



# Antioxidant and scolicidal activities of four Iranian *Mentha* species (Lamiaceae) in relation to phenolic elements

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## ABSTRACT

**Introduction:** There is a growing interest of industry to replace synthetic chemicals by natural products with bioactive properties from plant origin. The present study reported the antioxidant activity and scolicidal effects of the crude extracts from *Mentha spicata*, *M. aquatica*, *M. longifolia*, and *M. × piperita* var. *citrata* growing in Iran.

**Methods:** Total phenolic, flavonoid and flavonol contents of the four *Mentha* taxa were examined. Two antioxidant assays i.e. free radical scavenging activity (DPPH assay) and reducing power assay were used for determining the antioxidant capacity of the alcoholic extracts. Scolicidal activity of serial dilutions (15–200 mg/mL) of *Mentha* extracts was evaluated after 1, 5, 10, 20 and 30 minutes of exposure time.

**Results:** Methanol was the solvent yielding the highest values of total phenolic (TPC), flavonoid (TFC) and flavonol contents (TFvC). On the other hand, the extracts from *M. × piperita* var. *citrata* gave the highest total phenolic content (191.6 mg gallic acid equivalent per g; GAE/g), total flavonoid content (57.0 mg quercetin per g; QE/g) and total flavonol content (15.3 mg QE/g) values. The methanol extracts of *M. × piperita* var. *citrata* also gave the strongest DPPH radical scavenging activity (83.2%), whereas the reducing power yielded absorbance values between 0.189 in *M. spicata* and 1.16 in *M. × piperita* var. *citrata*. The highest scolicidal activity (99.54%) was observed in 200 mg/mL methanol extract of *M. aquatica* after 30 minutes of application.

**Conclusion:** Overall, *M. × piperita* var. *citrata* and *M. aquatica* could be the taxa of choice for future supplementary studies.

### Implication for health policy/practice/research/medical education:

Extracts of *Mentha* species exhibited potent antioxidant and scolicidal activities, which may have significant implications for the future development of anti-parasitic drugs derived from *Mentha* species.

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## Introduction

Oxidation is known to have negative effects, especially in industrial context. Oxidation reactions occur in foods and cosmetics, often because of the prolonged exposure to oxygen. Food preservation represents an issue of concern and requires assurance of protection from microbial spoilage and prolongation of shelf life (1). The damaging effects caused by oxidation have commonly been reduced by the usage of synthetic antioxidants. However, health critics and researchers are questioning the safety of such compounds, as the usage of synthetic chemicals has

increasingly become viewed as a threat to human health. Plant-derived compounds have gained considerable importance due to their increasing applications as additives in food and cosmetics technologies for the protection of easy oxidizing components (2). On the other hand, such compounds are of interest for the pharmaceutical industries to combat human diseases of, for example, microbial and parasitical origins. Phytochemicals may serve as key compounds to develop new drugs to be used against many chronic diseases recognized as complicated to handle. Hydatidosis caused by the larval stage of

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*Echinococcus* spp. is a major zoonotic infection that is detrimental to both humans and animal husbandries in many countries (3). Nowadays, parasitic diseases are usually dealt with synthetic drugs. However, such cures have some disadvantages, e.g. side effects, cost and risk of misuse leading to drug resistance, unavailability of treatments in developing countries, and environmental and food pollution (4). These problems stimulated the research of other potential therapeutic options, plants-based derivatives in particular. Accordingly, a large number of medicinal and aromatic plants contain chemical compounds, with antioxidant and therapeutic activities. Among these various kinds of natural substances, phenolic compounds including flavonoids, are receiving particular attention.

The genus *Mentha* L. (Lamiaceae) is widely distributed throughout the temperate regions of the world, and in Iran is represented by 6 species. *Mentha* species have been extensively used for treatment of various diseases as well as for application in cosmetic and food industries (5). They are well known worldwide for antioxidant, antimicrobial, and antiviral activities (2,6). In Iranian Traditional Medicine, these plants have found application as tonic, digestive, carminative, antispasmodic, stomachic, anti-inflammatory and flavoring remedies (6). However, literature data on the antiparasitic activity of the genus *Mentha* is scarce, and we know little about the scolicidal activity of the genus against protoscolices of hydatid cysts. Thus, the aim of the present work was to determine the biological activities, namely antioxidant and scolicidal effects, of the most common *Mentha* species growing in Iran (*M. spicata* L., *M. aquatica* L., *M. longifolia* L., *M. × piperita* L. var. *citrata*) in order to find further applications in the pharmaceutical industries.

