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Exploring Turkish biodiversity: A rich source of chemical diversity for drug leads discovery*

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Abstract: Bioresources offer tremendous potential by having excellent chemical diversity for drug discovery programs and by serving as templates for synthetic drugs. There are well-known examples of clinically important drugs derived from natural sources. The development of pharmaceutical, nutraceutical, agricultural, and industrial products from bioresources can be used to promote incentives for conservation by providing an economic return to innovative use. Of those sources, medicinal plants have a virtually untapped reserve of original drug molecules, which await determination and chemical and biological investigation. Marine organisms have also gained increasing attention from researchers worldwide due to their chemically diverse secondary metabolites with desirable biological activities.

There is still a great need for novel compounds with unique mechanisms of action to treat diseases such as cancer, Alzheimer's, arthritis, and diabetes. Besides, multiresistance development by the parasites to the present drugs also constitutes another problem for the treatment of parasitic diseases as well as tuberculosis.

In this article, 209 plant species belonging to 11 plant families were investigated for cholinesterase inhibitory activity by in vitro Ellman method at 10 μg/ml and 1 mg/ml doses. Among them, *Salvia, Rosmarinus*, and *Fumaria* species were found to have the most significant cholinesterase inhibitory activity.

Keywords: biodiversity; biological activity; drug discovery; medicinal chemistry; natural products; structure–activity.

INTRODUCTION

Plants produce a diverse range of structurally novel bioactive molecules, making them a rich source of different types of medicines. Traditional medicines are still the mainstay of about 75–80 % of the world population, mainly in the developing world. The history of drug discovery showed that plants are highly rich sources in the search for new active compounds, and they have become a source for the modern pharmaceutical industry. Many synthetic drugs owe their origin to plant-based complementary medicine. Most prescribed drugs in use have been obtained from bioresources. Many new natural product-originated bioactive compounds effective in treating several diseases have been isolated from different plants, fungi, and microorganisms. Sensitive bioassays for the high-throughput screening (HTS) methods have been developed to screen these plant extracts. The simplest assays are the ones based on the mechanisms of action of a known drug. The assays have also incorporated into efficient testing schemes

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that are useful to HTS. For example, one assay used for Alzheimer's disease (AD) is based on the inhibition of acetylcholinesterase (AChE). The development of new leads of AChE inhibitors has been realized by the Ellman method using enzyme-linked immunoabsorbent assay (ELISA) microplate-reader for screening of biological sources. The anticholinesterase potential of a number of selected Turkish medicinal plants with various ethnobotanical uses, aiming to discover new candidates for anticholinesterase compounds, are presented in this study.

AMARYLLIDACEAE

Chloroform (CHL):methanol (MET) extracts of the bulbs of five Amaryllidaceae plants, namely, *Galanthus elwesii* Hooker fil., *G. ikariae* L., *Narcissus tazetta* subsp. *tazetta* L., *Leucojum aestivum* L., and *Pancratium maritimum* L. growing in Turkey, were evaluated for their anticholinesterase activity by the Ellman method in comparison with galanthamine as the standard drug. Bioactivity-directed fractionation and isolation studies carried out on *G. ikariae* and *N. tazetta* subsp. *tazetta* extracts afforded eight Amaryllidaceae-type alkaloids in total. We found that the activity of both plant extracts was due to the synergistic interaction of the alkaloids isolated [1].

