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Antioxidant, anti-inflammatory, acetylcholinesterase and thioredoxin reductase inhibitory activities of nine selected Turkish medicinal plants

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Ethyl acetate, methanol, dichloromethane, petroleum ether and water extracts of nine selected plant species, which are commonly used as herbal medicines for anticancer and anti-inflammatory purposes in Turkey, were evaluated for their total phenolic and flavonoid contents and *in vitro* antioxidant potency with a thiobarbituric acid assay using the lipid peroxidation of phosphatidylcholine liposomes, DPPH• and ferric ion reducing antioxidant power assays. Inhibitory activity against cyclooxygenase (COX) was used to evaluate the anti-inflammatory activities of the extracts. As thioredoxin reductase (TrxR) has emerged as a new target for anticancer drug development, the extracts were investigated for their inhibitory activities on TrxR. The ability of the extracts to inhibit acetylcholinesterase (AChE), which is a target for cholinesterase inhibitors, used for the symptomatic treatment of Alzheimer's disease, was also examined. The results showed that the extracts of *C. coggygria* and *M. officinalis* subsp. *officinalis* are the most effective hydrogen and electron donors and contained the highest amounts of phenolic compounds; thus, they can be considered the best antioxidants among the nine plants selected for the study. All the plants showed inhibitory effects against AChE, COX-1 and COX-2, therefore may be of potential therapeutic interest for the treatment of neurodegenerative and inflammatory disorders. It was found that *M. officinalis* subsp. *officinalis, C. coggyria, S. aucuparia* and *P. major* subsp. *major* have a strong inhibitory effect on TrxR by up to 99 %, highlighting their potential as preventive therapeutics for cancer. This study confirmed the use of these plants in folk medicine as anti-inflammatory and anticancer agents.

Keywords: Antioxidant, Anti-inflammatory, Anti-cholinesterase, Phenolics, Thioredoxin reductase inhibitory activity

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The use of traditional medicinal plants is widespread practice in the Kırklareli, Manisa and Çanakkale Provinces located in the Western region of Turkey. Kültür[1] summarized and documented one hundred and twenty six plant species of traditional Kirklareli herbs on the basis of long-term folk practical experience for many kinds of diseases. Crude plant extracts in the form of decoctions or infusions are traditionally more commonly used by the population for the treatment of cancer, inflammation and neurological disorders[1,2]. In recent years, the roles of inflammatory mediators in various pathologies have been identified. Such findings have significantly increased the importance of identifying new targets for the development of innovative and safe therapeutic strategies to manage inflammatory diseases, such as Alzheimer's disease (AD), cardiovascular disorders, atherosclerosis, and cancer[3]. Inhibition of acetylcholinesterase (AChE) is considered a promising strategy for the treatment of neurological disorders, such as AD, senile dementia, ataxia and myasthenia gravis[4]. Cyclooxygenase (COX) inhibitors are being evaluated as therapeutic agents for the prevention and treatment of inflammation. The thioredoxin (Trx) system, composed of Trx reductase (TrxR), Trx, and NADPH, represents an effective target for the development of new anticancer agents[5]. In this study, the selected medicinal plants are commonly used as herbal medicines as well as anti-inflammatory and wound healing agents. It is to be expected that these activities

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might be related to possible antioxidant effects, i.e., their ability to quench reactive oxygen species and terminate free radical reactions.

The aim of this study was to evaluate whether the biological activities can support the reported traditional uses of these plants in those region of Turkey. For this purpose we investigated the antioxidant, AChE, COX, and TrxR inhibitory activities of ethyl acetate, methanol, dichloromethane, and petroleum ether extracts from *Urtica dioica* L., *Achillea millefolium* L. subsp. *pannonica* (Schelek) Hayek, *Malva sylvestris* L., *Stachys cretica* L. subsp. *lesbiaca* Rech fil., *Marrubium rotundifolium* Boiss., *Melissa officinalis* L. subsp. *officinalis*, *Cotinus coggygria* Scop., *Sorbus aucuparia* L., and *Plantago major* L. subsp. *major* and thus justify the traditional uses of these medicinal plants as anti-inflammatory, wound healing and anti-cancer agents.