## Material and Methods

### Plant material

Aerial parts of four *Mentha* species including *M. spicata*, *M. aquatica*, *M. longifolia*, *M. × piperita* var. *citrata* were collected in Mazandaran province, Iran, in 2017, at three different seasonal stages (5 April, 5 June and 5 August, hereafter April, June and August, respectively). The botanical identification was performed by the first author (M.R.) and voucher specimens were deposited at the Herbarium of the Department of Biology, Mazandaran University, Babolsar, Iran (IR-2017/345-348).

### Preparation of extracts

Aerial parts of each specimen (100 g) were powdered to a homogenous particle size, and macerated in either ethanol or methanol at room temperature for 48 hours. After filtration, the crude extract was evaporated to dryness under reduced pressure, and then dissolved in 80% methanol to the final dilution as required, except for determination of scolicidal activity, in which the dried

extract was dissolved in physiological serum.

### Total phenolic, flavonoid and flavonol contents

The concentrations of phenolic compounds in the extracts were determined according to the method published by Kim et al, with slight modifications, and results were expressed as mg gallic acid equivalents per gram dry weight of sample (GAE/g dw) (7). The total flavonoid and flavonol contents in the extracts (expressed in milligram quercetin per g of plant material (mg Q/g dw) were determined according to Moreno et al and Ložienė et al methods, respectively (8,9).

### DPPH radical scavenging and reducing power assays

The antioxidant activities of methanol and ethanol extracts were determined by the DPPH free radical scavenging assay method as previously described (10). The ability of the extracts to reduce ferric ions ( $Fe^{+3}$ ) was determined according to the method published by Yen and Chen (11,12).

### Scolicidal activity

Hydatid cysts protoscolices were collected from the livers of infected sheep in Northern Iran, at Mazandaran, Amol. The viability of protoscolices was assessed by 0.1% eosin staining under light microscopy. To determine the scolicidal activity of methanol and ethanol extracts against protoscolices of hydatid cysts, five dilutions (20, 40, 60, 90 and 120 mg/mL) of extracts were used for 1, 3, 5, 10, 20 and 30 minutes. 0.5 mL of the protoscolices solution was placed in test tubes and 0.5 mL of various concentrations of extracts was added to each test tube. Contents of the tubes were gently mixed and incubated at 37°C for 1, 3, 5, 10, 20 and 30 min. The upper parts of the solution were removed with a pipette and 1 mL of 0.1% eosin stain was added to protoscolices. The protoscolicidal activity of each solution was determined by counting of 250 protoscolices (13). In control, *Mentha* extracts were excluded from the medium containing protoscolices. Sterile control was a medium without the parasite. In positive control, the standard drug albendazole (Pourateb, Iran) was used (50 µg/mL) against protoscolices.

### Statistical analysis

All experiments were carried out in triplicate, and the data were analyzed using analysis of variance (ANOVA) and significant differences among means at ( $P < 0.05$ ) were determined by Duncan's test using SPSS 16 (SPSS Inc., USA) software.

## Results

### Extract yields

The extract yields of the four *Mentha* species were obtained by using methanol and ethanol evaluated at three harvest times (Table 1). Variations in the yields of

**Table 1.** Methanol/Ethanol extract yields of four *Mentha* species over growing season

Taxa	April		June		August	
	Methanol	Ethanol	Methanol	Ethanol	Methanol	Ethanol
<i>M. spicata</i>	12	8.5	18.8	12.9	23.3	17.2
<i>M. aquatica</i>	9.2	8.8	12.9	10.5	19.5	13.5
<i>M. longifolia</i>	18.9	11.5	19.5	12	20.75	14.5
<i>M. × piperita</i> var. <i>citrata</i>	13.6	9	15.2	9.8	17.4	11

extracts with respect to the species, the solvent type and harvest times were significant ( $P < 0.05$ ). Regardless of the species and harvest dates, the extract yields obtained by both methanol and ethanol more or less increased over the growing season. However, comparing extract yields obtained by using both solvents in all the species examined revealed that the methanolic extracts gave higher yields than ethanolic ones. This was ranged 12-23.3, 9.2-19.5, 18.9-20.75 and 13.6-17.4 in *M. spicata*, *M. aquatica*, *M. longifolia* and *M. × piperita* var. *citrata*, respectively. The methanol extract yield in all of the examined taxa showed a constant increase through the growing season, reaching the maximum value in August (Table 2).

