

## Antioxidant and anticholinesterase activities of *Heliotropium dolosum*, *H. lasiocarpum* and *H. hirsutissimum* growing in Turkey

Songül KARAKAYA<sup>1\*</sup>, Mehmet Koca<sup>2</sup>, Ceyda Sibel KILIÇ<sup>3</sup>

<sup>1</sup>Atatürk University, Faculty of Pharmacy, Department of Pharmacognosy, Erzurum, Turkey

<sup>2</sup>Ataturk University, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Erzurum, Turkey

<sup>3</sup>Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Ankara, Turkey

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### Abstract

Alzheimer's disease (AD) has encouraged science to find plants or biologic compounds which can be utilized for prophylaxis of AD. *Heliotropium* genus is presented by 16 taxa in Turkey and they contain pyrrolizidine alkaloids, and certain are known to have antioxidant and anticholinesterase activities. The paper displays the anticholinesterase and antioxidant capacities of the aerial parts lyophilized aqueous extracts of *H. dolosum*, *H. lasiocarpum* and *H. hirsutissimum*. Phenolic contents and antioxidant activity of examples were determined by Folin-Ciocalteu's, qualitative/quantitative DPPH and TBA. Anticholinesterase activity was assessed by Ellman's method. The total phenolic content of *H. lasiocarpum* extract was found the highest (738.39 mg/g), however the total phenolic content of *H. dolosum* extract was found the lowest (122.731 mg/g). Every example indicated that antioxidant activity with DPPH test and *H. lasiocarpum* presented the highest activities (69.99 µg/mL). Furthermore, the *H. lasiocarpum* extract indicated considerable inhibition towards acetylcholinesterase (5.90%) and butyrylcholinesterase (24.04%) enzymes. These findings suggest that these *Heliotropium* species can be effective for pharmaceutical products that achieve anticholinesterase and antioxidant activities.

**Keywords:** Antioxidant, Anticholinesterase, *Heliotropium*

### Türkiye'de yetişen *Heliotropium dolosum*, *H. lasiocarpum* ve *H. hirsutissimum*'un Antioksidan ve Antikolinesteraz Aktiviteleri

#### Öz

Alzheimer hastalığı (AD), bilimi AD'nin profilaksisinde kullanılacak bitkileri veya biyolojik bileşikleri bulmaya teşvik etmektedir. *Heliotropium* cinsi Türkiye'de 16 taksonla temsil edilir ve bu türler pirolizidin alkaloidleri içermektedir ve bazılarının antioksidan ve antikolinesteraz aktivitelere sahip olduğu bilinmektedir. Bu çalışmada, *H. dolosum*, *H. lasiocarpum* ve *H. hirsutissimum*'un toprak üstü kısımlarının liyofilize sulu ekstraktlarının antikolinesteraz ve antioksidan kapasiteleri incelenmiştir. Örneklerin fenolik içerikleri ve antioksidan aktivitesi Folin-Ciocalteu, kalitatif / kantitatif DPPH ve TBA ile belirlenmiştir. Antikolinesteraz aktivitesi Ellman metodu ile değerlendirilmiştir. En yüksek toplam fenolik içerik *H. lasiocarpum* ekstresinde (738.39 mg/g), ancak en düşük toplam fenolik içerik *H. dolosum* ekstresinde (122.731 mg/g) tespit edilmiştir. Her örnek DPPH testi ile antioksidan aktivite göstermiş ve en yüksek aktivite *H. lasiocarpum*'da (69.99 µg/mL) görülmüştür. Ayrıca, *H. lasiocarpum* ekstresi, sırasıyla asetilkolinesteraz (%5.90) ve butirikolinesteraz (%24.04) enzimlerine karşı önemli ölçüde inhibisyon göstermiştir. Bu sonuçlar, bu

*Heliotropium* türlerinin, antioksidan ve antikolinesteraz aktivitesine sahip farmasötik ürünler için etkili olabileceğini göstermektedir.

**Anahtar Kelimeler:** Antioksidan, Antikolinesteraz, *Heliotropium*

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## 1. Introduction

Alzheimer's disease (AD) is an impairment brain ailment and the most diffuse case for dementia. The ordinary indications of dementia are questions withal the mind, speech, issue-solving and other cognitive talents that impress a man's skill to have diurnal actions. These occasions come about for nerve cells in portions of the brain mingled in cognitive action have been dilapidated. In AD, neurons in other portions of the brain are at last endangered or demolished so long as, comprising those that authorizes a man to carry through basis personal functions such as walk and swallow. Mankind in the recent degrees of the ailment are bed-bound and take around-the-clock nursing and AD is in the consequence of mortal (Alzheimer's Association, 2018).