Methodology

Chemicals

AChE, acetylthiocholine iodide (ATChI), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), galantamine hydrobromide, L-α-phosphatidylcholine, 2,2-diphenyl-1-picrylhydrazyl (DPPH), TrxR assay kit and curcumin were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-ascorbic acid, 2,4,6-tripyridyl-s-triazine (TPTZ), thiobarbituric acid (TBA), trichloroacetic acid (TCA), iron (II) sulphate heptahydrate and ferric chloride were purchased from Merck (Darmstadt, Germany). COX enzyme immunoassay (EIA) kit and indomethacin were obtained from Cayman (Ann Arbor, MI, USA).

Plant materials

U. dioica, *A. millefolium* subsp. *pannonica*, *M. sylvestris*, *M. officinalis* subsp. *officinalis*, *C. coggygria*, *S. aucuparia* and *P. major* subsp. *major* were collected from Kırklareli province between June 2010 to June 2011, *S. cretica* subsp. *lesbiaca* was collected from Çanakkale province in June 2009, and *M. rotundifolium* was collected from Manisa province of Turkey in July 2011. These plants were identified and deposited in the Herbarium of the Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University (ISTE). The plants were collected during the flowering and/or fruiting stage and then, air-dried at room temperature (20-25 ^{o}C) for one week.

Preparation of extracts

The dried leaves or aerial parts were manually ground to a fine powder and extracted using solvents of increasing polarity: petroleum ether, dichloromethan, ethyl acetate and methanol. For extractions, the powdered leaves or aerial parts (1 g) were extracted with 100 mL of solvent in a Soxhlet apparatus for 4 h. The extracts were filtered and evaporated to dryness under reduced pressure at 40°C in a rotary evaporator. The crude extracts were transferred to vials and kept at -20 °C. These crude extracts were dissolved in solvents and used for the assessment of biological activities.

For water extracts, 100 mL of boiling water was added to 1 g of the herbal drug and the mixture was boiled for 5 min. After cooling at room temperature, the mixture was filtered and the obtained aqueous extract was lyophilized.

Biochemical assays

The total phenolic content of the extracts was analysed by using Folin-Ciocalteu's reagent[6]. The flavonoid content was determined according to the $AlCl_3$ method[7]. Antioxidant activity was estimated using TBA, based on the lipid peroxidation (LPO) of liposomes[6], DPPH free radical scavenging[6] and ferric ion reducing antioxidant power (FRAP)[6] assays.

The extracts were screened for their AChE inhibitory activity through the modified Ellman's spectrophotometric method[8]. The ability of the extracts to inhibit ovine COX-1 and human recombinant COX-2 was determined by calculating percent inhibition of PG-E2 production using EIA kit according to the manufacturer's instructions (Cayman Europe). For the assessment of TrxR activity, different concentrations of the extract were incubated with DTNB and recombinant TrxR in 96-microwell plate for 1 hr at room temperature, and then TrxR activity was determined by DTNB reduction assay[6].

Results and discussion

The results of the total phenolic content expressed as gallic acid equivalents (GAE)/g of the extracts from the aerial parts or leaves of nine medicinal plants obtained using five different solvents (ethyl acetate, methanol, dichloromethan, petroleum ether and water), are presented in Table 1. With the exception of *M. rotundifolium* and *P. major* subsp. *major*, extraction with ethyl acetate (from 19.4 to 435.6 mg GAE/g extract) resulted in the highest amount of phenolic compounds. Thus, it was concluded that ethyl acetate is a more efficient means of extracting phenolic compounds from medicinal plants than water and methanol. These results indicated that a less polar

solvent such as ethyl acetate could extract more phenolic compounds compared to solvents with greater polarity, including methanol and water. The greater efficiency of ethyl acetate in extracting phenolic compounds resulted in higher antioxidant activity of obtained extracts.