### Phenolic compounds

We evaluated the total phenolic, flavonoid and flavonol contents of the examined taxa. Sample content of phenolic

compounds (expressed in mg GAE/g dw) in methanol and ethanol extracts of four *Mentha* species are summarized in Table 3. Our study showed that the solvent type had a significant effect on the recovery of phenolics. According to Table 3, methanol was the solvent with higher efficiency for recovering total phenolics. The only exception was the values measured in June for *M. × piperita* var. *citrata* using ethanol, which was second in rank only to methanol in June for the same taxon. Moreover, the higher concentration of phenolic content for both solvents was recorded in June. Generally, the four *Mentha* species showed high TPC ranging from 61.2 (*M. longifolia*) to 191.6 (*M. × piperita* var. *citrata*) mg GAE/g, showing a difference up to 3-fold. The amount of TPC varied with respect to both species and the solvent used; the highest TPC was detected in *M. × piperita* var. *citrata* collected in June using both solvents (191.6 and 159.1 mg GAE/g, respectively), and the lowest

**Table 2.** Total phenolics of four *Mentha* species, as affected by harvest date and solvent type

Species	Total phenolic (mg GAE/g dw)					
	Methanol			Ethanol		
	April	June	August	April	June	August
<i>M. spicata</i>	91.8 <sup>i</sup>	122.2 <sup>c</sup>	87.1 <sup>l</sup>	74.5 <sup>n</sup>	87.2 <sup>l</sup>	67.6 <sup>p</sup>
<i>M. aquatica</i>	111.7 <sup>e</sup>	92.3 <sup>i</sup>	114.7 <sup>d</sup>	71.6 <sup>o</sup>	68.1 <sup>p</sup>	81.3 <sup>m</sup>
<i>M. longifolia</i>	92 <sup>j</sup>	98.4 <sup>h</sup>	87.9 <sup>k</sup>	63 <sup>q</sup>	67.6 <sup>p</sup>	61.2 <sup>r</sup>
<i>M. × piperita</i> var. <i>citrata</i>	111.4 <sup>e</sup>	191.6 <sup>a</sup>	109.8 <sup>f</sup>	89.8 <sup>j</sup>	159.1 <sup>b</sup>	97.2 <sup>h</sup>

Note: Means with the same letter are not significantly different ( $P < 0.05$ ).

**Table 3.** Total flavonoid and flavonol contents of four *Mentha* species, as affected by harvest season and solvent type

Taxa	Methanol			Ethanol		
	April	June	August	April	June	August
	Total flavonoid content (mg Q/g dw)					
<i>M. spicata</i>	29 <sup>m</sup>	34.6 <sup>h</sup>	37.4 <sup>f</sup>	23.2 <sup>q</sup>	31.1 <sup>k</sup>	33.4 <sup>l</sup>
<i>M. aquatica</i>	38.9 <sup>d</sup>	38.1 <sup>e</sup>	51.6 <sup>b</sup>	32.4 <sup>j</sup>	26.9 <sup>o</sup>	37.1 <sup>f</sup>
<i>M. longifolia</i>	24.2 <sup>p</sup>	28.1 <sup>n</sup>	30.1 <sup>l</sup>	17.8 <sup>u</sup>	18.6 <sup>t</sup>	20.6 <sup>c</sup>
<i>M. × piperita</i> var. <i>citrata</i>	36.3 <sup>h</sup>	46.12 <sup>c</sup>	57 <sup>a</sup>	21.8 <sup>r</sup>	36.1 <sup>g</sup>	37.7 <sup>ef</sup>
Total flavonol content (mg Q/g dw)						
<i>M. spicata</i>	9.1 <sup>i</sup>	11.1 <sup>f</sup>	12.1 <sup>d</sup>	6.7 <sup>m</sup>	8.1 <sup>k</sup>	9.6 <sup>h</sup>
<i>M. aquatica</i>	11.7 <sup>de</sup>	11.8 <sup>de</sup>	12.9 <sup>c</sup>	9.1 <sup>i</sup>	10.1 <sup>g</sup>	11.5 <sup>ef</sup>
<i>M. longifolia</i>	7.4 <sup>l</sup>	8.6 <sup>j</sup>	10.2 <sup>g</sup>	6.5 <sup>m</sup>	8.1 <sup>k</sup>	8.3 <sup>jk</sup>
<i>M. × piperita</i> var. <i>citrata</i>	11.1 <sup>f</sup>	14.2 <sup>b</sup>	15.3 <sup>a</sup>	8.4 <sup>kl</sup>	11.1 <sup>f</sup>	12.9 <sup>c</sup>

