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Original article

Astragalus neurocarpus Bioss. as a potential source of natural enzyme inhibitor associated with Alzheimer's and Parkinson diseases along with its rich polyphenolic content and antioxidant activities

Nazim Sekeroglu◆*, and Sevgi Gezici**,***

**Department of Food Engineering, Faculty of Engineering and Architecture, Kilis 7 Aralik University, 79000, Kilis, Turkey.*

***Advanced Technology Application and Research Center, Kilis 7 Aralik University, 79000, Kilis, Turkey.*

****Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Kilis 7 Aralik University, 79000, Kilis, Turkey.*

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Abstract

Astragalus neurocarpus Bioss. (AN), a member of the Fabaceae family, is a traditional medicinal plant that its root parts have been known as strengthening the immune system. As far as our literature survey could ascertain, enzyme inhibitory activity, free radical scavenging and reducing antioxidant power activities of AN have not been reported elsewhere previously. Therefore, the present study was performed to assess total polyphenolic contents, antioxidant activities and neuroprotective potentials of the ethanol and water extracts from aerial part and root of the plant.Total phenol and flavonoid contents of the extracts were determined spectrophotometrically. Antioxidant activity was analysed by using *in vitro* DPPH, ABTS, FRAP and CUPRAC assays. Neuroprotective activities of the extracts were assessed through enzyme inhibition on AChE, BChE and TYR. Regarding of total phenol and flavonoid quantities, all the extracts obtained from the aerial parts possessed higher polyphenolic contents, when compared to the root extracts. Ethanol extract prepared from the aerial parts of the plant were found the highest amount of total flavonoid quantity (179.34 ± 1.02 mg/g extract, as QE), while ethanol extract prepared from the root part possessed the lowest amount of total flavonoid quantity (49.73 ± 1.56 mg/g extract, as QE). The tested extracts were found to have higher inhibition against cholinesterase enzymes than those of the tyrosinase enzyme. Cholinesterase inhibitory activity assays on AChE and BChE enzymes were resulted in the superiority of the ethanol extracts obtained from the aerial parts (68.14 ± 0.03% and 59.44 ± 1.02% inhibition, respectively). According to antioxidant assays, all the extracts obtained from different parts of the plant exhibited remarkable activities on DPPH, ABTS and FRAP, whilst they were moderate effects on CUPRAC.

Keywords: *Astragalus neurocarpus* Bioss., neuroprotective, polyphenolic content, antioxidant, enzyme inhibition

1. Introduction

Among the people, there has been a widespread interest in usage of the natural antioxidants in order to prevent oxidative stress related to diseases recently. Fighting the free radicals occurring in the human body is one of the most effective prevention strategy in most oxidative-stress related diseases including cancer, cardiovascular disease, chronic kidney diseases, diabetes, rheumatoid arthritis atherosclerosis, and neurodegenerative diseases (Newman and Cragg, 2016; Gezici, 2018; Gezici, 2019; Gezici and Sekeroglu, 2019a).

There are many natural herbal products and formulations, obtained from medicinal plants, with high antioxidant capacities for fighting free radicals around the world (Iqbal, 2013; Biradar, 2015; Gezici and Sekeroglu, 2019a). Among these medicinal plants, *Astragalus* (Fabaceae family) species are well-known plant group and of important antioxidant source. This genus has many valuable species which have been used for immunostimulant, hepatoprotective, antiviral, antioxidative, wound-healing, anti-depressive, and anti-inflammatory properties in folk medicine (Block and Mead, 2003; Gülcemal *et al*., 2013; Roleira *et al*., 2018). Members of this genus are rich in secondary metabolites such as polysaccharides, saponins, indolizidine alkaloids, flavones and cycloartanes, which are closely linked to their wide range of biological activities (Rios and Waterman, 1997; Ionkova *et al*., 2014).