Age is one of the risk factors of AD. Accordingly the ageing inhabitants, it is awaited that AD will be a complexity socio-economic defiance in the nearing years (Karch et al., 2018). With respect to the World Health Organization record, AD, which impresses roundly 47 million mankind worldwide, is the most extensive of entire dementias (60-80% of whole incidents) (WHO, 2015) and AD is a pathology that impresses >46.8 million humans beings, the most extensive neurodegenerative ailment for an approximative worldwide cost of US\$818 billion (Sadaoui et al. 2018). Additionally, oxidative stress occurs owing to the detriment to nerves or metal acumulation is severely connected to the pathogenesis of AD besides, it is rather prominent to own-

both of cholinesterase inhibition and antioxidant potencies for a medicament applicant towards AD (Ustun et al., 2012).

Diversification of AD, the deficit of extensive insight on the pathogenesis of AD and limited therapeutical approaches advance novel searches for nominees against AD (Davinelli et al., 2012). The antioxidants may clean the reactive oxygen species (ROS) and inflammatory pathways are prevented by antioxidants. In AD progressive utilisation of antioxidants is beneficial (Ferreira et al., 2006). Several searches have been accomplished on biological effects of herbs which are applied conventionally as mind increasings (Luz et al., 2016; Perry et al., 1998).

*Heliotropium* L. is a genus of flowering herbs belonging to the Boraginaceae family and is generally known as "don't forget me". The genus *Heliotropium* has 250-300 species called "heliotropes". Up to 100 species spread throughout the Mediterranean and temperate regions (Shah, 2012). *Heliotropium* species are annual or rarely two-year-old shrub-like plants that vegetate in the driest period of summer. They are known "siğil otu" in Turkey. Juice obtained by crushing the fresh leaf is applied to the warts to destroy the warts (Ceribasi 2010).

Certain species of the *Heliotropium* genus have significant therapeutic activity, and these species have extensive use in the world. Many species are utilized in the treatment of inflammation, fever, throat infection, rheumatism, ring worm, ulcer, wounds and

expectorant. For example; *H. strigosum* is traditionally utilised as laxatives and diuretic. Fruit juice prepared from the herb is utilized in gums, eye pain and also nettle, insect and snake bites (Khan et al., 2013). Another species, *H. foertherianum*, is used by the public in the Ciguatera region of the Pacific for the treatment of fish poisoning (Rossi et al. 2012). In India, *H. marifolium* is known as ‘choti santari’, has commonly used for the treatment of ulceration, wounds and inflammation, snake and dog bites, eye pain, gums and ear pain (Singh and Dubey, 2001). *H. indicum* is known as “cock’s comb” and ‘ake-onfem atiko’ in Ghana. This plant has extensive uses in West Africa, India and the Philippines. Leaves of the plant are used in children's abortion, ectopic, antidote (snake-scorpion bites), arthritis, rheumatism, eye diseases, fever reducer, analgesic, wolf-lowering. It has been used in the treatment of epilepsy and convulsions. It is registered that the whole plant is used against cancer, diarrhea, dysentery, tumors. The plant is widely used in Ghana to treat hypertension (Koffuor et al., 2012).

The biological significance of genus *Heliotropium* is attributed mainly to pyrrolizidine alkaloids (PAs), which are considered the primary constituent of this genus and the chemotaxonomic marker of family Boraginaceae (Aboelmagd et al., 2018). *Heliotropium* has been utilized in curation of inflammation, fever, throat infection, bronchitis, eczema, antipyretic, nail problems, rheumatism, fungus, ulcerations, anticancer, gastritis, stomachic, asthma, wounds, and as an expectorator (Khan et al., 2016; Arihan et al., 2018).

*Heliotropium* L. (Boraginaceae) is represented 16 species in Turkey and they are

known as “bambulotu” in Turkey (Güner, 2012). *H. dolosum* De Not, *H. hirsutissimum* Grauer and *H. lasiocarpum* Fisch. & C.A.Mey. are cosmopolit perennial species, growing in North Turkey, South & Inner Anatolia; Inner & North-East Anatolia and North-West & Inner Anatolia Turkey, respectively (Davis, 1972).