APIACEAE

The AChE and butyrylcholinesterase (BChE) inhibitory activities of 19 essential oils obtained from the cultivated plants; counting *Anethum graveolens* L. (organic fertilizer), two from *Foeniculum vulgare* Mill. collected at fully mature and flowering stages (organic fertilizer), by the Ellman method at 1 mg/ml concentration. In addition, a number of single components widely encountered in most of the essential oils (γ-terpinene, 4-allyl-anisole, (–)-carvone, dihydrocarvone, (–)-phencone, cuminyl alcohol, cumol, 4-isopropylbenzaldehyde, *trans*-anethole, camphene, *iso*-borneol, (–)-borneol, L-bornyl acetate, 2-decanol, 2-heptanol, methyl-heptanol, farnesol, nerol, *iso*-pulegol, eucalyptol, citral, citronellal, citronellol, geraniol, linalool, α-pinene, β-pinene, piperitone, *iso*-menthone, menthofurane, linalyl oxide, linalyl ester, geranyl ester, carvacrol, thymol, menthol, vanilline, and eugenol) were also screened for the same activity in the same manner. The results showed that almost all of the essential oils showed a very high inhibitory activity (over 80 %) against both enzymes, whereas the single components were not as active as the essential oils [2].

The genus *Heptaptera* (Apiaceae) possesses 10 species in the world mainly distributed from Europe to the Middle East, including Italy, the Balkans, Turkey, Syria, and Palestine. It is represented by four species in Turkey, one of which is endemic: *H. anatolica* (Boiss.) Tutin, *H. anisoptera* (DC.) Tutin, *H. cilicica* (Boiss. & Balansa) Tutin (endemic), and *H. triquetra* (Vent.) Tutin. The ethyl acetate and MET extracts prepared from the fruits, aerial parts, and roots of *H. anatolica* (Boiss.) Tutin, (Apiaceae), *H. anisoptera* (DC.) Tutin, *H. cilicica* (Boiss. & Balansa) Tutin (endemic), and *H. triquetra* (Vent.) Tutin were tested for their AChE inhibitory and antioxidant activities. AChE inhibition was evaluated using the Ellman method at 500, 1000, and 2000 μg/ml.

Antioxidant activity was determined by 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging test and $Fe+^{2}$-ferrozine test system for metal chelating power at the same concentrations. Total phenol contents of the extracts were determined using Folin-Ciocalteu reagent. At 2000 μg/ml, only the aerial parts and fruits of *H. anatolica* showed moderate anti-AChE effect (61.97 and 49.80 %, respectively), while the aerial parts and fruits of *H. triquetra* had the highest DPPH scavenging effect (80.48 and 86.19 %, respectively). All of the MET extracts exhibited significant ferrous ion-chelating effect varying between 72.97 and 92.36 %, whereas only four of the ethyl acetate extracts exerted a chelating effect over 70 %. These results indicate that *Heptaptera* species could be a good source for antioxidant compounds [3]. *Heptaptera* species are known to be rich in coumarin derivatives, sesquiterpene coumarin ether types in particular. Coumarins having a basic benzo-2-pyrone skeleton are one of the simplest groups of phenolic compounds and have been reported to have a strong antioxidant effect by

various mechanisms including DPPH and hydroxyl radical scavenging activity, inhibition of lipid peroxidation, metal chelating, etc. Therefore, it is most likely that coumarin-type of compounds in *Heptaptera* species could be responsible for antioxidant and mild anticholinesterase activity. In fact, we have recently published a research article about anticholinesterase activity of several coumarins (umbelliferone, 4-methylumbelliferone, 4-hydroxycoumarin, scopoletin, 8-methoxypsoralen, bergapten, and *iso*-bergapten) and only 4-methylumbelliferone, scopoletin, and bergapten showed inhibition against AChE over 50 % at 1 mg/ml. However, synergistic interactions can frequently occur in a single herb due to existence of dozens of bioactive compounds, and, therefore, it is very important to elucidate the active component(s). In conclusion, our results showed that *H. triquetra* showed the best radical scavenging and iron chelating effects among the extracts and could be a potential source for antioxidant compounds; while *H. anatolica* may be worthy of further phytochemical characterization for its anticholinesterase effect. To the best of our knowledge, this is the first report on anticholinesterase and antioxidant activities of *Heptaptera s*pecies [3].