Previously, root extracts of *A. memdranaceus*, *A. sieversianus* and *A. mongholicus* have been screened for their biological activities and their protective effects against liver damage were reported using *in vivo* rat and mouse models (Zhang *et al*., 1992; Wang and Han, 1992). There are valuable scientific reports performed both *in vitro* and *in vivo* conditions, approving good biological activities, *Astragalus* species with distinguished phytochemical compositions. Liu *et al*. (2018) demonstrated in animal model that *Astragalus*

Author for correspondence: Dr. Nazim Sekeroglu
Professor, Department of Food Engineering, Faculty of Engineering and Architecture, Kilis 7 Aralýk University, 79000, Kilis, Turkey.
E-mail: nsekeroglu@gmail.com, sekeroglu@kilis.edu.tr
Tel.: +90-5324015512

polysaccharides (APS) had good therapeutic effect in the treatment of neurodegenerative diseases and nerve injury disease. Labed *et al*. (2016) studied phytochemical composition and biological activities of *Astragalus armatus* Willd. subsp. *numidicus* (Fabaceae) pods, an endemic shrub of Maghreb. They found that ethyl acetate extract afforded a flavonoid (1) while the n-butanol extract gave four flavonoids (2-5), a cyclitol (6) and a cycloartane-type saponin (7). Highest antioxidant activity in DPPH (IC_{50}= 67.90 ± 0.57 lg/ml), ABTS (IC_{50}= 11.30 ± 0.09 lg/ml) and CUPRAC (A0.50:50.60 ± 0.9 lg/ml) determined in the ethyl acetate extract. Chloroform extract gave the best antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, each with 80 lg/ml MIC values. The n-butanol extract resulted in higher phagocytic activity. However, many *Astragalus* species have not been screened their phytochemical compositions and biological activities.

In our previous study, aerial parts and root extracts of *A. elongatus* subsp. *nucleiferus* were analysed for their anticancer, antiproliferative and lactate dehydrogenase releasing effects (Sekeroglu *et al*., 2019, in press). Considering our previous results, we aimed to investigate total polyphenolic contents, *in vitro* antioxidant activities and neuroprotective potentials of ethanol and water extracts of the aerial parts and roots of *Astragalus neurocarpus* (AN) in the current research. Although, there is considerable evidence about pharmacological properties and traditional uses of *Astragalus* species, there has been no study so far determining biological activities of aerial parts and root extracts from AN. From this point of view, the results presented in this research could be the first report for the literature.

2. Materials and Methods

2.1 Plant material

The plant samples, roots and aerial parts, used in laboratory analyses were collected from Gaziantep (Turkey) during the months of April - May 2017. The plant samples were taxonomically identified by a senior taxonomist, FatihYayla, from the Department of Biology, Gaziantep University (Gaziantep, Turkey). The herbarium voucher (herbarium number: GAUN1708) was deposited at the Department of Biology, Gaziantep University, Turkey.

2.2 Preparation of plant extracts

AN samples were dried under the shade at laboratory conditions and powdered individually as aerial and root parts before the extraction. Each plant parts were extracted with ethanol (EtOH) and distilled water (dH_2O) by the method of maceration as described in our previous publication (Gezici and Sekeroglu, 2019b; Gezici, 2019). After maceration period, plant samples were filtered and EtOH was evaporated under vacuum using a rotary evaporator, and then the extracts were kept at 4°C until further analysis. Extract yields of the EtOH and dH_2O extracts from the aerial parts and roots of AN are given as w/w % in Table 1.

2.3 Total phenol and flavonoid contents

Phenolic compounds in total were determined in accordance with slightly modified Folin-Ciocalteau's method (Singleton and Rossi, 1965; Gezici and Sekeroglu, 2019b). Absorption was measured at 760 nm at a using a 96-well microplate reader (Versa Max Molecular Devices, USA). Total flavonoid content of the extracts was calculated by Aluminum Chloride Colorimetric method (Woisky and Salatino, 1998; Gezici and Sekeroglu, 2019b). A number of dilutions of quercetin were obtained to prepare a calibration curve. Absorbance of the reaction mixtures was measured at wavelength of 415 nm with a using a 96-well microplate reader (VersaMax Molecular Devices, USA). The total phenol and flavonoid contents of the extracts were expressed as gallic acid and quercetin equivalents (mg g^{-1} extract), respectively.

