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Memory-vitalizing effect of twenty-five medicinal and edible plants and their isolated compounds

F. Sezer Senol [a], Ilkay Erdogan Orhan [a,*], Ufuk Ozgen [b], Gulin Renda [b], Gokhan Bulut [c], Leyla Guven [c], Esen Sezen Karaoglan [c], Handan G. Sevindik [c], Krystyna Skalicka-Wozniak [d], Ufuk Koca Caliskan [a], Nazim Sekeroglu [e]

[a] Faculty of Pharmacy, Department of Pharmacognosy, Gazi University, Ankara 06330, Turkey
[b] Faculty of Pharmacy, Department of Pharmacognosy, Karadeniz Technical University, Trabzon 610800, Turkey
[c] Faculty of Pharmacy, Department of Pharmacognosy, Ataturk University, Erzurum 25240, Turkey
[d] Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, Lublin 20-093, Poland
[e] Medicinal and Aromatic Plants Program, Vocational School, Kilis 7 Aralik University, Kilis 79000, Turkey

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ABSTRACT

The extracts from 25 plant species from *Salvia*, *Hypericum*, *Onosma*, *Thymus*, *Origanum*, *Rosa*, and *Prunus* with eight isolated compounds were screened for their memory-vitalizing potential against the enzymes, i.e., acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and tyrosinase (TYR) along with DPPH, DMPD, and NO radicals and iron-chelation capacity using spectrophotometric microtiter assays. The *n*-hexane:dichloromethane (1:1) extract of *Onosma nigricaule* roots (63.18 ± 0.56%) and the ethyl acetate extract of the aerial parts of *Hypericum capitatum* var. *capitatum* (50.79 ± 3.88%) were the most active toward AChE and BChE, respectively. The highest iron-chelating capacity was found in the *n*-hexane extract of *Thymus sipyleus* subsp. *sipyleus* var. *sipyleus*, where mostly Labiatae species exhibited better radical scavenging effect. Among the extracts, the roots of *O. nigricaule*, the aerial parts of *Hypericum capitatum* var. *capitatum* and *T. sipyleus* subsp. *sipyleus* var. *sipyleus* could be further evaluated for their memory-vitalizing properties based on different mechanisms.
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1. Introduction

Neurodegeneration is a complex procedure causing neuronal death associated with many devastating diseases including Alzheimer's disease (AD), Parkinson's disease (PD), prion diseases, amyotrophic lateral sclerosis (ALS), etc. Among them, AD is a progressive neurological disorder as the most common type of dementia, which destroys memory and behavioral skills particularly in elderly population progressive degeneration of the cerebral cortex. Some mechanisms have been proposed for pathogenesis of the disease, which are metal dysregulation leading to oxidative damage, deficit in acetylcholine (ACh) level as well as aggregation of toxic amyloid fibrils on amyloid beta (Aβ) peptide (Rowinska-Zyrek et al., 2015). According to cholinergic hypothesis, severe deficit in ACh level, hydrolyzed by acetylcholinesterase (AChE, EC 3.1.1.7), has been verified as the pathological hallmarks in the brains of AD patients, and consequently, AChE inhibitors have become the most prescribed drug class for the AD treatment (Orhan, 2012). On the other hand, butyrylcholinesterase (BChE, EC 3.1.1.8, also known as psedocholinesterase or plasma cholinesterase) has been suggested to be involved in the pathogenesis of AD despite of its blurred role, which can also breaks down ACh (Darvesh et al., 2003). Tyrosinase (TYR, EC 1.14.1.8.1, syn. polyphenol oxidase) is a copper-containing enzyme essential for tyrosine-melanin pigmentation and the role of toxic quinones in dopamine-induced neuronal damage catalyzed by TYR has been cleared in a number of studies (Miyazaki and Asanuma, 2009). At the same time, AD is often seen in PD patients, and therefore, the inhibition of TYR as well as cholinesterases is also critical for the treatment of these diseases. Based on these reports, it can be suggested that multi-target drug approaches may be more appropriate for the amended treatment of multifaceted brain diseases and cholinesterase inhibitors still stand as the prominent alternatives for AD treatment.

In our ongoing project on discovering novel cholinesterase or TYR-inhibiting medicinal plants, we have now aimed to screen twenty-four

Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; Aβ, amyloid beta; BChE, butyrylcholinesterase; DMPD, *N*,*N*-dimethyl-*p*-phenylendiamine; DMSO, dimethyl sulfoxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DTNB, 5,5′-dithiobis(2-nitrobenzoic)acid; EDTA, ethylenediaminetetraacetic acid; EtOAc, ethyl acetate; EtOH, ethanol; MeOH, methanol; NO, nitric oxide; PD, Parkinson's disease; SEM, standard error of the mean; TYR, tyrosinase.