ASTERACEAE

Echinacea sp. (coneflower) is well known for its immunostimulant effects, and its standardized extracts are widely used in various types of phytotherapeutic formulations for flu and cough treatment. The dichloromethane, ethyl acetate, ethanol, and aqueous extracts of *Echinacea purpurea* (EPU) and *E. pallida* (EPA) were assessed for their AChE and antioxidant activities. AChE inhibition was estimated using the spectrophotometric Ellman method. Antioxidant activity was evaluated by DPPH radical scavenging and ferrous ion-chelating power tests. Ferric-reducing antioxidant power (FRAP) of EPU and EPA were also tested. The CHL extract of the aerial parts of EPU displayed the highest ferrous ion-chelating effect. Alkanmides did not show scavenging effect, whereas the scavenging activity of the extracts correlated well with their cichoric acid contents [4].

BRASSICACEAE

Antioxidant activity of the ethanolic extracts of the aerial parts of *Turritis laxa* (Sibth & Sm.) Hayek (Brassicaceae) was screened by DPPH radical scavenging and FRAP assays. Total *phenolic* contents of the extracts were determined using Folin-Ciocalteau reagent. *T. laxa* was also tested for its anti-AChE activity. The extracts were analyzed by liquid chromatography–diode array detection–mass spectroscopy (LC–DAD–MS) for their flavonoid content, and the ethanolic extract of *T. laxa* has been found to contain rutin in appreciable amounts (7.63 ± 0.2 %). It was also the most active in the antioxidant tests.

The radical scavenging effect of the ethanol extract and MET fraction of the plant may result from rutin, although rutin itself showed a much higher antioxidant activity in both assays as flavonoids, in particular, are well known to have antioxidant effect. Since *T. laxa* is a member of the Brassicaceae family, whose genera have been known to be rich in isothiocynates, it could be considered to contain isothiocynates containing –SH groups in an appreciable amount. Despite its prosperous rutin quantity, lower FRAP effect of the plant might be dependent on its rich isothiocynate content. On the other hand, the anticholinesterase effect of the plant probably results from isothiocynate derivatives widely found in Brassicaceae [5].

BUXACEAE

The CHL:MET (1:1) extracts of *Buxus sempervirens* L. (Buxaceae), were screened for their anticholinesterase activity on AChE and BChE enzymes by in vitro Ellman method at 10 μg/ml and 1 mg/ml concentrations. The extracts did not show any noticeable inhibitory activity against both of the

enzymes at 10 μg/ml. *B. sempervirens* showed remarkable inhibitory activity above 50 % inhibition rate on AChE at 1 mg/ml. *B. sempervirens* extracts were the most active against BChE having 93.45 ± 0.88 % inhibition rate. The extracts those of *B. sempervirens*, which had lower activity against AChE, exhibited much higher activity against BChE. This may suggest that these extracts might be interacting with the enzymes in different mechanisms [6].

ERICACEAE

The CHL:MET (1:1) extracts of *Rhododendron ponticum* L. subsp. *ponticum* and *Rhododendron luteum* Sweet. (Ericaceae) were screened for their anticholinesterase activity on AChE and BChE enzymes by in vitro Ellman method at 10 μg/ml and 1 mg/ml concentrations. The extracts did not show any noticeable inhibitory activity against both of the enzymes at 10 μg/ml. The extracts of *R. ponticum* subsp. *ponticum* and *R. luteum* showed remarkable inhibitory activity above 50 % inhibition rate on AChE at 1 mg/ml. Among them, *R. ponticum* subsp. *ponticum* was the most active extracts against BChE having 95.46 ± 1.03 % inhibition rate [6].

