) of *M. xanthioides* leaf and stem extracts and ascorbic acid at different concentrations (0–1 mg/mL).

Table 1
Total phenol and flavonoid amounts and antioxidant activity of *M. xanthioides* extracts.

Sample		Total phenol content (mg/g dw)	Total flavonoid content (mg/g dw)	DPPH free radical scavenging	
				IC_{50} (mg/mL)	Average (%)
M. xanthioides	Stem	3.74 ± 0.17[b]	3.59 ± 0.46[a]	0.119 ± 0.006	88.88
	Leaf	10.26 ± 0.32[a]	3.63 ± 0.12[a]	0.133 ± 0.009	89.16
Ascorbic acid		–	–	0.142 ± 0.002	71.26

Experiment was performed in triplicate and expressed as mean ± SD. Values in each column with different superscripts are significantly different ($P < 0.05$).

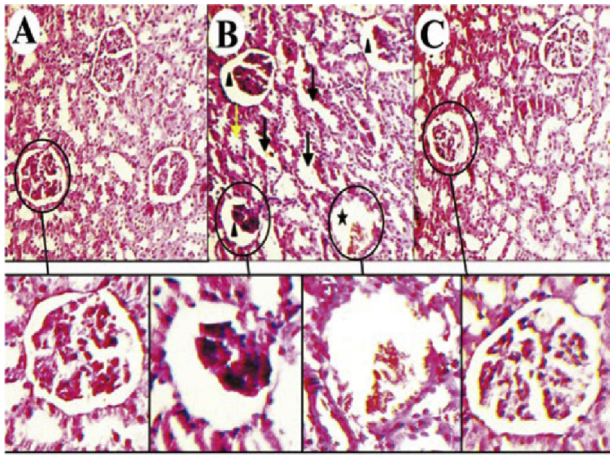

Figure 2. Histological changes in kidney of rats after ethanol and *M. xanthioides* leaf extract administration.
(A) Control group (without treatment). (B) Ethanol-treated rats showed tubular necrosis (yellow arrow), epithelial desquamation (black arrow), glomerular atrophy (black arrowhead) and capillary congestion (black star). (C) Ethanol + leaf extract-treated rats showed regenerate kidney tissue to near normal condition.

(black arrow in Figure 2B), peritubular congestion and epithelial desquamation (black star in Figure 2B) were increased in ethanol-treated group. However, almost all injuries were improved by the leaf extract (group 3) (Figure 2C).

3.3.2. Biochemical assay

Results from biochemical assays of kidney tissue are represented in Table 2. Total protein content and superoxide dismutase (SOD) activity were decreased significantly ($P < 0.05$) in ethanol-treated rats (group 2) in comparison to control (group 1) and ethanol + extract-treated rats (group 3). In other biochemical analyses, group 2 showed significant increase ($P < 0.05$) in the contents of H_2O_2 and MDA in comparison to other groups (Table 2).

3.4. Antibacterial activity

Results from antibacterial assessment of the extracts and standards (positive and negative) are presented in Table 3. The studied extracts were dissolved in DMSO at different concentrations (0.01, 0.1 and 1.0 mg/mL). DMSO solution was also assessed as negative control against all bacteria taken in this study and no activity was found. The extracts represented a concentration-dependent inhibition against Gram positive bacteria in comparison to reference antibiotics, especially penicillin (Table 3). But negative bacteria especially *E. coli* (−) showed more resistance. The leaf extract showed a higher inhibition activity than the stem extract against bacteria tested.

4. Discussion

Plant extracts with antioxidant potential such as free radical scavenging capacity is very important due to the deleterious role of reactive oxygen species (ROS) and reactive nitrogen species [12,14,26]. Polyphenols that possess pharmaceutical activity have been characterized as phenolic acids and flavonoids [26]. Phenolic compounds especially flavonoids with hydroxyl groups in their structure are powerful hydrogen donors because they can react with the most of ROS and reactive nitrogen species act as scavengers, reducing or quencher agents to prevent from the free radical damages in organism [11–14,27,28]. These compounds have high capacity to strongly interact with the most of proteins because they give them the ability to inhibit some oxidative enzymes involved in ROS generation, such as lipoxygenases, cyclooxygenase, xanthine oxidase and various cytochrome P_{450} isoforms. Activities of

Table 2
Results from biochemical assay of the kidney tissue in different groups of rats.