Among the ethyl acetate extracts, the highest total phenolic levels were detected in the extracts from *C. coggyria*, followed by *M. officinalis* subsp. *officinalis*, *S. aucuparia*, *S. cretica* subsp. *lesbiaca*, *A. millefolium* subsp. *pannonica*, *U. dioica*, *M. rotundifolium* and *M. sylvestris*, whereas the lowest amount was found in *P. major* subsp. *major*.

Water and methanol extracts also contained high levels of phenolics. The extraction procedure with a water was more efficient means of extracting the phenolic compounds of *M. rotundifolium* and *P. major* subsp. *major*.

The present study revealed that dichloromethane is the least effective at extracting phenolic compounds due to its low polarity. The petroleum ether extracts of the plants showed no detectable phenolic content, with the exception of *C. coggyria*. Phenolic compounds are often polar, however, due to non-polar groups, they also may be extracted in non-polar solvents. Flavonoids are the important secondary metabolites, which act as antioxidant agents[9]. The concentrations of flavonoids in ethyl acetate extracts ranged from 9.23 to 382.72 mg of CE/g extract followed by methanol (from 5.93 to 196.48 mg CE/g extract) and water (from 25.03 to 178. 95 mg CE/g extract) extracts, while the content of flavonoids in dichloromethan extracts was the lowest (from 2.27 to 23.07 mg CE/g extract). With the exception of *C. coggyria*, the petroleum ether extracts of the plants contained almost no flavonoids. Based on the flavonoid contents of the extracts, ethyl acetate was the best extraction solvent to extract the flavonoids from the leaves of *M. officinalis* subsp. *officinalis*. In contrast, the flavonoid concentration of the ethyl acetate extract obtained from *C. coggygria* was the lowest together with the extracts obtained from *M. sylvestris*, *M. rotundifolium* and *P. major* subsp. *major*. Methanol was the best extraction solvent to extract flavonoids from *C. coggygria*. Water and ethyl acetate extracts from *U. dioica*, *A. millefolium* subsp. *pannonica* and *S. aucuparia* also contained high levels of flavonoids. Ethyl acetate and methanol were the best extraction solvents for *S. cretica* subsp. *lesbiaca* and *S. aucuparia* (Table 1). Thus, estimation of total phenolics revealed that these species are rich sources of phenolic compounds. The results appear to

Table 1 — Total phenolic compounds (as gallic acid equivalents) and total flavonoids (as catechin equivalents) in ethyl acetate, methanol, dichloromethane, petroleum ether and water etracts of the nine selected Turkish medicinal plants.