PAPAVERACEAE-FUMARIOIDEAE

The CHL:MET (1:1) extracts of 14 *Fumaria* species were screened for their anticholinesterase activity AChE and BChE enzymes by in vitro Ellman method at 10 μg/ml and 1 mg/ml concentrations. During prescreening, alkaloid extracts of *Fumaria* species were found to exhibit significant in vitro AChE inhibitory activity at 1 mg/ml concentration. While galanthamine, the standard drug used in this study, showed 48.80 ± 0.36 % inhibitory activity, all of the extracts had much higher activity compared to galanthamine, ranging between 84.98 ± 1.07 and 96.89 ± 0.17 % [6]. Out of these extracts, *F. vaillantii*, having 94.23 ± 0.47 % inhibitory activity, was selected for bioactivity-directed fractionation and isolation studies. The alkaloid extract of *F. vaillantii* was subjected to column chromatography, leading to collection of 70 fractions. These fractions were combined according to thin-layer chromatography (TLC) monitoring and seven subfractions were obtained. Their AChE inhibitory activities were determined at 1 mg/ml concentration. Except for the first and second subfractions, the remaining of them had inhibitory activity, ranging between 74.58 ± 0.37 and 89.31 ± 0.64 %. Through the active fractions, six isoquinoline alkaloids were isolated by preparative TLC. The alkaloids isolated and their AChE inhibitory activities are as follows: canadine (56.75 ± 0.58 %), hydrastine (10.08 ± 0.78 %), bulbocapnine (65.23 ± 0.42 %), fumarophycine (37.90 ± 0.99 %), corydaldine (17.11 ± 0.89 %), and protopine (80.53 ± 0.59 %). Among the alkaloids, protopine (IC_{50} = 1.8 μM) had the most potent inhibitory activity; hydrastine, an alkaloid having phthalideisoquinoline skeleton, and corydaldine, an alkaloid with dihydroisoquinolone structure, were the least potent alkaloids, leading to the suggestion that both types of alkaloids do not have remarkable AChE inhibitory activity. On the other hand, bulbocapnine (IC_{50} = 2.0 μM) with aporphine skeleton, which has a better IC_{50} value than galanthamine (IC_{50} = 5.8 μM), could be considered to contribute to the activity of the extract. Canadine has also strong inhibitory activity (IC_{50} = 2.6 μM). In conclusion, the present results provide that the compounds responsible for the activity of *F. vaillantii* extract were determined as the alkaloids, namely, protopine, bulbocapnine, and canadine. Moreover, the activity may be due to the synergistic interaction between these alkaloids, which may be of therapeutic value in the treatment of AD [7].

The CHL:MET (1:1) extracts of *Corydalis solida* (L.) Swartz subsp. *solida* and *Glaucium corniculatum* (L.) J. H. Rudolph (Papaveraceae), were screened for their anticholinesterase activity on AChE and BChE enzymes by in vitro Ellman method at 10 μg/ml and 1 mg/ml concentrations. The extracts of *Corydalis solida* subsp. *solida* and *Glaucium corniculatum* showed remarkable inhibitory activity above 50 % inhibition rate on AChE at 1 mg/ml. Among them, *C. solida* subsp. *solida* showed 93.08 ± 0.97 % inhibition rates [6].

LAMIACEAE

Rosemary, used also as an aromatic tea and in aromatherapy, has been examined for its influence on mood cognition; it was concluded that its essential oil produced a significant enhancement of performance and overall quality of memory in healthy adults. AChE and BChE inhibitory activities of the petroleum ether (PE), ethyl acetate, CHL, and MET extracts, rosmarinic acid as well as the essential oil obtained from *Rosmarinus officinalis* L. growing in Turkey were investigated by Ellman method at 0.2, 0.5, and 1.0 mg/mL concentrations. In addition, quantification of rosmarinic acid, a common phenolic acid found in rosemary, was carried out by reverse-phase high-performance liquid chromatography (HPLC) in the methanolic extract of the plant, which was found to have 12.21 ± 0.95 % (122.1 ± 9.5 mg/g extract) of rosmarinic acid. Rosmarinic acid was also tested for its AChE and BChE inhibitory effect and found to cause 85.8 % of inhibition against AChE at only 1.0 mg/mL. The methanolic extract of *R. officinalis* L. showed a remarkable BChE-inhibitory effect. Besides, the essential oil was analyzed by GC–MS technique, which was shown to be dominated by 1,8-cineol (44.42 %) and followed by α-pinene (12.57 %) as major compounds in our rosemary oil, and we conclude that anticholinesterase activity of *R. officinalis* L. essential oil most likely depends on a synergistic mechanism between a number of oil components, whereas rosmarinic acid seems to be responsible for strong anti-BChE effect of the methanolic extract of *R. officinalis* L. [8].