Note: Means with the same letter are not significantly different ( $P < 0.05$ ).

in *M. longifolia* collected in August and April using ethanol (61.2 and 63 mg GAE/g, respectively) (Table 3).

The TFvC of the crude extracts from *Mentha* species are summarized in Table 4. The maximum content was recorded in the methanol extracts. The highest level of TFvC was recorded in *M. × piperita* var. *citrata* (15.3 and 14.2 mg Q/g dw in August and June, respectively), followed by *M. aquatica* (12.9 mg Q/g dw in August), while the minimum levels were found in *M. longifolia* methanol extract (7.4 mg Q/g dw in April) and *M. spicata* ethanol extract (8.1 mg Q/g dw in June).

#### Antioxidant activity

Two antioxidant assays i.e. free radical scavenging activity (DPPH assay) and reducing power assay were used for determining the antioxidant capacity of four *Mentha* species extracts.

#### Free radical scavenging activity (DPPH assay)

DPPH radical scavenging activity of *Mentha* species extracts are shown in Table 5. The addition of different dilutions of *Mentha* extracts to the DPPH solution induced a rapid decrease in the optical density at 517 nm. In all of the tested materials, increase in the concentration of the solvent ranging from 10 to 100 µg/mL, resulted in the significantly better free radical scavenging activity over 2-fold. Furthermore, all extracts obtained by using methanol gave stronger radical scavenging capacity than those prepared with ethanol. The extracts obtained by methanol yielded DPPH radical scavenging activity ranging from 3.3% (August, 10 µg/mL, *M. spicata*) to 89.6% (June, 100 µg/mL, *M. × piperita* var. *citrata*). This was between 1.8% (August, 10 µg/mL, *M. spicata*) and 83.2 % (June, 100 µg/mL, *M. × piperita* var. *citrata*) for ethanol extracts.

Calculation of extract concentrations providing 50%

inhibition (IC<sub>50</sub>) for both methanol and ethanol extract are also presented in Table 5. In methanolic *Mentha* extracts, the least IC<sub>50</sub> value was recorded in *M. × piperita* var. *citrata* (June, 52.7 µg/mL), while the extracts obtained from the August harvest of *M. longifolia* gave the highest value (78.9 µg/mL). The lowest and the highest IC<sub>50</sub> values yielded by ethanolic *Mentha* extracts were recorded in *M. × piperita* var. *citrata* (June, 60.9 µg/mL) and *M. longifolia* (April, 110.5 µg/mL).

#### Ferric reducing antioxidant power assay

The reducing power of the extracts, which reflects their antioxidant activity, was determined using a Fe<sup>3+</sup> to Fe<sup>2+</sup> reduction assay. The reducing power of the alcoholic (methanol & ethanol) extracts from four *Mentha* species was measured using three levels of concentration (100, 200 and 400 µg/mL), and results are summarized in Table 6 as absorbance values (AV) at 700 nm.