Taxon	Phenolic compounds (mg/g extract)				
	Ethyl acetate	Methanol	Dichloromethane	Petroleum ether	Water
U. dioica	72.71 ± 4.86^{a}	25.38 ± 1.72^{a}	3.91 ± 0.22^{a}	N.d.	44.09 ± 3.03^{a}
A. millefolium subsp. *pannonica*	82.38 ± 9.31^{a}	25.66 ± 1.53^{a}	$31.23 \pm 3.51^{b,f}$	N.d.	45.31 ± 2.92^{a}
M. sylvestris	31.38 ± 3.34^{b}	11.90 ± 0.72^{b}	20.19 ± 1.45^{c}	N.d.	27.50 ± 1.06^{b}
S. cretica subsp. *lesbiaca*	107.38 ± 9.89^{c}	77.28 ± 2.61^{c}	$31.18 \pm 2.67^{b,f}$	N.d.	32.92 ± 2.44^{c}
M. rotundifolium	58.32 ± 4.89^{d}	15.50 ± 0.78^{d}	$27.47 \pm 3.35^{b,d}$	N.d.	82.43 ± 1.80^{d}
M. officinalis subsp. *officinalis*	397.51 ± 30.54^{e}	214.35 ± 16.06^{e}	10.82 ± 0.24^{e}	N.d.	195.72 ± 4.19^{e}
C. coggygria	435.63 ± 27.53^{e}	402.75 ± 35.01^{f}	35.38 ± 2.48^{f}	45.53 ± 1.63	327.33 ± 3.28^{f}
S. aucuparia	170.24 ± 6.29^{f}	144.46 ± 16.38^{g}	$23.92 \pm 2.18^{c,d}$	N.d.	73.68 ± 2.88^{g}
P. major subsp. *major*	19.48 ± 1.47^{g}	22.78 ± 1.02^{a}	14.88 ± 0.79^{g}	N.d.	40.65 ± 0.71^{a}
Taxon	Flavonoids (mg/g extract)				
	Ethyl acetate	Methanol	Dichloromethane	Petroleum ether	Water
U. dioica	57.10 ± 3.73^{a}	21.85 ± 2.64^{a}	2.27 ± 0.44^{a}	N.d.	42.35 ± 3.78^{a}
A. millefolium subsp. *pannonica*	60.77 ± 4.28^{a}	N.d.	15.99 ± 1.10^{b}	N.d.	47.70 ± 2.02^{a}
M. sylvestris	17.26 ± 1.26^{b}	5.93 ± 0.73^{b}	7.09 ± 0.51^{c}	N.d.	25.03 ± 2.34^{b}
S. cretica subsp. *lesbiaca*	61.35 ± 2.57^{a}	57.63 ± 1.63^{c}	14.53 ± 0.87^{b}	N.d.	28.61 ± 1.20^{b}
M. rotundifolium	29.76 ± 2.46^{c}	9.73 ± 1.45^{d}	6.60 ± 0.71^{c}	N.d.	73.33 ± 4.96^{c}
M. officinalis subsp. *officinalis*	382.72 ± 14.79^{d}	196.48 ± 10.16^{e}	$7.87 \pm 0.74^{c,e}$	N.d.	178.95 ± 3.29^{d}
C. coggygria	29.01 ± 2.33^{c}	118.15 ± 10.08^{f}	23.07 ± 2.38^{d}	18.99 ± 1.30	48.20 ± 2.15^{a}
S. aucuparia	83.87 ± 4.59^{e}	115.83 ± 18.30^{f}	8.77 ± 0.61^{e}	N.d.	71.60 ± 1.35^{c}
P. major subsp. *major*	9.23 ± 0.35^{f}	21.19 ± 2.28^{a}	6.21 ± 1.31^{c}	N.d.	44.58 ± 1.26^{a}

Values were the means of three replicates ± standard deviation. Values with different letters in the same column were significantly ($p < 0.05$) different. N.d.-Not determined

be in reasonable agreement with the literature. *U. dioica*[10,11], *A. millefolium*[12-17], *M. sylvestris*[18-20], *S. cretica*[21-23], *M. officinalis*[24-29], *C. coggygria*[30,31], *S. aucuparia*[12,32,33] and *P. major*[34,35] from different regions across the globe were reported to be rich in bioactive phenolics, which significantly contribute to the antioxidant activity of these plants. There were no reports on the phytochemical composition and *in vitro* antioxidant activity of *M. rotundifolium*, *A. millefolium* subsp. *pannonica* and *S. cretica* subsp. *lesbiaca*.

The antioxidant activity of ethyl acetate, methanol, dichloromethane, petroleum ether and water extracts prepared from nine medicinal plants were assayed by the three different methods including the TBA test for the determination of anti-LPO activity, DPPH for free radicals scavenging activity and FRAP assays.

Ethyl acetate, methanol and water extracts of the plants were more effective in scavenging DPPH radicals than the dichloromethan extracts. With the exception of *C. coggyria*, no such activity was detected in petroleum ether extracts. As shown in Table 2, among the ethyl acetate extracts, the most effective DPPH radical scavengers were *C. coggyria*, *M. officinalis* subsp. *officinalis*, *S. aucuparia*, *S. cretica* subsp. *lesbiaca* and *A. millefolium* subsp. *pannonica* with EC_{50} values below 1 mg/ml, followed by the extract of *U. dioica*; extracts of *M. rotundifolium*, *M. sylvestris* and *P. major* subsp. *major* were the least effective. Moreover, the methanol extract of *C. coggyria* was a more potent DPPH radical scavenger than the reference antioxidant, quercetin. These findings are in agreement with our observation on the phenolic contents of the extracts and seem to suggest that phenolics are important contributors to antioxidant activity. The results revealed that only ethyl acetate, methanol and water extracts from *M. officinalis* subsp. *officinalis* and *C. coggygria,* ethyl acetate and water extracts from *S. aucuparia,* ethyl acetate and methanol extracts from *S. cretica* subsp. *lesbiaca*, ethyl acetate extract from *A. millefolium* subsp. *pannonica*, and water extracts from *M. rotundifolium* and *P. major* subsp. *major* were capable of inhibiting the production of thiobarbituric acid reactive substances (TBARS) produced from LPO of the soybean phosphatidylcholine (lecithin) liposomes induced by the Fe^{3+}/ascorbate model system. Dichloromethan and petroleum ether extracts had no inhibitory activity on LPO. As shown in Table 2, the