Salvia genus (Lamiaceae) is represented by totally 95 species in Turkey, which are classified into seven sections including Eusphace Benth., Hymenosphace Benth., Aethiopis Benth., Plethiosphace Benth., Horminum (Moench) Dumort Drymosphace Benth., and Hemisphace Benth. Later, sect. Eusphace was changed to sect. *Salvia* by Hedge. In Anatolian folk medicine, the leaves of *Salvia triloba* are used as infusion (1–5 %) for simple disorders, and it is one of the plants used for memory enhancement in Anatolia recorded by an Ottoman herbalist-physician who lived at the time of the ruler Sultan Mehmed the fourth (1641–1693). On the other hand, *Salvia* species have been also recorded to be used against memory loss in Europe. In the current study [9], we aimed to screen another 55 taxa of the *Salvia* genus growing in Turkey for their anti-AChE activity by the Ellman method as well as antioxidant activity by two methods of DPPH and iron-chelating capacity.

The dichloromethane, ethyl acetate, and MET extracts prepared from 55 *Salvia* taxa were tested for their AChE inhibitory activity at 25, 50, and 100 μg/ml using an ELISA microplate reader. The extracts were also screened for their scavenging effect against DPPH radical and iron-chelating capacity. Total phenol and total flavonoid contents of *Salvia fruticosa* were determined. Among the 165 *Salvia* extracts screened, only the dichloromethane extract of *S. fruticosa* showed inhibition toward AChE at 100 μg/ml having 51.07 % of inhibition, while only the dichloromethane and ethyl acetate extracts of *Salvia cilicica* had a notable iron-chelating capacity at 100 μg/ml having 54.71 % of chelating capacity. Most of the extracts showed remarkable scavenging effect against DPPH radical. Since oxidative stress is one of the reasons for neurodegeneration and iron dysregulation being reported to be associated with AD, the extracts were screened for their antioxidant activity by these two methods. It is an advantage for the *Salvia* extracts to possess anticholinesterase activity besides antioxidant activity. In fact, among all 165 extracts from 55 *Salvia* taxa screened in this work, the dichloromethane extract of *S. fruticosa* appeared to be the most promising one. Additionally, total phenol and total flavonoid contents of the dichloromethane extract of *S. fruticosa* (syn. *S. triloba*) were determined. Thus, *S. fruticosa* is worth to be investigated phytochemically further, and phytochemical examination through activity-guided fractionation and isolation of the dichloromethane extract of *S. fruticosa* for its anticholinesterase activity is in progress in our laboratory in order to elucidate its active component(s).

We also examined in vitro anticholinesterase and antioxidant activities of 56 extracts prepared with PE, CHL, ethyl acetate, and MET obtained from 14 *Salvia* species growing in Turkey [10]. The antioxidant activities were assessed by both chemical and enzymatic methods against DPPH radical-scavenging and xanthine/xanthine oxidase (XO) system generated superoxide anion radical inhibition. Anticholinesterase effect of the extracts was tested against both AChE and BChE at concentrations of

