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A mechanistic investigation on anticholinesterase and antioxidant effects of rose (*Rosa damascena* Mill.)

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ABSTRACT

In the current study, neuroprotective effect of the essential oil and aromatic waters of rose was investigated by in vitro and in silico methods. The samples were tested for their inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Since oxidative damage is associated with neurodegeneration, antioxidant activity of the samples was determined by DPPH radical scavenging, metal-chelation, and ferric-reducing antioxidant power (FRAP) assays. Their chemical composition was elucidated by GC–MS. Anti-cholinesterase effects of the main components (citronellol, geraniol, nerol, and phenylethyl alcohol) were also examined. The rose oil showed a noteworthy inhibition against AChE (60.86 ± 1.99%) and BChE (51.08 ± 1.70%) at 1000 μg/mL and low activity in DPPH radical scavenging and FRAP tests. Phenylethyl alcohol exerted higher cholinesterase inhibition than other components and applied further to molecular docking simulations. Docking and binding energies supporting experimental results show that the compound is more selective towards BChE than AChE.
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1. Introduction

Rosa damascena Mill. (Rosaceae) has been used in medicine since ancient times. Dioscorides (c.40–c.90 B.C.), a famous Anatolian physician and botanist, mentioned about rose oil (*Oleum Rosae*) as an ointment in his De Materia Medica (Altintas, 2009). Turkey and Bulgaria are the leading producers of the rose oil from *R. damascena* in the world. Rose is one of the most important aromatic plants in Turkey, the main producer of 70% of the rose concrete from *R. damascena* (damask rose) in the world and one of the most important rose germplasm centers (Ercisli, 2005). On the other hand, a sum of rose flowers is used by the food industry for making jams and liqueurs and rose extracts have been suggested to be potential sources of antioxidants which can be used in food preservation (Gokturk Baydar & Baydar, 2013; Kumar, Bhandari, Singh, & Bari, 2009; Lee, Lee, & Choung, 2011).

Alzheimer's disease (AD) is characterized by progressive loss of memory and abnormal social behaviors. It has become one of the major health issues especially in the countries with increasing aging population. Several mechanisms have been suggested to be associated with pathogenesis of AD, which is a multi-factorial disease. Among them, "cholinergic hypothesis" is the most accepted mechanism since a deficit in acetylcholine (ACh) level has been demonstrated in the brains of AD patients and thus, inhibitors of acetylcholinesterase (AChE), the key enzyme that hydrolyzes ACh, have become the most prescribed drug class for the treatment of AD (Orhan, Orhan, Subutay-Oztekin, Ak, & Sener, 2009). Other factors playing role in AD etiology are formation of senile β-amyloid-containing plaques called "amyloid hypothesis" as well as mitochondrial dysfunction and oxidative stress, which are also prominent evidences in the brains of AD patients (Maruszak & Żekanowski, 2011; Sheng et al., 2012). The theories targeting aging mechanisms have suggested that accumulative oxidative stress might possibly cause mitochondrial dysfunction and oxidative damage (Lin & Beal, 2006). Besides, many reports have underlined that formation of free radicals and oxidative stress is among the triggering and inducing factors for neurodegenerative diseases (Gilgun-Sherki, Melamed, & Offen, 2001). Nevertheless, cholinesterase inhibitors are only successful in symptomatic treatment of this disease and current inhibitors exert several side effects. Thus, drug discovery studies on novel inhibitors from natural or synthetic sources are being conducted comprehensively.

In the light of the traditional use of rose relevant to memory, the current study was undertaken to investigate inhibitory effects of the essential oil and two aromatic water samples from *R. damascena* against AChE and BChE, which are linked to pathogenesis AD, at

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100, 200, 500, and 1000 μg/mL. Chemical composition of the rose oil and aromatic water (*Eau de rose*) samples was analyzed by gas chromatography–mass spectrometry (GC–MS) and the principal components including geraniol, nerol, (−)-β-citronellol, (+)-β-citronellol, and phenylethyl alcohol were also tested solely or in various combinations against the afore-mentioned enzymes in the same manner. Since oxidative damage is strongly associated with Alzheimer's disease, antioxidant activity of the essential oil was also tested by three in vitro methods; 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, metal-chelation, and ferric-reducing antioxidant power (FRAP) assays at 1, 2, 5, and 10 mg/mL concentrations.

2. Materials and methods

2.1. Plant material

The essential oil and aromatic water sample no. 1 (Eau de rose) were provided by "SEBAT Rose Oil and Essential Oils Ltd." located in Isparta province (Turkey) in 2010. The aromatic water sample no. 2 was purchased in 2010 as a commercial product produced by "Rosense Cosmetic Products Company" (Isparta, Turkey), which is the world's largest producer and exporter of rose oil (www.rosense.com).

2.2. Tested compounds

The chemical compounds tested in this study as the principal components in rose; (−)-β-citronellol, (+)-β-citronellol, geraniol, nerol, and phenylethyl alcohol, were from Sigma-Aldrich Co. (St. Louis, Mo, USA).