The highest reducing power was exhibited by methanol extracts at the concentration of 400 µg/mL, and the ethanol at the concentration of 100 µg/mL showed the least activity. The reducing power of the methanol extracts yielded AVs between 0.189 in *M. spicata* (August, 100 µg/mL) and 1.16 in *M. × piperita* var. *citrata* (June, 400 µg/mL). AV was ranged between 0.04 in *M. spicata* and *M. longifolia* (5 April, 100 µg/mL) and 1.13 in *M. × piperita* var. *citrata* (June, 400 µg/mL) in the methanol extracts. In general, reducing power for all species, growth seasons and solvent types enhanced by nearly 2-fold, as the concentration of the solvent was increased. In general, the methanol extracts of *M. × piperita* var. *citrata* at the concentration of 400 µg/mL gave the best reducing power.

#### Scolicidal activity

The scolicidal activity of methanol extracts from four *Mentha* species against protoscolices of hydatid cysts was

**Table 4.** Free-radical scavenging activity (DPPH assay) (%) under different solvent type, solvent concentrations and *Mentha* species

Taxa		Concentration (µg/mL)							
		Methanol			IC <sub>50</sub>	Ethanol			IC <sub>50</sub>
		10	40	100		10	40	100	
<i>M. spicata</i>	April	9.7	31.9	71.8	68.3	8.7	28	63.3	78.2
	June	7.9	33.7	80.6	62.7	7.5	28.9	77.1	65.9
	August	3.3	24.8	58	86.4	1.8	22.5	56.7	88.7
<i>M. aquatica</i>	April	10.3	36.5	79.86	61.3	7	21.6	55.5	90.9
	June	8.2	23.2	77.5	68.3	5.2	20.2	48.7	103.6
	August	13.3	37.1	81.6	58.3	7	26	63.7	79.4
<i>M. longifolia</i>	April	10.9	26.6	65.7	76.4	5.3	15.2	46.4	<b>110.5</b>
	June	12.6	38	77.2	61.6	6	29.9	69.7	72.3
	August	9	22.5	64.7	<b>78.9</b>	6	20.9	53.3	94.8
<i>M. × piperita</i> var. <i>citrata</i>	April	10.7	35.7	76.2	62.9	8.1	29.1	73.6	68.6
	June	16.9	37.9	89.6	<b>52.7</b>	13.1	30.2	83.2	<b>60.9</b>
	August	13.9	37.6	81.7	58.1	7	29.4	75.7	67.5

**Table 5.** Reducing power (absorbance at 700 nm) of different extracts from four *Mentha* species

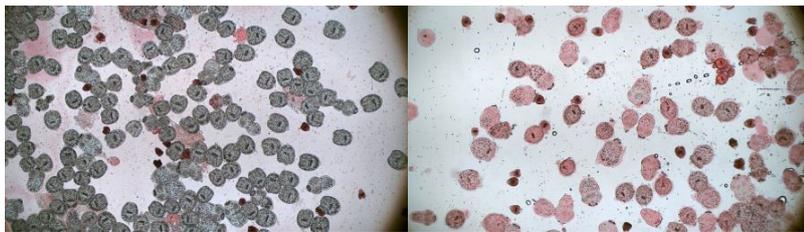
Taxa		Concentration ( $\mu\text{g/mL}$ )					
		Methanol			Ethanol		
		100	200	400	100	200	400
<i>M. spicata</i>	April	0.205	0.284	0.549	0.04	0.11	0.259
	June	0.255	0.442	0.621	0.145	0.385	0.711
	August	0.189	0.224	0.494	0.1	0.269	0.599
<i>M. aquatica</i>	April	0.242	0.353	0.612	0.11	0.237	0.499
	June	0.247	0.365	0.623	0.06	0.194	0.497
	August	0.313	0.451	0.725	0.11	0.243	0.551
<i>M. longifolia</i>	April	0.199	0.244	0.544	0.04	0.128	0.388
	June	0.281	0.4	0.703	0.11	0.266	0.575
	August	0.286	0.328	0.565	0.06	0.193	0.529
<i>M. × piperita</i> var. <i>citrata</i>	April	0.305	0.386	0.782	0.125	0.288	0.688
	June	0.404	0.623	1.16	0.234	0.583	1.13
	August	0.343	0.394	0.791	0.188	0.287	0.645

also examined. The reference synthetic drug, Albendazole, was also applied (50  $\mu\text{g/mL}$ ) as positive control, and the parasitocidal activity of the plant extracts was compared with that of this drug. Our results demonstrated that 50  $\mu\text{g/mL}$  albendazole killed 97.3% and 100% of the scolices in 10 and 20 minutes, respectively. Table 6 and Figure 1 show the scolical activity of the alcoholic extracts from *Mentha* species prepared in various dilutions (15–200 mg/mL) and applied at four different (1–30 minutes) exposure times against protoscolices of *E. granulosus*.