0.2 and 1 mg/ml using a microplate-reader assay based on the Ellman method. Most of the extracts did not show any activity against AChE at 0.2 mg/ml, while the CHL extracts had noticeable inhibition against BChE between 47.7 and 74.7 %. The most active extracts at 1 mg/ml for AChE inhibition were observed to be PE extract of *Salvia albimaculata* (89.4%) and CHL extract of *Salvia cyanescens* (80.2 %), whereas ethyl acetate extracts of *Salvia frigida* and *Salvia migrostegia*, CHL extracts of *Salvia candidissima* ssp. *occidentalis* and *Salvia ceratophylla*, as well as PE extract of *Salvia cyanescens* were found to inhibit potently BChE (92.2, 89.6, 91.1, 91.3, and 91.8 %, respectively). Particularly, the ethyl acetate and MET extracts were observed to be highly active against both DPPH and XO. Our data indicates that nonpolar extracts of *Salvia* species for anticholinesterase activity and the polar extracts for antioxidant activity are worth further phytochemical evaluation for identifying their active components [10].

The essential oils of *Melissa officinalis* L. (cultivated using organic and chemical fertilizers), *Mentha piperita* L. and *M. spicata* L. (organic fertilizer), *Lavandula officinalis* Chaix ex Villars (cultivated using organic and chemical fertilizers), *Ocimum basilicum* L. (green and purple-leaf varieties cultivated using only organic fertilizer), *Origanum onites* L., *O. vulgare* L., *O. munitiflorum* Hausskn., and *O. majorana* L. (cultivated using organic fertilizer), *Salvia sclarea* L. (organic and chemical fertilizers), *S. officinalis* L. (organic fertilizer), and *Satureja cuneifolia* Ten. (organic fertilizer) were screened for enzyme inhibitory activity by the Ellman method at 1 mg/ml concentration. In addition, a number of single components widely encountered in most of the essential oils (γ-terpinene, 4-allyl-anisole, (–)-carvone, dihydrocarvone, (–)-phencone, cuminyl alcohol, cumol, 4-isopropylbenzaldehyde, *trans*-anethole, camphene, *iso*-borneol, (–)-borneol, L-bornyl acetate, 2-decanol, 2-heptanol, methyl-heptanol, farnesol, nerol, *iso*-pulegol, eucalyptol, citral, citronellal, citronellol, geraniol, linalool, α-pinene, β-pinene, piperitone, *iso*-menthone, menthofurane, linalyl oxide, linalyl ester, geranyl ester, carvacrol, thymol, menthol, vanilline, and eugenol) were also screened for the same activity in the same manner. The results showed that almost all of the essential oils showed a very high inhibitory activity (over 80 %) against both enzymes, whereas the single components were not as active as the essential oils [2].

The members of *Scutellaria* L. (Lamiaceae) are known to be rich particularly in flavonoids and among them, *S. baicalensis* has been recorded to be used for memory-enhancing purpose. *S. baicalensis*, a famous Chinese medicinal plant, has been recorded to be prescribed for treatment of memory deficit in traditional Chinese medicine. To date, there have been a number of studies performed on *S. baicalensis* and their components for their memory-augmenting effects. However, no relevant study has been reported on the other species of this genus. Actually, the ethanol extract of the roots of *S. baicalensis* of Korean origin was shown to possess neuroprotective effect tested by passive avoidance test in ibotenic acid-induced amnesia in rats. This record prompted us to investigate anticholinesterase and antioxidant activities of the Turkish *Scutellaria* species. Therefore, we initiated a study to screen the MET extracts prepared from the aerial parts of 33 Turkish *Scutellaria* species for their AChE and BChE inhibitory activities, which are the key enzymes taking place in pathogenesis of AD. The MET extracts of 33 *Scutellaria* species growing in Turkey were tested for their enzyme inhibitory activity against AChE, BChE, and tyrosinase at 250, 500, and 1000 μg/ml concentrations. All of the extracts were inactive against AChE, while only *S. orientalis* subsp. *macrostegia* showed some degree of inhibition against BChE having 29.08, 33.92, and 39.44 % of inhibition at the tested concentrations.