Thereof, the aim of current search is the preliminary paper on inhibition towards AChE and BuChE enzymes together with antioxidant capacities of extracts from aerial parts of *H. dolosum*, *H. lasiocarpum* and *H. hirsutissimum*.

## 2. Material and Methods

### 2.1. Plant material

The plants were gathered by authors from the below forenamed locality and described by Prof. Dr. Hayri Duman. Voucher specimens are stored in AEF (Herbarium of Ankara University Faculty of Pharmacy):

*Heliotropium dolosum*: B4: Ankara, Ankara University Tandoğan Campus, roadsides, 863 m, 18/9/2013, AEF 26354

*H. hirsutissimum*: C2: Antalya, Manavgat, Çolaklı, 1 km to A.Ü. Örsem facilities, roadsides, 31 m, 14/9/2013, AEF 26355

*H. lasiocarpum*: B1: İzmir, Dikili Bankacılar Sitesi, roadsides, 28 m, 2/8/2013, AEF 26353.

General appearances and herbarium specimens of *H. hirsutissimum*, *H. dolosum* and *H. lasiocarpum* are presented in Figure 1.



**Figure 1.** General appearances and herbarium specimens of *Heliotropium hirsutissimum*, *H. dolosum* and *H. lasiocarpum*.

## 2.2. Preparation of extracts

50 g of aerial parts from these plants were grinded and macerated with 300 mL of distilled water for 8 hours/3 days at room temperature, in severalty. The aqueous extracts were filtered, frozen and lyophilized to gain aqueous extracts of aerial parts. The amount of the starting materials, the quantity of the obtained aqueous extracts and extract yield in % are given in Table 1.

**Table 1.** Amounts of starting materials and extracts obtained from *Heliotropium* species and their yields in %.

<i>Heliotropium</i> species	Starting material (g)	Aqueous lyophilized extracts (g)
<i>H. dolosum</i>	50	10.1047
<i>H. hirsutissimum</i>	50	10.1047
<i>H. lasiocarpum</i>	50	8.5374

### 2.3. Total phenolic content

The total polyphenol content was colourimetric with Folin-Ciocalteu reagent as gallic acid equivalents (Singleton and Rossi, 1965; Slinkard and Singleton 1977; Mavi et al., 2004). The indicator of 250  $\mu$ L Folin-Ciocalteu, 50  $\mu$ L sample and 20% water solution of 500%  $\text{Na}_2\text{CO}_3$  were blended, vortexed and consummated with 5 mL of water. The absorbance of the admixture was read at 765 nm. It was then incubated for 30 minutes at room temperature and a reference curve was generated with gallic acid. Total polyphenols were exhibited as gallic acid equivalent (GAE) and mg GAE/g specimen (dw) were expressed as  $\pm$  standard deviation (SD) (Karakaya et al., 2018).

### 2.4. Antioxidant activity

#### 2.4.1. Quantitative DPPH

The free radical scavenging activity was determined by Brand-Williams et al. (Brand et al., 1995). 0.1 mL of the examples were consumed as a free radical source in 2.9 ml of DPPH solution. The admixtures were capital shaken, incubated in a water bath at 30  $^{\circ}\text{C}$  for 30 minutes, and the absorbance of each sample was recorded at 517 nm. Rutin, propyl gallate,

and chlorogenic acid were utilised as reference. Results were expressed as the percentile of radical scavenging activity (%) of DPPH and calculated by the formula  $[(A_o - A_s) / A_o] \times 100$ , where  $A_o$  is the absorption in the presence of examples or references (Karakaya et al., 2018).

#### 2.5. Anti-lipid peroxidation activity

Thiobarbituric acid (TBA) test was used to perform the activity of the samples in the protection of liposomes from lipid peroxidation (Dinis et al., 1994). The specimens, rutin, chlorogenic acid, propyl gallate (as standard) were prepared at 7 different concentrations range. 0.5 mL of phosphate buffer, 0.2 mL of brain extract, 0.1 mL of ascorbic acid, 0.1 mL of ferric chloride, and samples were blended and incubated at 37  $^{\circ}\text{C}$  for twenty minutes. Then, 0.5 mL of 1% TBA, 0.5 mL of 25% HCl, and 0.1 mL of 2% BHT were annexed to the admixture, shaken and incubated at 85  $^{\circ}\text{C}$  for 30 minutes. The admixture was cooled and 2.5 mL of n-butanol solvent was supported. After centrifugation, the absorbance of examples enregistered at 532 nm (Karakaya et al., 2018).