Table 2 — DPPH radical scavenging and anti-LPO activities of ethyl acetate, methanol, dichloromethane, petroleum ether and water extracts of the nine selected Turkish medicinal plants.

Taxon	DPPH EC_{50} (mg/mL)				
	Ethyl acetate	Methanol	Dichloromethane	Petroleum ether	Water
U. dioica	1.16 ± 0.15^{a}	2.97 ± 0.15^{a}	N.d.	N.d.	2.46 ± 0.34^{a}
A. millefolium subsp. *pannonica*	0.98 ± 0.07^{a}	2.61 ± 0.13^{b}	$6.81 \pm 1.24^{a,c}$	N.d.	1.44 ± 0.10^{b}
M. sylvestris	5.01 ± 0.61^{b}	11.39 ± 1.35^{c}	11.49 ± 1.01^{b}	N.d.	5.05 ± 0.24^{c}
S. cretica subsp. *lesbiaca*	0.71 ± 0.01^{c}	0.97 ± 0.18^{d}	8.57 ± 1.29^{a}	N.d.	2.56 ± 0.17^{a}
M. rotundifolium	3.42 ± 0.32^{d}	6.97 ± 1.02^{e}	5.62 ± 0.19^{c}	N.d.	$1.20 \pm 0.21^{b,f}$
M. officinalis subsp. *officinalis*	0.17 ± 0.002^{e}	0.31 ± 0.03^{f}	13.80 ± 0.15^{d}	N.d.	0.53 ± 0.11^{d}
C. coggygria	0.08 ± 0.004^{f}	0.02 ± 0.01^{g}	1.90 ± 0.07^{e}	1.22 ± 0.16	0.13 ± 0.009^{e}
S. aucuparia	0.34 ± 0.03^{g}	0.56 ± 0.06^{h}	$12.08 \pm 1.68^{b,d}$	N.d.	1.06 ± 0.017^{f}
P. major subsp. *major*	7.89 ± 0.35^{h}	4.27 ± 0.56^{i}	N.d.	N.d.	2.24 ± 0.18^{a}
Quercetin			0.063 ± 0.002^{j}		
Taxon	Anti-LPO EC_{50} (mg/mL)				
	Ethyl acetate	Methanol	Dichloromethane	Petroleum ether	Water
U. dioica	N.d.	N.d.	N.d.	N.d.	N.d.
A. millefolium subsp. *pannonica*	1.24 ± 0.18^{a}	N.d.	N.d.	N.d.	N.d.
M. sylvestris	N.d.	N.d.	N.d.	N.d.	N.d.
S. cretica subsp. *lesbiaca*	2.85 ± 0.17^{b}	1.22 ± 0.09^{a}	N.d.	N.d.	N.d.
M. rotundifolium	N.d.	N.d.	N.d.	N.d.	1.57 ± 0.06^{a}
M. officinalis subsp. *officinalis*	0.71 ± 0.05^{c}	1.15 ± 0.01^{a}	N.d.	N.d.	1.35 ± 0.05^{b}
C. coggygria	0.72 ± 0.09^{c}	0.24 ± 0.01^{b}	N.d.	N.d.	1.02 ± 0.16^{c}
S. aucuparia	1.48 ± 0.10^{a}	N.d.	N.d.	N.d.	2.87 ± 0.20^{d}
P. major subsp. *major*	N.d.	N.d.	N.d.	N.d.	4.77 ± 0.18^{e}
Quercetin			0.057 ± 0.002^{f}		

Values were the means of three replicates ± standard deviation.