According to our results, the extracts screened showed weak AChE and BChE inhibition, which led us to make a conclusion that memory-enhancing property of *Scutellaria* species does not result from AChE/BChE inhibition, but by some other mechanisms as suggested in different reports. In fact, several antioxidant activity studies have been carried out to date, which were limited to only a few *Scutellaria* species such as *S. baicalensis* and. *S. barbata*.

Although those species are not included in our screening as they do not grow in Turkey, results of the antioxidant studies relevant to those species are in accordance with ours [11].

LYCOPODIACEAE

The genus *Lycopodium* L. (syn. Huperzia Bernh. and Diphasia Presl.) from the clubmoss family (Lycopodiaceae) consists of low terrestrial or epiphytic plants with approximately 100 species, occurring over most of the earth. In Turkey, this genus is represented by five species (*Lycopodium clavatum* L., *L. selago* L., *L. annotinum* L., *L. alpinum* L. and *L. complanatum* L. ssp. *chamaecyparissus* (A. Br.) Do¨ ll), distributed only in the northern Black Sea region . On the other hand, previous investigations have shown that *Huperzia serrata* (Thunb.) Trev. (syn. *Lycopodium serratum* Thunb.), a plant used for improvement of memory and learning in Chinese traditional medicine for a long time, afforded the alkaloid Huperzine A, which has been proved to be effective in treatment of AD via inhibiting AChE. AD is a progressive and neurodegenerative disorder illustrated by memory insufficiency and cognition loss and its pathogenesis is still unclear. For this purpose, we herein examined the anticholinesterase potential of the PE, CHL, and MET extracts as well as the alkaloid fraction of *L. complanatum* L. ssp. *chamaecyparissus* (A. Br.) Do¨ ll (LCC), growing in Turkey, against both AChE and BChE using the Ellman method. Galanthamine, used as the reference drug, exhibited 100 % of inhibitory activity against AChE at both 0.2 and 1 mg/ml concentrations, while it showed 98.6 and 100 % of inhibition against BChE at 0.2 and 1 mg/ml. Among the extracts screened, LCC-PE had a quite significant activity against AChE with 76.5 % of inhibition at 1 mg/ml concentration, whereas the rest of the extracts were found to be ineffective against AChE at 0.2 and 1 mg/ml. Interestingly, LCC-PE also seemed to be considerably active against BChE at the two tested concentrations (51.3 and 69.6 %, respectively). Although LCC-CHL extract was shown to moderately inhibit BChE at 1 mg/ml with 48.7% of inhibition, rest of the extracts did not exhibit any noticeable inhibition on AChE and BChE. The extracts were also screened for their antioxidant capacity by DPPH radical-scavenging activity. BHA, a well-known antioxidant, was used as reference drug. The results demonstrated that all of the extracts did not present any notable radical-scavenging activity, ranging between 22.7 and 38.6 %, while BHA displayed 92.7 % of scavenging activity against DPPH [12,13].