2.4 Antioxidant activity analyses

Antioxidant activities of the extracts were performed using *in vitro* methods including 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and cupric ion reducing capacity (CUPRAC) (Chaturvedi, 2012; Gezici *et al*., 2017; Sekeroglu *et al*., 2017; Gundogdu *et al*., 2018; Gezici and Sekeroglu, 2019b). The extracts and commercial antioxidant standards were dissolved in DMSO at different concentrations, ranging from 100 μg ml^{-1} to 1000 μg ml^{-1} for the antioxidant assays.

2.5 Enzyme inhibition assays

Neuroprotective activities of the extracts were evaluated against AChE (acetylcholinesterase), BChE (butyrylcholinesterase), and TYR (tyrosinase) in the current study. For the enzyme inhibition assays, the extracts were dissolved in DMSO at different concentrations, ranging from 100 μg ml^{-1} to 800 μg ml^{-1}. The percentage of enzyme inhibition was calculated as following equation:

$$\text{Percent of enzyme inhibition (\%)} = [(Abs_{control} - Abs_{sample}) / Abs_{control} \times 100]$$

2.5.1 AChE and BChE inhibitory activity

AChE and BChE inhibitory activity of the samples was measured by slightly modified spectrophotometric method of Ellman *et al*. (1961). Electric eel AChE (EC 3.1.1.Sigma, St. Louis, MO, USA) and horse serum BChE (EC 3.1.1. Sigma, St. Louis, 7 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, conditions and calculations were same as described in our previous publication (Senol *et al*., 2018; Gezici and Sekeroglu, 2019b), and the final concentration of the tested samples was adjusted to 800 μg ml^{-1}. Galanthamine hydrobromide (Sigma, St. Louis, MO, USA) was employed as the reference anti-cholinesterase, and the final concentration of the reference was adjusted to 100 μg ml^{-1}.

2.5.2 TYR inhibitory activity

The modified dopachrome method with L-DOPA as substrate was used for determination the inhibition of tyrosinase (EC 1.14.1.8.1, 30 U, mushroom tyrosinase, Sigma) (Sekeroglu *et al*., 2012). α - Kojic acid (Sigma, St. Louis, MO, USA) was used as the reference, and the final concentration of the tested samples and reference was adjusted to 800 μg ml^{-1} and 100 μg ml^{-1}, respectively.

2.6 Data analysis

The data were taken from three independent experiments, and expressed as mean and standard deviation of mean (Mean ± SD). The measurements and calculations were evaluated by using Softmax PRO 4.3.2.LS software. *p* value of <0.05 was considered to be

statistically significant, p <0.01 and p <0.001 were considered to be very significant.

3. Results

3.1 Total phenolicand flavonoid contents

Total phenolicand flavonoid compositions of the extracts were identified spectrophotometrically in the current research. Gallic acid (GA) and quercetin (Q) equivalent were used as commercial standards for total phenolic and flavonoid contents, and the results were presented in Table 1.

Table 1: Extraction yields (w/w%) and total polyphenolic contents of *A. neurocarpus* extracts

Plant part	Extract type	Extract yield (w/w%)	Total polyphenolic content[a]	
			TPC [a,b]	TFC[a,c]
Aerial	EtOH	2.62	117.15 ± 1.06	179.34 ± 1.02
	dH_2O	2.58	91.34 ± 0.84	108.22 ± 0.45
Root	EtOH	2.34	102.84 ± 0.12	49.73 ± 1.56
	dH_2O	2.21	110.75 ± 1.68	42.08 ± 0.73

[a]The values were expressed as Mean ± standard deviation (n=3).
[b]TPC: total phenolic content; mg equivalent of gallic acid (GAE) per g extract.
[c]TFC: total flavonoid content; mg equivalent of quercetin (QE) per g extract.

According to polyphenolic contents of AN extracts, EtOH extract obtained from aerial part of the plant was found to have the richest in total phenols and flavonoids quantities (117.15 ± 1.06 mg/g extract, as GAE and 179.34 ± 1.02 mg/g extract as QE). In addition, dH_2O extract prepared from the aerial parts of the plant was demonstrated the lowest amount of total phenol content (91.34 ± 0.84 mg/g extract as GAE), whereas dH_2O-root extract of the plant was showed the lowest total flavonoid content (42.08 ± 0.73 mg/g extract, as QE) (Table 1).