The viability of protoscolices was significantly affected in the experiment. In all of the tested samples, the scolical activity showed a constant increase, as the concentration of the extract intensified; with respect to exposure time and concentration of the extract, the lowest/highest activities were observed at concentrations 15 and 200 mg/mL of the methanol extracts after 1 and 30 minutes of application, respectively. Our results also indicated that with the exception of 1 minute of exposure in *M. aquatica*, *M. longifolia* and *M. × piperita* var. *citrata*, in all of the

**Table 6.** Scolical activity (%) of the metanolic extracts from four *Mentha* species against protoscolices of hydatid cysts

Taxa	Time (min)	Concentration (mg/mL)					
		15	45	70	100	150	200
<i>M. spicata</i>	1	7.32	9.15	14.57	32.56	34.64	92.08
	5	9.62	9.49	25.31	34.3	52.62	93.3
	10	12.03	12.3	30.65	40.52	58.83	95.55
	20	13.37	13.79	32.64	45.01	61.52	98.3
	30	14.03	15.81	34.54	53.74	80.1	98.98
<i>M. aquatica</i>	1	14.91	23.8	24.32	26.28	54.77	84.97
	5	15.35	24.75	25.29	26.55	55.97	96.56
	10	16.01	25.76	26.3	26.9	58.25	99.25
	20	16.69	26.87	27.4	27.42	63.1	99.31
	30	17.4	27.94	28.6	28.77	64.72	99.54
<i>M. longifolia</i>	1	11.04	11.55	20.93	25.38	48.52	88.2
	5	11.11	11.6	23.37	26.63	51.03	92.73
	10	11.15	11.62	24.85	38.48	57.74	92.85
	20	11.24	11.71	25.42	40.35	58.45	97.48
	30	11.35	11.76	25.69	41.08	60.8	99.11
<i>M. × piperita</i> var. <i>citrata</i>	1	9.46	11.33	14.25	25.93	44.41	84.14
	5	11.92	11.98	14.69	26.58	46.94	97.2
	10	25.31	26.61	26.78	43.47	48.68	97.7
	20	27.11	40.05	47.39	48.17	72.48	97.7
	30	28.72	52.5	60.4	62.88	93.22	98.9



**Figure 1.** Scolicidal activity of *Mentha aquatica* methanolic extract against protoscolices of hydatid cysts at concentration of 200 mg/mL; intact and viable protoscolices (Left), ex-posed protoscolices (Right).

taxa, the methanol extracts at concentration 200 mg/mL showed the most potent (over 90%) scolicidal activity. The highest activity was observed with 200 mg/mL of methanol extract from *M. aquatica* after 30 (99.54%), 20 (99.31%) and 10 (99.25%) minutes of application, followed by 98.98% and 98.3% of inhibition for *M. spicata* extract after 30 and 20 minutes of application, respectively. At this concentration, the lowest values were recorded in *M. × piperita* var. *citrata* (84.14%), *M. longifolia* (88.2%) and *M. aquatica* (84.97%) after 1 minute of application. For all of the taxa and exposure times, the mortality given by extracts at concentrations below 200 mg/mL remained below 90%, except for the extract of *M. × piperita* var. *citrata*, which yielded 93.22% of mortality in 30 minutes treatment and at concentration of 150 mg/mL.