MORACEAE

Various *Ficus* species have been reported to be used ethnopharmacologically in different parts of the world. Among them, *F. religiosa* was reported to be used traditionally for improving memory in India. Thus, this information prompted us to investigate ChE inhibitory activity of *F. carica* since *F. religiosa* does not grow in our country. *Ficus carica* var. *domestica* Tsch. & Rav. (common fig) is widely grown in Turkey and exported for its edible fruits. The *n*-hexane, CHL, acetone, MET, buthanol, and water extracts of the leaves of *F. carica* var. *domestica* were screened for their cholinesterase inhibitory and antioxidant activities. Cholinesterase inhibition against AChE and BChE was measured by the Ellman method at 25, 50, and 100 μg/ml concentrations, while antioxidant activity was tested using three in vitro methods: DPPH radical-scavenging activity, metal-chelation capacity, and FRAP. Total phenol and flavonoid contents of the extracts were determined spectrophotometrically. Our results revealed that the *n*-hexane and acetone extracts exerted a notable inhibition against both AChE (62.9 ± 0.9 and 50.8 ± 2.1 %, respectively) and BChE (76.9 ± 2.2 and 45.6 ± 1.3 %, respectively). However, they had low activity in the antioxidant tests. The CHL extract was found to be the richest in total flavonoid content (252.5 ± 1.1 mg/g quercetin equivalent), while the buthanol extract had the highest total phenol amount (85.9 ± 3.2 mg/g extract gallic acid equivalent). In particular, the *n*-hexane and acetone extracts, which exerted a remarkable ChE inhibition, might be considered as the potential sources containing anticholinesterase compound(s) and worth further investigation. Besides, our antioxidant results obtained from the leaf extracts from *F. carica* var. *domestica* differed from those reported previously, which might depend on the fact that the collection site of the plant may play a critical role in affecting the phytochemistry and relevant bioactivity of the same species [14].

ZYGOPHYYLLACEAE

The CHL:MET (1:1) extracts of *Tribulus terrestris* L. and *Zygophyllum fabago* L. (Zygophyllaceae) were screened for their anticholinesterase activity on AChE and BChE enzymes by in vitro Ellman method at 10 µg/ml and 1 mg/ml concentrations. The extracts did not show any noticeable inhibitory activity against both of the enzymes at 10 µg/ml. *T. terrestris* and *Z. fabago*, which had lower activity against AChE, exhibited much higher activity against BChE. This may suggest that these extracts might be interacting with the enzymes in different mechanisms [6].

CONCLUSION

As well-known cholinesterase inhibitors are the most prescribed drug class currently for the treatment of AD, which is a progressive-nature neurodegenerative disease affecting the senior population over the age of 60, particularly in industrialized countries. However, the ChE inhibitors are not only important for their therapeutic effects for AD, but also their use due to cholinergic action extends to glaucoma and myasthenia gravis, even to the treatment of an intellectual disability such as Down syndrome. Besides, many insecticidal agents such as organophosphates show their efficiency through ChE inhibition, which leads to paralysis and finally death in the insect. Interestingly, it is also the mechanism of action for some nerve gases used as chemical warfare agents [15]. Based on their utilizations for diverse medical cases, ChE inhibitors have become a hot topic for research worldwide. There is growing interest in using and discovering new antioxidants from natural sources since synthetic antioxidants possess some unwanted effects. Therefore, a huge amount of research is being carried out on plants in order to find new antioxidant alternatives. On the other hand, AD is a kind of neurodegeneration which is also connected with oxidative mechanism. At the moment, AChE inhibitors (e.g., donepezil, rivastigmine, and galanthamine), and *N*-methyl-D-aspartate (NMDA) receptor antagonists (e.g., memantine) as well as antioxidants (e.g., *Ginkgo biloba* extract, EGb761) are the only drug classes in clinical practice for AD treatment [16].

Turkey is one of the rich countries in the world for plant sources depending on different geographical, ecological, and aquatic environments as well as passageway between Europe, Asia, and Africa. The floristic diversity provides a wide choice of species representing 12 000 taxa of which 3700 are endemic. Turkish flora is the richest of any country in Europe, North Africa, and the Middle East [17].

The plants have been used in the treatment of memory dysfunction in some folk medicines for centuries. The present study was undertaken to evaluate the anticholinesterase potential of a number of selected Turkish medicinal plants with various ethnobotanical uses, aiming to discover new candidates for anticholinesterase compounds. Among them, *Salvia, Rosmarinus*, and *Fumaria* species were found to have the most significant cholinesterase inhibitory activity. In addition, alkaloids [18,19], phenolic acids and flavonoids [20,21], lignans [22], coumarins, anthraquinones, and stilbene derivatives [23], along with marine organisms [24], were also investigated.

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