Values with different letters in the same column were significantly ($p < 0.05$) different. N.d.-Not determined

methanol extract from *C. coggygria* possessed the most potent antioxidative potential for chain-breaking inhibition of LPO, followed by *M. officinalis* subsp. *officinalis* and *S. cretica* subsp. *lesbiaca*, which were comparable ($p > 0.05$). These data confirmed the greater antioxidant activity of the methanol extract of *C. coggygria* in the DPPH assay. The ethyl acetate extract of *C. coggygria* and *M. officinalis* subsp. *officinalis* showed similar ($p > 0.05$) degrees of efficacy in their inhibitory activities, which were the highest among the ethyl acetate extracts, followed by *A. millefolium* subsp. *pannonica* and *S. aucuparia*, which were comparable ($p > 0.05$); *S. cretica* subsp. *lesbiaca* showed the lowest activity. None of the extracts of *U. dioica* and *M. sylvestris* were able to inhibit phospholipid peroxidation. The inhibitory activity of water extracts was lower compared to that of ethyl acetate and methanol extracts. All of the extracts were significantly less effective ($p < 0.05$) than the reference antioxidant, quercetin. Similarly, a strong antioxidant activity against LPO has been reported for *A. millefolium*[15-17], *M. sylvestris*[18], *M. officinalis*[36,37], *C. coggygria*[30,31] and *S. aucuparia*[12,32] from different regions of the world.

The antioxidant activity of plant extracts is often attributed to their redox effects. The greater efficiency of ethyl acetate and methanol in extracting the phenolic compounds would be expected to result in higher reducing power. The effectiveness in the reducing powers of methanol extracts was, in descending order: *C. coggyria* > *M. officinalis* subsp. *officinalis* > *S. aucuparia* > *S. cretica* subsp. *lesbiaca* > *A. millefolium* subsp. *pannonica* ≥ *U. dioica* > *P. major* subsp. *major* > *M. rotundifolium* > *M. sylvestris* (Table 3). This order of reducing power of the extracts was the same order as the total phenolic contents in these extracts. Moreover, the reducing power of the methanol extract from *C. coggygria* was comparable to the reducing power of quercetin. Our results were in accordance with other investigators who also reported that the reducing ability of the *U. dioica*[10,38,39], *M. sylvestris*[19,20], and *M. officinalis*[25,27], partly contributes to their antioxidant activity. Similarly, a strong reducing power, DPPH and ABTS scavenging activities have been reported for *A. multifida*[40]. The results showed that the extracts of *C. coggygria* and *M. officinalis* are the most effective hydrogen and electron donors and contained the highest amounts of phenolic compounds and thus can be considered the best antioxidants among the nine plants selected for the study.

The inhibition af AChE has been one of the most used strategies for the treatment of AD. Nowadays, much attention has been paid to medicinal plants with anticholinergic properties and they have been considered for memory loss therapy[41]. The water extracts of the plants were tested for their *in vitro* AChE inhibitory activities using galantamine as a positive control. The results were obtained with three concentrations of all plant extracts via the microplate assay and were expressed as EC_{50} values, as summarized in Table 4. As seen from the EC_{50} values, *C. coggygria* was found to be the most potent AChE inhibitor with an EC_{50} value of 1.44 ± 0.04 mg/mL, followed by *M. rotundifolium*, *P. major*, *A. millefolium*, *U. dioica*, *M. officinalis* subsp. *officinalis* and *S. aucuparia*.

Table 3 — Ferricion reducing antioxidant powers (FRAP) of ethyl acetate, methanol, dichloromethane, petroleum ether and water extracts of the nine selected Turkish medicinal plants, expressed in FRAP values.