### Discussion

Overall, our findings indicated that the extraction could vary in quantity and quality according to climate as well as age and vegetative cycle (14). Medicinal plants synthesize and accumulate phenolics in response to environmental stress and other corresponding factors (15,16). In relation to our study, synthesis of phenolic derivatives e.g. flavonoids is reported to be upregulated when plants are overexposed to light (17). Moreover, we concluded that the variation in the recovery yields of crude extracts prepared from *Mentha* species could be explained by the difference in the solubility of certain compounds in the solvents employed. Bioactive compounds from plants belong to various chemical groups (e.g. terpenes, phenolics, alkaloids, glycosides, etc), and therefore, exhibit different solubility in certain solvents. Methanol in most cases is used in the extraction of polar bioactive components from plants. Numerous non-polar components are also dissolved in methanol and can be extracted, except for instances where only non-polar compounds are required to be extracted, and then it will surely require the use of solvents that are strictly non-polar. Therefore, methanol is commonly used for extraction of a wide range of bioactive compounds, as it is relatively inexpensive, easily evaporated and dissolves a wide range of compounds from polar to non-polar. In general, our results suggested that absolute methanol could be the solvent of choice for yielding high levels of extractable compounds. These findings were in line with previous studies on e.g. *Limnophila aromatica*

(Lam.) Merr. (13) and *Phoenix dactylifera* L. (18).

It has been widely proposed to prepare herbal medicines as mixtures (extracts) rather than isolates, as their activity is potentiated when delivered in mixtures (19). Phenolic compounds are a vital part of the human diet, and are of considerable interest due to their biological and physiological activities (e.g. antioxidant, anticarcinogenic, antimutagenic and anti-inflammatory effects) (20). They can basically be categorized into several classes, of which flavonoids are bioactive substances occurring widely in food and industrial plants. Flavonols are the most prevalent group of flavonoids in plants and occur in many fruits, vegetables and medicinal plants (21). The extraction of polyphenols in plants can be difficult, as these compounds are extremely different in structure; they may occur in plant tissues combined with sugars, proteins or they may give rise to polymerized derivatives showing various levels of solubility. Their chemical structure and interactions with other food components, which are not fully known, are a very important information to select the appropriate solvent and extraction conditions. Therefore, the recovery of polyphenols from plant materials is influenced by their solubility in the solvent, polarity of the solvent, degree of polymerization of phenols, interaction with other plant constituents and formation of insoluble complexes. According to the results obtained by a number of researchers worldwide (22-25), we found out that application of the more polar solvent, i.e. methanol, led to a higher recovery rate of phenolic compounds in *Mentha* species. Along with such matters, the content of phenolic compounds in *Mentha* species was obviously under the influence of both genetic makeup (genotype) and developmental stage (harvest date) of plants. Scientific evidence reveals that biosynthesis of SMs is not a random process, but rather highly ordered with respect to plant development (26). Many researchers worldwide have evaluated the effect of physiological development stage on the productivity of medicinal plants. To name a few, Kiani et al reported that the distribution of SMs might change during plant development, in relation to climatic conditions of the plant's habitat, which stimulate the biosynthesis of active substances (27). Besides, Medini et al argued that the quantity and the biological activity of phenolic compounds in *Limonium delicatulum* (Girard) Kuntze were significantly affected by physiological

stages at which the plants were harvested (22). Moreover, Mikami-Konishide et al reported that the antioxidant capacity and phenol content of six crops cultivated in Japan were strongly affected by the growing season (28).

The antioxidant capacities of the plant extracts cannot be fully described with only one method, but it is necessary to perform more than one type of antioxidant capacity measurement to consider the occurrence of various mechanisms of antioxidant activity (29). The most commonly used methods for the determination of antioxidant activity are DPPH radical scavenging and ferric reducing power assays (30). Results of our study revealed that either methanol or ethanol extracts of *Mentha* species had high DPPH activity and ferric reducing power. Generally, the antioxidant capacities of the plant extracts largely depend on the composition of the extracts and conditions of the test system. Therefore, all the aforementioned factors (e.g. agroclimatic conditions, laboratory conditions etc) influencing the recovery of plant extracts, would accordingly affect the antioxidant capacity of plants. Today, we know that additive and synergistic effects of phytochemicals in plants are responsible for their potent antioxidant activity, and that the benefit of a diet rich in fruit and vegetables is attributed to the complex mixture of phytochemicals present in the body of the plants, not a single compound (31). A large number of medicinal plants contain chemicals with antioxidant activity, among which phenolics and their derivatives are receiving particular attention (32). The largest and best studied natural phenolics in plants are flavonoids, which include numerous compounds e.g. anthocyanidins, flavonols, flavones, flavanones, flavan-3-ols and isoflavonoids (33). The antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations (34). For example, the phenolic moiety in flavonoids can accept an electron to form relatively stable phenoxyl radicals, thereby disrupting chain oxidation reactions in cellular components (33). This might also be associated with the free radical scavenging activity of a variety of enzymes namely superoxide dismutase, catalase, peroxidase, etc as well as the presence of carotenoids, tocopherol and ascorbic acid. The synergistic effects of polyphenols, flavonoids, phenolic acids etc should also be taken into account.