## 2.6. Detection of AChE and BuChE inhibition capacities

Anticholinesterase activity of examples were estimated through colorimetric Ellman's method (Ellman 1961), with slight modifications by the way of commercially available donepezil as the reference (Yerdelen and Tosun 2015). Acetylthiocholine, thioester is utilised in place of the oxy ester of acetylcholine used in the Ellman method. Examples were dissolved in DMSO and then diluted to diversified concentrations in Tris buffer solution (50 mM, pH 8.0). 25 µL different of four different concentrations of inhibitory compounds, 50 µL Tris buffer solution, 125 µL DTNB (3 mM) solution, were mixed with AChE or BuChE enzymes. 25 µL was then annexed to the wells and the admixture was incubated at 37 °C for 15 minutes. After 15 minutes of incubation, a solution of substrate (ATCI

or BTCl) at a concentration of 15 mM in a volume of 25 µL volume was annexed to each well. The absorbance of the reaction mixtures was enregistered at 405 nm with a microplate reader (Karakaya et al., 2018).

## 2.7. Statistical analysis

Entire indications are denoted as mean ± SE and differentnesses were statistically analyzed via ANOVA one-way analysis dogged via way of complementary analysis of Bonferroni ( $p < 0.05$ ), conceived to demonstrated statistic meaningfulness.

## 3. Result and Discussion

*Heliotropium hirsutissimum*, *H. dolosum* and *H. lasiocarpum* extracts were carried out as regards antioxidant capacity potential. The results of examples as regards the content of total phenolics are displayed in Table 2.

**Table 2.** Total phenolic contents of the extracts from *Heliotropium dolosum*, *H. hirsutissimum* and *H. lasiocarpum*

Tested samples	Total phenolic contents (mg/g) ± SD*		
	<i>H. dolosum</i>	<i>H. hirsutissimum</i>	<i>H. lasiocarpum</i>
	Aerial part	Aerial part	Aerial part
Aqueous lyophilized extracts	122.731 ± 4.16	678.55 ± 2.70	738.39 ± 2.09

\*SD:Standart deviation

*H. lasiocarpum* and *H. hirsutissimum* extracts showed the highest total phenolic content (738.39 ± 2.09 and 678.55 ± 2.70 mg GAE/g dry weight, respectively)

however, *H. dolosum* extract presented the lowest total phenolic content (122.731 ± 4.16 mg GAE/g dry weight). Quantitative DPPH analysis indications were presented as IC<sub>50</sub> (µg/mL) in Table 3.

**Table 3.** DPPH radical scavenging activity of the extracts from *Heliotropium dolosum*, *H. hirsutissimum* and *H. lasiocarpum* ( $\mu\text{g/mL}$ ).

Tested samples	IC <sub>50</sub> values ( $\mu\text{g/mL}$ ) $\pm$ SD*		
	<i>H. dolosum</i>	<i>H. hirsutissimum</i>	<i>H. lasiocarpum</i>
	Aerial part	Aerial part	Aerial part
Aqueous lyophilized extracts	89.32 $\pm$ 3.89	76.61 $\pm$ 3.87	69.99 $\pm$ 1.92
Chlorogenic acid	2.41 $\pm$ 0.58		
Propyl gallate	0.005 $\pm$ 0.21		
Rutin	3.05 $\pm$ 0.89		

\*SD: Standard deviation

**Table 4.** Antioxidant activities of the extracts from *Heliotropium dolosum*, *H. hirsutissimum* and *H. lasiocarpum* in TBA test.

Tested samples	IC <sub>50</sub> values ( $\mu\text{g/mL}$ ) $\pm$ SD*		
	<i>H. dolosum</i>	<i>H. hirsutissimum</i>	<i>H. lasiocarpum</i>
	Aerial part	Aerial part	Aerial part
Aqueous lyophilized extracts	278.31 $\pm$ 3.03	209.64 $\pm$ 2.81	169.93 $\pm$ 2.90
Chlorogenic acid	12.98 $\pm$ 4.89		
Propyl gallate	3.44 $\pm$ 2.05		
Rutin	9.65 $\pm$ 3.09		

\*Standard deviation. The data display the mean  $\pm$  SD of four independent testings ( $p < 0.05$ ).