* Corresponding author. Tel.: +90 312 2023186; fax: +90 312 2235018.
E-mail address: iorhan@gazi.edu.tr (I.E. Orhan).

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plant species using spectrophotometric microtiter assays. The plant species screened herein have been selected from the genera (*Salvia*, *Hypericum*, *Onosma*, *Thymus*, *Origanum*, *Rosa*, and *Prunus*) were tested for their AChE or BChE inhibitory activity due to their memory enhancing potential. Selection has been based on some previous studies carried out on other species of the genera studied herein related to their memory-improving effect (Orhan et al., 2007, 2009, 2011, 2012, 2013; Altun et al., 2013; Senol et al., 2013). Since oxidative damage and metal accumulation, i.e., iron are strongly linked to neurodegenerative diseases as a triggering factor (Nunomura, 2013), antioxidant potential of the plants screened was tested in the same manner against DPPH, DMPD, and nitric oxide radicals along with iron-chelation capacity. Total phenol and flavonoid contents of the extracts were determined spectrophotometrically. In addition, deoxyshikonin, acetylshikonin, and β,β-dimethylacrylshikonin from *Onosma nigricaule*, apigenin 7-*O*-neohesperidoside from *Onosma obtusifolium*, ursolic acid and luteolin 5-*O*-β-D-glucoside from *Thymus pseudopulegioides*, thymoquinone-2,5-diglucoside from *Thymus praecox* subsp. *grossheimii* var. *grossheimii*, and ursolic acid–oleanolic acid mixture from *Origanum micranthum* were also tested in the same manner.

2. Materials and methods

2.1. Plant materials

Among the species studied in this study, the blooming parts of the *Salvia* species (*Salvia amplexicaulis* Lam., *Salvia azurea* Michx. ex Lam., *Salvia barrelieri* Ettling, *Salvia cadmica* Boiss., *Salvia deserta* Schangin ex Ledeb, *Salvia nemorosa* L., *Salvia plebeian* R. Br., *Salvia przewalskii* Maxim., *Salvia* × *superba* Stapf, *Salvia tesquicola* Klokov, *Salvia tomentosa* Mill., and *Salvia verticillata* L.) were collected from the medicinal plant garden belonging to Medical University in Lublin (Poland) in 2012, where the corresponding voucher species (29a/7, 29a/4, 29b/10, 29a/5, 29a/6, 29a/10, 27a/23, 29b/16, 29q/15, 29b/30, and 29a/17, respectively) are preserved at the Department of Pharmacognosy with Medicinal Plant Unit of the same university. The samples of *Hypericum capitatum* Choisy var. *capitatum* Choisy and *Prunus mahaleb* L. were collected from Mardin province (Turkey) in 2013 and identified by the biologist Kamil Aydin whose voucher specimens are kept at the Herbarium of Department of Biology, Kilis 7 Aralik University (Turkey) (Herbarium no: KHB-14-1-1 and KHB-28-2-3, respectively). *O. nigricaule* H. Riedl, *O. obtusifolium* Hausskn. et Sint. ex H. Riedl, *Onosma tauricum* Pallas ex Willd., *Onosma armeniacum* Klokov, *Thymus sipyleus* Boiss. subsp. *sipyleus* var. *sipyleus*, and *Rosa pimpinellifolia* L. were collected from Erzurum province (Turkey) in 2011 and the samples of *T. pseudopulegioides* Klokov et Des Shost from Trabzon province (Turkey) in 2013. The samples of *O. nigricaule* (AEF 25874), *O. obtusifolium* (AEF 25875), *O. tauricum* (not recorded, yet), and *O. armeniacum* (AEF 23796), *T. pseudopulegioides* (AEF 23176), *T. praecox* subsp. *grossheimii* var. *grossheimii* (AEF 23861), *Origanum rotundifolium* (AEF25947), *Origanum minutiflorum* (AEF25949), and *R. pimpinellifolia* (AEF 21147) are kept at the Herbarium belonging to Faculty of Pharmacy, Ankara University (Ankara, Turkey), while the voucher specimen of *T. sipyleus* subsp. *sipyleus* var. *sipyleus* is preserved at the Herbarium of Faculty of Pharmacy, Ataturk University (Erzurum, Turkey) under the code of ATA 9718.