Sample	Protein (mg/g.W.t)	MDA (mg/g.W.t)	H_2O_2 (μmol/L·g.W.t)	SOD (%)
Ethanol	0.205 ± 0.002[a]	0.118 ± 0.007[a]	18.50 ± 0.53[a]	65.76 ± 0.18[a]
Ethanol + Extract	0.234 ± 0.006[b]	0.085 ± 0.006[b]	3.79 ± 0.12[b]	72.24 ± 0.42[b]
Control	0.236 ± 0.003[b]	0.076 ± 0.009[b]	0.84 ± 0.05[c]	73.25 ± 0.53[b]

Experiment was performed in triplicate and expressed as mean ± SD. Values in each column with different superscripts are significantly different ($P < 0.05$).

Table 3
Antibacterial activity of the studied extracts and standards (positive and negative controls).

Sample		Concentration (mg/mL)	Inhibition zone (mm)			
			Ralstonia solanacearum (−)	*E. coli* (−)	*Staphylococcus aureus* (+)	*Bacillus cereus* (+)
M. xanthioides	Stem	1	16.00 ± 0.31[a]	8.00 ± 0.52[a]	13.00 ± 0.42[a]	18.00 ± 0.21[a]
		0.1	11.00 ± 0.28[b]	7.00 ± 0.10[b]	11.00 ± 0.25[b]	12.00 ± 0.18[b]
		0.01	8.00 ± 0.12[c]	NA	10.00 ± 0.16[c]	9.00 ± 0.32[c]
	Leaf	1	18.00 ± 0.26[a]	NA	20.00 ± 0.61[a]	17.00 ± 0.15[a]
		0.1	14.00 ± 0.14[b]	NA	15.00 ± 0.32[b]	12.00 ± 0.36[b]
		0.01	12.00 ± 0.57[c]	NA	13.00 ± 0.15[c]	10.00 ± 0.13[c]
Control (+)		Gentamicin	30.00 ± 0.10	NA	33.00 ± 0.23	23.00 ± 0.11
		Streptomycin	26.00 ± 0.00	18.00 ± 0.16	27.00 ± 0.21	20.00 ± 0.32
		Penicillin	NA	NA	NA	NA
Control (−)		DMSO	NA	NA	NA	NA

Experiment was performed in triplicate and expressed as mean ± SD. Values in each column with different superscripts are significantly different ($P < 0.05$). NA: no active.

these enzymes increase after heavy alcohol exposure [29]. Also, regulation of intracellular glutathione amounts and synergistic or antagonistic effects of polyphenols with other antioxidants agents have also been described [30,31]. In this research, a higher total phenol amount was detected in the leaf extract, but the extracts were not significantly different ($P < 0.05$) in total flavonoid amount. Among *in vitro* antioxidants agents, polyphenols especially flavonoids have been shown to have more activity than vitamins E and C on molar basis [32]. Results from DPPH free radical scavenging showed that both extracts have antiradical activity more than ascorbic acid (vitamin C). It has been demonstrated a positive correlation between phenolic compounds and their biological activities in plant extracts by some researches, while others reported a poor or no relationships [33–35]. Generally, differences in antioxidant properties could be related to the types of polyphenols such as flavonoids and also their synergistic or antagonistic effects in minor percentages [36]. However, literature search did not reveal any references to previous study on assessment of polyphenol amount and antioxidant activity of the studied species.