Taxon	FRAP mM Fe^{2+}*				
	Ethyl acetate	Methanol	Dichloromethane	Petroleum ether	Water
U. dioica	0.67 ±0.04[a]	0.25 ±0.02[a,g]	N.d.	N.d.	0.37 ± 0.01[a]
A. millefolium subsp. *pannonica*	0.83 ±0.04[b]	0.27 ± 0.01[a]	0.33 ±0.013[a]	N.d.	0.31 ± 0.01[b]
M. sylvestris	0.33 ±0.03[c]	0.11 ± 0.01[b]	0.18 ±0.004[b]	N.d.	0.21± 0.02[c]
S. cretica subsp. *lesbiaca*	1.01 ±0.05[d]	0.79 ± 0.01[c]	0.35 ± 0.013[a]	N.d.	0.24 ± 0.02[c]
M. rotundifolium	0.40 ±0.04[c]	0.14 ± 0.02[b]	0.34 ±0.028[a]	N.d.	0.52 ± 0.05[d]
M. officinalis subsp. *officinalis*	4.48 ±0.19[e]	2.66 ± 0.16[d]	0.07 ± 0.006[c]	N.d.	1.04 ± 0.01[e]
C. coggygria	3.72 ± 0.23[f]	3.45 ±0.22[e,i]	0.34 ± 0.030[a]	0.18 ± 0.017	1.17 ± 0.03[f]
S. aucuparia	1.56 ± 0.11[g]	1.27 ± 0.25[f]	0.11 ±0.008[d]	N.d.	0.53 ± 0.01[d]
P. major subsp. *major*	0.13 ± 0.01[h]	0.21 ± 0.02[g]	N.d.	N.d.	0.37 ± 0.02[a]
Quercetin			3.24 ± 0.13[i]		

Values were the means of three replicates ± standard deviation. Values with different letters in the same column were significantly ($p < 0.05$) different. N.d.-Not determined

* FRAP values of the extracts, quercetin and methanol extract of *C. coggygria* were determined at 0.625, 0.25 mg/mL and 0.16 mg/mL, respectively.

However, when compared to the EC_{50} value obtained for the galantamine (10.49 ± 0.27 μg/mL), the AChE inhibitory activities of the above mentioned extracts were found to be significantly lower ($p < 0.05$). The present study confirmed and extended the results of other studies showing that *M. officinalis*[29,42-45], *A. millefolium*[46] and *P. major*[47] demonstrated AChE inhibitory activity and thus may be relevant to the treatment of neurodegenerative disorders such as AD. The AChE inhibitory activity of *U. dioica*, *S. cretica* subsp. *lesbiaca*, *M. rotundifolium*, *C. coggygria* and *S. aucuparia* has been reported for the first time in this study.

The inhibitory activity against COX was used to evaluate the anti-inflammatory activity of the water extracts. From the EC_{50} values it was observed that *C. coggygria* showed the highest COX-1 inhibitory activity, followed by *M. officinalis* subsp. *officinalis*, *M. rotundifolium*, *M. sylvestris*, *U. dioica*, *P. major* subsp. *major*, and *S. cretica* subsp. *lesbiaca*. The EC_{50} values of all the extracts were significantly different ($p < 0.05$) from the EC_{50} values obtained for indomethacin (Table 4).The extracts also showed inhibitory activities against COX-2, which is a target for many current anti-inflammatory and cancer-preventive drugs. From the EC_{50} values it was seen that *C. coggygria* and *U. dioica* were the most active COX-2 inhibitors. *M. rotundifolium*, *M. sylvestris* and *M. officinalis* showed similar degrees of efficacy as shown by the small differences in their EC_{50} values. *S. aucuparia* and *S. cretica* which were similar ($p > 0.05$), were the least active inhibitors (Table 4). However, no plant extract had greater inhibitory activity than the positive control, indomethacin. These results showed similarity to the literature reports on the effectiveness of *U. dioica*[48] and *M. officinalis* subsp. *officinalis*[27] in the inhibition of COX-1 and COX-2. More recently, *U. dioica*[49-51], *A. millefolium* subsp. *pannonica*[52,53], *M. sylvestris*[18] and *C. coggygria*[31] were found to be capable of inhibiting the inflammatory response in several *in vitro* cell culture and *in vivo* models. *Plantago* species has been reported to have antioxidant and anti-inflammatory effects[54].