There is a growing interest of industry to replace synthetic chemicals by natural products with bioactive properties of plant origin (35). Despite increasing interest in phytochemical research, a few attempts have been made to identify antiparasitic agents from medicinal plants. The potential of the mint family to disturb the viability of protoscolices has been investigated and some show significant activity. In relation to our study, for example, *Mentha piperita*, *Mentha pulegium*, *Salvia officinalis*, *Satureja khuzestanica*, *Thymus vulgaris* and *Zataria*

*multiflora*, ethnobotanically known as anthelmintics, have been employed to combat *Echinococcus granulosus* (36). Two pure phenolic compounds menthol and thymol, the principal components of *Mentha arvensis* and *Thymus vulgaris* essential oils (EOs), respectively, were tested by Yones et al and showed excellent scolicidal activity (37). The major products of *Mentha pulegium* and *Mentha piperita* EOs, which were successfully used by Maggiore et al against *Echinococcus granulosus* were piperitone oxide and isomenthol, respectively (38). Elissondo et al used the phenolic compound thymol against the parasite and the same result was obtained (35). Likewise, Mahmoudvand et al reported that thymol and carvacrol, the main constituents of *Zataria multiflora* EO, were completely effective to kill protoscolices (39). As with abovementioned cases, the bioactivity of a given plant is mostly decided by either one or two of its main components. However, sometimes overall activity cannot be attributed to any of the major constituents, and additive or even synergistic effects in the presence of other compounds within a crude mixture modifies the activity to exert significant effect (40). There is evidence that crude plant extracts often have greater biological activity than isolated constituents at an equivalent dose. In traditional medicine whole plants or mixtures of plants are used rather than isolated compounds (41,42). Many traditional systems of medicine have several crude formulations having promising effects on parasites, but unfortunately none of these formulations has been characterized till date. The literature data on the bioactivity of plants crude extract against protoscolices is scarce, and the present work is the first attempt made to investigate the scolicidal potential of crude extracts from taxa belonging to the genus *Mentha*. As it can be seen through the latter studies, in nearly all cases a phenolic substance is responsible for scolicidal activity of the tested plants. According to Verma et al, the loss in viability of the parasite is associated with a severe morphological alteration to the surface of the parasite leading to the disturbance in osmoregulatory system of protoscolices (43). Interestingly, a similar mode of action has been proposed for antimicrobial activity of phenolic compounds and the reference synthetic anthelmintic drugs praziquantel, benzimidazole and ivermectin (43). Taking all these into consideration, main mechanisms involved in scolicidal effects of phytochemicals remain poorly understood and elucidation of these mechanisms awaits further studies.

## Conclusion

To sum up, the current study is an attempt to determine the antioxidant effects of the most common *Mentha* species growing in Iran, and to screen out a new source of natural drugs having promising effects against protoscolices. The initial results obtained are quite encouraging since crude extracts of *Mentha* exhibited potent antioxidant

effect, and scolicidal activity close to some synthetic drugs. The tested taxa can be further investigated for chemical characterization leading to the understanding of mechanism of action. These results may have significant implications for the future development of anti-parasitic drugs derived from *Mentha* species.

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### Authors' contributions

MR and AN conceived and designed the analysis. MR and MK conducted the experiment and collected the data. AN performed the analysis. MK and MR interpreted the data and wrote the paper. All read and confirmed the final version of manuscript for publication

### Conflict of interest

All authors confirm that there is no conflict of interest to disclose.

### Ethical considerations

This study protocol was approved by the ethics committee of Amol University of Special Modern Technologies (Ethical code: ir.ausmt.rec.961110). The ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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