Industrial Crops & Products 111 (2018) 179–184

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Research Paper

Onosma heterophyllum: Phenolic composition, enzyme inhibitory and antioxidant activities

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ARTICLE INFO

Keywords:
Onosma heterophyllum
Antioxidant
Enzyme inhibitory
HPLC
Phenolics

ABSTRACT

Onosma species have frequently been used for their colouring and dyeing properties in foods and medicinal preparations. The antioxidant and enzyme inhibitory activities of the ethyl acetate, methanol and water extracts of *Onosma heterophyllum* (Griseb.) are described. Phytochemical compositions of these extracts were also determined. The water extract showed not only remarkable antioxidant activity in all assays but also considerable inhibitory activity on tyrosinase and α-glucosidase (112.44 μmol KAEs/g dry plant and 984.36 μmol ACEs/g dry plant, respectively). The methanol extract exhibited the highest inhibitory activity on acetylcholinesterase (AChE) and α-amylase (79.18 μmol GALAEs/g dry plant and 10.42 μmol ACEs/g dry plant, respectively). Chromatographic analyses revealed that the water extract was found to be rich in phenolic and flavonoid contents. On the basis of the correlation coefficients calculated separately for all experimental parameter pairs, protocatechuic acid, *p*-hydroxybenzoic acid, syringic acid, *p*-coumaric acid and luteolin were found to be highly in correlation with the antioxidant and enzyme inhibitory activities. This study demonstrates that *O. heterophyllum*, contained in food preparations with various purposes for many years, could be used for the treatment of diabetes as well as its skin whitening effect.

1. Introduction

Lipid peroxidation, which is one of the main reasons of food deterioration, causes the formation of undesirable reactions in foods (Antolovich et al., 2002). Degradation of biological molecules as a result of free radical reactions is known to be associated with various diseases (Achour et al., 1997). In order to decrease the adverse effects of reactive oxygen species on normal physiological functions, antioxidants are added into food products (Laguerre et al., 2009). For many years, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxyltoluene (BHT) have been used in food preservation although they have potential risks on human health (Qian et al., 2008; Su et al., 2016). Therefore, the scientists have focused on finding new and alternative antioxidants with less or no side effects. One of the main goals of this study is to explore the preservative potential of the extracts of *Onosma heterophyllum* (Griseb.) on foods.

Reducing or eliminating the hazardous effects of reactive oxygen species (especially on the brain) is among the most efficient treatment strategies of Alzheimer's disease (AD). Dementia, memory loss and other cognitive impediments are known to be among the symptoms of this disease (Townsend, 2011). Low levels of acetylcholine, accumulation of β-amyloid (Aβ) plaques and oxidative damage are the key pathological signs of the disease (Luo et al., 2011). As the main treatment strategy, scientists are currently trying to improve the cholinergic neurotransmission in the brain (Gualtieri et al., 1996). Cholinesterase (ChE) inhibitors are the agents that offer promising results in the treatment of the disease (Huang et al., 2012). Tacrine, Donepezil, Rivastigmine and Galantamine are known to be the acetylcholinesterase (AChE) inhibitors that have been approved by the European and US regulatory authorities (Huang et al., 2010). They effectively decrease the cognitive, functional and behavioral symptoms (Luo et al., 2016).

The plant-derived compounds can be used for not only their antioxidant activities as food additive agents, but also to prevent the

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http://dx.doi.org/10.1016/j.indcrop.2017.10.026
Received 3 June 2017; Received in revised form 11 October 2017; Accepted 12 October 2017

browning of the fruits. Browning causes the loss of fruits and vegetables. Tyrosinase causes the browning of fruits and vegetables. In this process, essential amino acids are being destroyed and a significant decrease occurs in nutritional and market values of the foods (Artes et al., 1998). Tyrosinase inhibitors are the frequently used to control the enzymatic browning reactions (Dong et al., 2016).

Plants are also the sources of the compounds that possess anti-diabetic activity. In diabetes mellitus, glucose excessively accumulates in blood. High glucose level causes to the vascular diabetic complications, generation of free radicals and oxidation-related damage on various organs (King and Loeken, 2004). α-Amylase and α-glycosidase are known as the key enzymes that digest the carbohydrates in small intestine (McDougall and Stewart, 2005). Inhibition of these enzymes prevents the digestion of starch and causes to decrease in blood glucose level in diabetic patients. In order to control hyperglycemia in type II diabetic patients, synthetic inhibitors are being used clinically. On the other hand, synthetic anti-diabetic drugs cause some unwanted side effects in humans (Hemalatha et al., 2016). Therefore, the scientists are still trying to explore novel alternative compounds (Bischoff et al., 1985). Phenolic compounds are known to have α-amylase and α-glucosidase inhibitory activities (Hemalatha et al., 2016; Kim et al., 2011; Qin et al., 2013; Ranilla et al., 2009; Shobana et al., 2009; Sreerama et al., 2012).