2.2. Extraction procedure

All of the plant species studied were dried in shade and powdered mechanically. The parts used and the correspondent solvents (ethanol-EtOH, methanol-MeOH, ethyl acetate-EtOAc, dichloromethane-CH_2Cl_2, chloroform-$CHCl_3$, and distilled water-H_2O) used in extraction at room temperature for each plant are listed in Table 1. For all species, the filtered and combined either organic or aqueous phases were concentrated to dryness under reduced pressure to give the crude extracts.

2.3. Isolation of the compounds

Isolation and structure elucidation of deoxyshikonin, acetylshikonin, and β,β-dimethylacrylshikonin from *O. nigricaule*, apigenin 7-*O*-neohesperidoside from *O. obtusifolium*, ursolic acid from *T. pseudopulegioides*, thymoquinone-2,5-diglucoside from *Thymus praecox*, and ursolic acid–oleanolic acid mixture from *O. micranthum* was described in detail in our previous publications (Ozgen et al., 2011a,b; Bulut et al., 2012).

2.4. Microtiter assays for enzyme inhibition

2.4.1. AChE and BChE inhibitory activity

AChE and BChE inhibitory activity of the extracts was determined by modified spectrophotometric method of Ellman et al. (1961). Electric eel acetylcholinesterase (Type-VI-S, EC 3.1.1.7, Sigma) and horse serum butyrylcholinesterase (EC 3.1.1.8, Sigma) were used as the enzyme sources, while acetylthiocholine iodide and butyrylthiocholine chloride (Sigma, St. Louis, MO, USA) were employed as substrates of the reaction. 5,5′-Dithio-bis(2-nitrobenzoic) acid (DTNB, Sigma, St. Louis, MO, USA) was used for the measurement of the cholinesterase activity. All the other reagents and conditions were the same as described in our previous publication (Orhan et al., 2013), and the final concentration of the tested samples and reference was adjusted to 100 μg mL^{-1}. In brief, 140 μL of 0.1 mM sodium phosphate buffer (pH 8.0), 20 μL of 0.2 M DTNB, 20 μL of sample solutions, and 20 μL of 0.2 M AChE/BChE solution were added by multichannel automatic pipette (Gilson pipetman, France) in a 96-well microplate and incubated for 15 min at 25 °C. The reaction was then initiated with the addition of 10 μL of 0.2 M acetylthiocholine iodide/butyrylthiocholine chloride. The hydrolysis of acetylthiocholine iodide/butyrylthiocholine chloride was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at a wavelength of 412 nm utilizing a 96-well microplate reader (VersaMax, Molecular Devices, USA). Galanthamine, the anti-cholinesterase alkaloid-type of drug isolated from the bulbs of snowdrop (*Galanthus* sp.), was purchased from Sigma (St. Louis, MO, USA) and was employed as reference.

2.4.2. Tyrosinase inhibition

The inhibition of tyrosinase (TYR) (EC 1.14.1.8.1; 30 U, mushroom tyrosinase, Sigma) was determined using the modified dopachrome method with *L*-DOPA as substrate (Masuda et al., 2005). The assays were conducted in 96-well microplate using ELISA microplate reader (VersaMax Molecular Devices, USA) to measure absorbance at 475 nm. An aliquot of the extracts dissolved in DMSO with 80 μL of phosphate buffer (pH 6.8), 40 μL of TYR, and 40 μL of *L*-DOPA were put in each well. The final concentration of the tested samples and reference was adjusted to 100 μg mL^{-1}. Results were compared with control (DMSO). α-Kojic acid (Sigma, St. Louis, MO, USA) was used as the reference.

2.4.3. Data processing for enzyme inhibition assays

The measurements and calculations were evaluated by using Softmax PRO 4.3.2.LS software. The percentage of the inhibition of AChE/BChE was determined by comparison of rates of reaction of test samples relative to blank sample (ethanol in phosphate buffer pH 8). Extent of the enzymatic reaction was calculated based on the following equation: $I\% = (C - T) / C \times 100$, where $I\%$ is the activity of the enzyme as percent inhibition. *E* value expresses the effect of the test sample or the positive control on AChE and BChE enzyme activity articulated as the percentage of the remaining activity in the presence of test sample or positive control. *C* value is the absorbance of the control solvent (blank) in the presence of enzyme, where *T* is the absorbance of the tested sample (plant extract or positive control in the solvent) in the presence of enzyme.

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