Liver and kidney are very important organs for their functions including detoxification of xenobiotics and also are very sensitive to damage, especially against free radicals [37–40]. All different exogenous agents produced free radicals such as UV irradiation, smoking and both acute and chronic alcohol exposures can cause increase oxidative stress [5]. Yet exact mechanism, by which ethanol induces kidney damage is unclear. But, it has been recently indicated that ethanol by producing xenobiotic compounds induces alterations in physiological and biochemical conditions of cells such as increase of cytochrome P450 system activity that leads to more production of ROS, results in function and structure changes [9,10]. There are some reports on nephrotoxity induced by different compounds such as wine, chromium, ethanol, cadmium and gentamicin [41–52]. Previous studies showed that alcohol exposure causes desquamation of epithelial cells and degeneration of renal tubules epithelia, atrophy of glomeruli and cortical parts with mononuclear cell infiltrates in kidney tissue of rats [42]. According to our histological observations the ethanol-treated rats showed tissue injuries including tubular necrosis, glomerular atrophy, capillary congestion and epithelial desquamation in comparison to control group. However, administration of the leaf extract to ethanol-treated rats caused a marked histological protection.

In most cases, oxidative stress induces lipid peroxidation and cell destruction, due to the interaction between free radicals and cell membrane fatty acids. This process leads to MDA level increasing in tissue. In normal conditions, the important detoxification pathway against oxidative stress including SOD catalyzing the first step and then, catalase and also glutathione peroxidase removing the H_2O_2 generated [48–52]. Results from biochemical assay indicated a significant ($P < 0.05$) increasing in MDA and H_2O_2 levels, but decreasing in total protein content in the kidney tissue of rats treated with ethanol alone that could verify the lipid peroxidation and cell injuries. This situation coupled with a significant ($P < 0.05$) decrease in SOD activity in the kidney tissue. Previous studies on lipid peroxidation induction in rat kidneys tetrad with ethanol are controversial. Remarkable increase in MDA level in kidney tissue after chronic ethanol consumption was reported by some researchers, which is in agreement with our results [46,47]. In contrast, other studies have indicated that there are no remarkable changes in kidney MDA amount in rats fed ethanol [48,49]. Previous studies indicated that alcohol consumption causes decrease in SOD activity in different organs especially kidney [48–54]. According to our results, increasing in SOD activity in ethanol and leaf extract-treated rats may be due to the antioxidant properties of polyphenols compounds, particularly flavonoids in *M. xanthioides* leaf extract that act as scavenger, reducing, quencher agents and/or activators of cellular antioxidant enzymes to prevent from the ROS damages [11–14]. Our findings for the first time showed that *M. xanthioides* leaf extract has positive effect on the antioxidant system and can regenerate kidney of ethanol-treated rats to near normal condition.

Today most of bacteria are resistant to classical antibiotics and the discovery of new compounds with natural source is a matter of urgency. The most interesting area for application of plant metabolites is the inhibition of growth and reduction in numbers of more serious food-borne pathogens [53,54]. It is well known that *Staphylococcus aureus* (−), *E. coli* (−) and *Bacillus* species are agents of food poisoning [55]. On the other hand, *Ralstonia solanacearum* (−) is a plant pathogen and a soil inhabitant that attacks its host by entering through the root and grows in the plant xylem. This pathogen causes blocking the vascular system and prevents transport of water and nutrients that eventually can cause severe losses of crops and death of plants have great economic importance [56,57]. The studied extracts showed remarkable antibacterial activity in comparison to some standards. Although all bacteria tested were resistant to penicillin, the inhibitory effect of the extracts on resistant species was higher than that of the mentioned antibiotic. However, mechanism of inhibitory effect of plant extracts on microorganisms is unknown. It is possibly related to the effect of extracts on the important components of microbial cells including cell wall, cell membrane, DNA and proteins especially enzymes. Literature search did not reveal any references to previous study on the assessment of antibacterial effect of the investigated plant. Results from present research for the first time demonstrated that *M. xanthioides* methanolic extract contains important compounds represent biological activity. So, this plant can use as a novel therapeutic agent to prevent the progress of various diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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