The genus *Onosma* L. is represented by 150 species distributed worldwide. According to literature data, less than 10 species were evaluated for their chemical constituents and clinical potentials (Kumar et al., 2013). *Onosma* species are found to contain mainly aliphatic ketones, lipids, naphthazarins, alkaloids, and phenolic compounds. They have frequently been used for their colouring and dyeing properties in foods and medicinal preparations. Due to their distinct colour, *Onosma* species have been used as adulterant agents in red chili powder and other food preparations (Chakraborti et al., 2001). They have traditionally been used as laxative and anthelmintic agents as well as their uses in eye and blood diseases, bronchitis, abdominal pain, strangury, thirst, itch, leucoderma, fever, wounds, burns, piles, and urinary calculi. The flowers and leaves of *Onosma* species have also been used as stimulant, cardiotonic, and purgative (Maskovic et al., 2015).

The aim of this study is to evaluate the *in vitro* antioxidant and enzyme inhibitory activities of ethyl acetate, methanol and water extracts of *O. heterophyllum*. Antioxidant activity was analyzed by using phosphomolybdenum, metal chelating, radical scavenging, and reducing power assays. Inhibitory activities of the extracts were tested on AChE, butyrylcholinesterase (BChE), tyrosinase, α-amylase, and α-glucosidase. Additionally, the relationship between the phytochemical composition and biological activity were also discussed. As far as our literature survey could ascertain, Yildirim et al. (2013) have reported the antibacterial and antitumoral activities of *O. heterophyllum*. But antioxidant and enzyme inhibitory activities of *O. heterophyllum* have not previously been reported elsewhere. Therefore, data presented here could be assumed as the first report on this species.

2. Materials and methods

2.1. Chemicals

Ferric chloride, Folin–Ciocalteu's reagent, and methanol were purchased from Merck (Darmstadt, Germany). 1,1-diphenyl-2-picrylhydrazyl (DPPH), 5,5-dithio-bis-2-nitrobenzoic acid (DTNB), AChE (Electric ell acetylcholinesterase, Type-VI-S, EC 3.1.1.7), BChE (horse serum butyrylcholinesterase, EC 3.1.1.8), 3,4-dihydroxy-L-phenylalanine (L-DOPA), tyrosinase, acetylthiocholine iodide (ATCI), butyrylthiocholine chloride (BTCl), and all phenolic standards were purchased from Sigma Chemical Co. (Sigma–Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents were of analytical grade.

2.2. Plant material

Aerial parts of *O. heterophyllum* were collected from Gencali village, Suhut-Senirkent highway, Isparta-Turkey on 12 June 2014 (between 40.–50. km, 1366 m, 38° 14′ 26″N 30° 39′ 58″E). Taxonomic identification of the plant material was carried out by a senior taxonomist, Dr. Olcay Ceylan, from the Department of Biology, Mugla University, Mugla-TURKEY. The voucher specimen was deposited at the Herbarium of the Department of Biology, Mugla University, Mugla-Turkey (Voucher number: O.5000).

2.3. Preparation of the extracts

To prepare the extracts, air-dried samples (20 g) obtained from the aerial parts of *O. heterophyllum* were individually extracted with 250 ml of ethyl acetate and methanol for 5 h by using a Soxhlet extractor. To obtain the water extract, the air-dried sample (20 g) was extracted with 400 ml of boiling deionized water for 15 min. Ethyl acetate and methanol were then removed by using a rotary evaporator (Zengin et al., 2014). Then, the water extract was freeze-dried. All the extracts were stored at +4 °C until analyzed. Yields of the ethyl acetate, methanol and water extracts were determined as 0.85%, 8.06%, and 23.78% (w/w), respectively.

2.4. Quantification of phenolic compounds by reversed-phase high-performance liquid chromatography (RP-HPLC)

Phenolic compounds were evaluated by using RP-HPLC (Shimadzu Scientific Instruments, Kyoto, Japan). Detection and quantification were carried out with a LC-10ADvp pump, a Diode Array Detector, a CTO-10Avp column heater, SCL-10Avp system controller, DGU-14A degasser, and SIL-10ADvp auto sampler (Shimadzu Scientific Instruments, MD, USA). Separations were conducted at 30 °C on Agilent® Eclipse XDB C-18 reversed-phase column (250 mm × 4.6 mm in length, 5 μm in particle size). Phenolic compositions of the extracts were determined according to the method of Zengin et al. (2014). *(−)*-Epicatechin, *(+)*-catechin, apigenin, benzoic acid, caffeic acid, chlorogenic acid, eriodictyol, ferulic acid, gallic acid, hesperidin, kaempferol, luteolin, *o*-coumaric acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, protocatechuic acid, quercetin, rosmarinic acid, rutin, sinapinic acid, syringic acid, *trans*-cinnamic acid, and vanilin were used as standards. Analytical characteristics of the method used for the quantification of phenolics were reported in section S.1. Identification of the compounds was carried out by separate injections of each standard solution and the injection of stock solution containing all standards. Thus, the resolution peak and the run time were determined for each compound. In order to verify the identification of compounds, a chromatographic run was performed with the extracts. Phenolic and flavonoid compounds in the extracts were identified through the comparison of their retention times and spectrums with those obtained by the injection of standard solution under the same conditions. For the quantitation of compounds, peak areas were measured by using internal standard calibration. Chromatographic profiles of the standard phenolics and the extracts were given in section S. 2 and S. 3–5, respectively.

2.5. Determination of total phenolic and flavonoid compounds

Total phenolic and flavonoid contents of the extracts were determined according to the method of Sarikurkcu et al. (2015).

2.6. Antioxidant activity

Antioxidant activity of the ethyl acetate, methanol and water extracts of *O. heterophyllum* were evaluated by using