The water extracts of the plants were investigated for their inhibitory effects on TrxR. The Trx system is overexpressed in cancer cells, and on this basis, there is an active search for TrxR inhibitors. Because of published evidence showing that flavonoids possess strong inhibitory effects on mammalian TrxR[55], the extracts with the greatest concentrations of flavonoids were expected to be the most active. As summarized in Table 4, the results showed that *M. officinalis* subsp. *officinalis*, *C. coggygria* and *P. major* subsp. *major* exert a remarkable inhibitory effect on TrxR. However, the inhibitory activity of *P. major* subsp. *major* was not concomitant with the flavonoid content. This can be explained by the different phytochemical compositions of the extracts. The extracts were less active than curcumin, a major yellow pigment and active component of turmeric, which has been shown to possess anticancer and antiangiogenic properties. Inhibition of TrxR, which will directly affect the many redox functions of Trx, was proposed to be an important mechanism to explain the antitumor effects of curcumin[5]. Inhibition of TrxR by these plants might offer perspectives for

Table 4 — AChE, COX-1, COX-2 and TrxR reductase inhibitory activities of water extracts of the nine selected Turkish medicinal plants.

Taxon	EC_{50} (mg/mL)			
	AChE inhibition	COX-1 inhibition	COX-2 inhibition	TrxR inhibition
U. dioica	15.58 ± 0.43[a]	7.10 ± 0.08[a]	4.31 ± 0.23[a]	4.51 ± 0.09[a]
A. millefolium subsp. *pannonica*	14.79 ± 0.41[a]	N.d.	15.36 ± 0.04[b]	N.d
M. sylvestris	N.d.	6.38 ± 0.20[b]	10.88 ± 0.22[c]	N.d
S. cretica subsp. *lesbiaca*	N.d.	8.60 ± 0.12[c]	16.44 ± 0.31[d]	N.d
M. rotundifolium	11.44 ± 0.40[b]	5.91 ± 0.12[d]	9.63 ± 0.23[e]	N.d
M. officinalis subsp.*officinalis*	19.88 ± 0.48[c]	4.47 ± 0.24[e]	10.90 ± 0.39[c]	0.71 ± 0.02[b]
C. coggygria	1.44 ± 0.04[d]	2.21 ± 0.18[f]	4.10 ± 0.27[a]	0.49 ± 0.06[c]
S. aucuparia	28.72 ± 1.19[e]	7.21 ± 0.10[a]	16.51 ± 0.60[d]	2.18 ± 0.03[d]
P. major subsp. *major*	13.22 ± 0.46[f]	N.d.	10.22 ± 0.23[c]	1.14 ± 0.07[e]
Standard	Galantamine (μg/mL)	Indomethacin (μg/mL)	Indomethacin (μg/mL)	Curcumin (μg/mL)
	10.49 ± 0.27[g]	2.60 ± 0.02[g]	19.9 ± 1.11[f]	2.78 ± 0.04[f]

Values were the means of three replicates ± standard deviation.
Values with different letters in the same column were significantly ($p < 0.05$) different. N.d.-Not determined

future tumour therapies. As far as we know, there are no reports on the TrxR inhbitory activities of the plants in this study.

In our screening program for the potential biological activities of different extracts obtained from plants native to Kirklareli, Manisa and Çanakkale regions of Turkey, it was found that *C. coggyria* and *M. officinalis* has the strongest antioxidant, COX and TrxR inhibitory effects.

Conclusion

Considering the important role of oxidative stress and inflammation in the pathogenesis of neurological diseases and cancer, the medicinal plants from the Kırklareli, Manisa and Çanakkale provinces of Turkey may be used for supportive treatment of inflammation, AD and cancer. Our findings on the role of these plants as potent inhibitors of TrxR may be of potential interest for their use as anticancer agents.

Conflicts of interest

All contributing authors declear no conflicts of interest.

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