2.3. Cholinesterase inhibition assay

AChE and BChE inhibitory activity was measured by slightly modified spectrophotometric method of Ellman, Courtney, Andres, and Featherstone (1961). Electric eel AChE (Type-VI-S, EC 3.1.1.7, Sigma, St. Louis, MO, USA) and horse serum BChE (EC 3.1.1.8, Sigma, St. Louis, MO, USA) were used, 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 anticholinesterase activity. All reagents and conditions were same as described in a relevant previous publication (Senol, Orhan, Yilmaz, Cicek, & Sener, 2010). 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 412 nm utilizing a 96-well microplate reader (VersaMax Molecular Devices, USA). The measurements and calculations were evaluated by using Softmax PRO 4.3.2.LS software. Percentage of inhibition of AChE/BChE was determined by comparison of rates of reaction of samples relative to blank sample (ethanol in phosphate buffer pH = 8) using the formula $(E - S) / E \times 100$, where E is the activity of enzyme without test sample and S is the activity of enzyme with test sample. The experiments were done in triplicate. Donepezil, the anticholinesterase drug kindly provided by "Refik Saydam Central Hygiene Institute, The Ministry of Health of Republic of Turkey, Ankara", was used as the reference.

2.4. Antioxidant activity by radical-formation methods

2.4.1. DPPH radical scavenging activity

The stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined by the method of Blois (1958), which was later modified by Hatano (1995). The samples dissolved in ethanol (80%) were mixed with DPPH solution (1.5×10^{-4} M). Remaining DPPH amount was measured at 520 nm using a Unico 4802 UV–visible double beam spectrophotometer (Dayton, NJ, USA). Gallic acid (Sigma-Aldrich Co., St. Louis, MO, USA) was employed as the reference. Inhibition of DPPH in percent (I%) was calculated as given below:

$$I\% = \left[\left(A_{blank} - A_{sample}\right) / A_{blank}\right] \times 100,$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test sample), and A_{sample} is the absorbance of the extracts/reference. Analyses were run in triplicate and the results were expressed as average values with S.E.M. (standard error mean).

2.4.2. Metal-chelating effect

The ferrous ion-chelating effect of the samples was estimated by the method of Chua, Tung, and Chang (2008). Briefly, various dilutions of the samples dissolved in ethanol (80%) were incubated with 2 mM $FeCl_2$ solution. The reaction was initiated by the addition of 5 mM ferrozine into the mixture and left standing at ambient temperature for 10 min. The absorbance of the reaction mixture was measured at 562 nm using a Unico 4802 UV–visible double beam spectrophotometer (Dayton, NJ, USA). The ratio of inhibition of ferrozine–Fe^{2+} complex formation was calculated as follows:

$$I\% = \left[\left(A_{blank} - A_{sample}\right) / A_{blank}\right] \times 100,$$

where A_{blank} is the absorbance of the control reaction (containing only $FeCl_2$ and ferrozine), and A_{sample} is the absorbance of the samples/reference. Analyses were run in triplicate and the results were expressed as average values with S.E.M. The reference was ethylenediamine tetraacetic acid (EDTA, Merck KGaA, Darmstadt, Germany) in this assay.

2.4.3. Ferric-reducing antioxidant power (FRAP) assay

The ferric-reducing power was tested using the assay of Oyaizu (1986). Different concentrations of the samples were mixed with 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide. Later, the mixture was incubated at 50 °C for 20 min and, then, trichloroacetic acid (10%) was added. After the mixture was shaken vigorously, this solution was mixed with distilled water and ferric chloride (0.1%). After 30 min of incubation, absorbance was read at 700 nm using a Unico 4802 UV–visible double beam spectrophotometer (Dayton, NJ, USA). Analyses were achieved in triplicates. Increased absorbance of the reaction meant increased reducing power and compared to that of chlorogenic acid (Sigma-Aldrich Co., St. Louis, Mo, USA) as the reference.

2.5. Statistical analysis of data from bioactivity experiments

Data obtained from in vitro enzyme inhibition and antioxidant experiments were expressed as the mean standard error (±S.E.M.). Statistical differences between the reference and the sample groups were evaluated by ANOVA (one way). Dunnett's multiple comparison tests were used as post hoc tests. $p < 0.05$ was considered to be significant [$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$, $^{****}p < 0.0001$].

2.6. GC/GC–MS analysis and identification of the essential oil and aromatic waters

The essential oil and aromatic water samples were analyzed using an Agilent 6890N Network GC System with 5973 Network Mass Selective Detector. An HP-Innowax FSC column (60 m × 0.25 mm *i.d.*, with 0.25 μm film thickness) was used for separation of components in the oil. Helium (0.8 mL/min) was used as carrier gas. The GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min and then kept constant at 220 °C for 10 min and programmed to 240 °C at a rate of 1 °C/min. The mass range was recorded at m/z 35 to 450. The split flow was adjusted at 40 mL/min with a 40:1 split ratio. The injector temperature was at 250 °C. MS data were recorded at 70 eV.

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