**Table 5.** *In vitro* acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activities of the extracts from *Heliotropium dolosum*, *H. hirsutissimum* and *H. lasiocarpum* at 100  $\mu\text{g/mL}$ .

Tested samples	Enzyme type	Percentage of inhibition $\pm$ S.E.M <sup>a</sup> against AChE and BuChE		
		<i>H. dolosum</i>	<i>H. hirsutissimum</i>	<i>H. lasiocarpum</i>
		Aerial part	Aerial part	Aerial part
Aqueous lyophilized extracts	AChE	b	b	5.90 $\pm$ 3.94
	BuChE	c	15.93 $\pm$ 2.91	24.04 $\pm$ 2.96
Donepezil	AChE	82.45 $\pm$ 2.64		
	BuChE	90.33 $\pm$ 4.16		

<sup>a</sup> Standard error mean

<sup>b</sup> No activity.

<sup>c</sup> Not determined due to turbidity in the wells of microplates. The data display the mean  $\pm$  SD of three independent testings ( $p < 0.05$ ).

Also, *H. lasiocarpum* and *H. hirsutissimum* (69.99 and 76.61  $\mu\text{g/mL}$ , respectively) displayed notablens radical scavenging effect when compared the references chlorogenic acid, rutin and propyl gallate. Although, *H. dolosum* ( $\text{IC}_{50} = 59.27 \mu\text{g/mL}$ ) displayed the least DPPH radical scavenging potentials. The findings of TBA were presented as  $\text{IC}_{50}$  ( $\mu\text{g/mL}$ ) in Table 4. *H. lasiocarpum* presented the highest antioxidant effect ( $\text{IC}_{50} = 169.93 \mu\text{g/mL}$ ) in TBA assay. Numerous of the examples displayed antioxidant activity on liposome by comparison the chlorogenic acid and rutin. A correl between DPPH, TBA assay and total phenol content was revealed.

The acetylcholinesterase potentials of examples at 100  $\mu\text{g/mL}$  were presented at Table 5. Dependently enzyme inhibition findings *H. lasiocarpum* demonstrated significantive stoppage activities towards to acetylcholinesterase and butyrlcholinesterase, it has been indicated considerable inhibition against AChE ( $5.90 \pm 3.94\%$ ) and BuChE ( $24.04 \pm 2.96\%$ ) at 100  $\text{mg/mL}$ . Only, *H. lasiocarpum* indicated inhibition against AChE. On the other side, *H. dolosum* did not show inhibition against both two enzymes.

The *H. lasiocarpum* extract has been characterised by substantial higher content of total phenolics than *H. hirsutissimum* and *H. dolosum* extracts. In advance, it was seen that the crude extracts of *H. indicum* exhibited significant ( $P < 0.05$ ) stability to plasma cholinesterase (Boye, 2010).

It was detected a substantial correlation between antioxidant potential and total phenolics content in former searches as

well (Syta et al., 2015; Granato et al., 2018)

Alzheimer, a neurodegenerative sickness caused by oxidative stress, is a cholinergic decrement in brain. In particular, a disruption in acetylcholine summation unleashed from cholinergic synapses has been characterized. A method of healing has been developed to augment or sustain the sum amount of acetylcholine by inhibiting acetylcholinesterase. The search represented that the *H. dolosum*, *H. lasiocarpum* and *H. hirsutissimum* extracts have inhibitory activity on AChE and BuChE enzymes and antioxidant capacity. At healing of AD, the utilise of antioxidants can be worthy (Senol et al., 2011). According to the literature survey, the paper is the preliminary exploratory on anticholinesterase activity of *H. dolosum*, *H. lasiocarpum* and *H. hirsutissimum*.

#### 4. Conclusion

In 2050, a new AD case is estimated to develop every 33 seconds; this results in about 1 million novel cases per year, with an estimated prevalence of 11 million to 16 million. Between 2000 and 2013, deaths caused by stroke, heart disease, and prostate cancer reduced by 23%, 14%, and 11%, respectively, while deaths from AD enhanced by 71%. Among the species, *Heliotropium lasiocarpum* exhibited the best anticholinesterase and antioxidant effects. For this reason, *H. lasiocarpum* can be utilised in AD and a herbal alternating to synthetical medications which should be further confirmed.



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