3.2 Antioxidant activity results

Free radical scavenging activities of AN extracts were assessed against DPPH and ABTS radicals, and the results were given as inhibition (%) ± standard deviation (Table 2). As presented in the Table 2, all the extracts displayed higher ABTS scavenging effects as compared to the DPPH scavenging effects at the tested concentrations. The highest DPPH scavenging activity was found to belong to the EtOH extract obtained from the aerial part (82.51 ± 1.64% inhibition), whilst the highest ABTS scavenging activity was determined in the dH_2O extract obtained from aerial part of the plant (94.01 ± 1.16% inhibition), followed by the EtOH-aerial part extract (88.96 ± 0.34% inhibition).

In the presented research, the AN extracts were also tested for their reducing power capacities on FRAP and CUPRAC. Almost, all the extracts obtained from different parts of *A. neurocarpus* exerted remarkable ferric reducing antioxidant power (FRAP), and moderate cupric ion reducing antioxidant power (CUPRAC) capacities, as summarized in the Table 3 with respect to their absorbance values ± standard deviation (Table 3). However, a remarkable variation was not observed in the FRAP values of the extracts, in which the dH_2O extract of aerial part from AN possessed the highest FRAP value, closely followed by root-EtOH extract of the plant (3.372 ± 0.082 and 3.236 ± 0.041, respectively). Among the tested extracts, the EtOH extract of aerial part was determined to have the highest CUPRAC value (1.095 ± 0.036). In all cases, the extracts prepared from the aerial parts of the plant exerted higher antioxidant activity than those of the root extracts (Table 2 and Table 3).

Table 2: Free radical scavenging activities of *A. neurocarpus* extracts at 1000 μg ml^{-1}

Plant part	Extract type	DPPH[a]	ABTS[a]
Aerial	EtOH	82.51 ± 1.64***	88.96 ± 0.34***
	dH_2O	67.40 ± 1.01**	94.01 ± 1.16***
Root	EtOH	46.08 ± 0.52***	57.92 ± 0.09**
	dH_2O	33.61 ± 0.49**	81.36 ± 0.58***
Ascorbic acid[b]		72.94 ± 0.06	–
Trolox[c]		–	76.28 ± 0.15

[a]The values were given as inhibition (%) ± standard deviation.
[b]Ascorbic acid is a commercial standard for DPPH assay.
[c]Trolox is a commercial standard for ABTS assay.
**p value of < 0.01; ***p value of < 0.001

Table 3: Reducing power capacities of *A. neurocarpus* extracts at 1000 μg ml^{-1}

Plant part	Extract type	FRAP[a]	CUPRAC[a]
Aerial	EtOH	3.182 ± 0.006***	1.095 ± 0.036***
	dH_2O	3.372 ± 0.082**	0.576 ± 0.102**
Root	EtOH	3.236 ± 0.041**	0.324 ± 0.085***
	dH_2O	3.173 ± 0.010***	0.301 ± 0.002***
Trolox[b]		__	2.136 ± 0.008
Chlorogenic acid[c]		3.497 ± 0.001	__

[a]The values were given as absorbance values ± standard deviation.
[b]Trolox is a commercial standard for CUPRAC assay.
[c] Chlorogenic acid is a commercial standard for FRAP assay.
**p value of < 0.01; ***p value of < 0.001

3.3 Enzyme inhibition results

Neuroprotective activity of the extracts obtained from different parts of AN were evaluated through enzyme inhibition assays on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), and tyrosinase (TYR) enzymes at 100, 200, 400, and 800 μg ml^{-1} concentrations. As can be seen in Figure 1, the AN extracts were inhibited AChE and BChE enzymes from moderate level to high level, in which the higher cholinesterase inhibitory activity was observed against AChE, compared to BChE, whilst they did not demonstrate significantly inhibition against TYR enzyme at the tested concentrations.

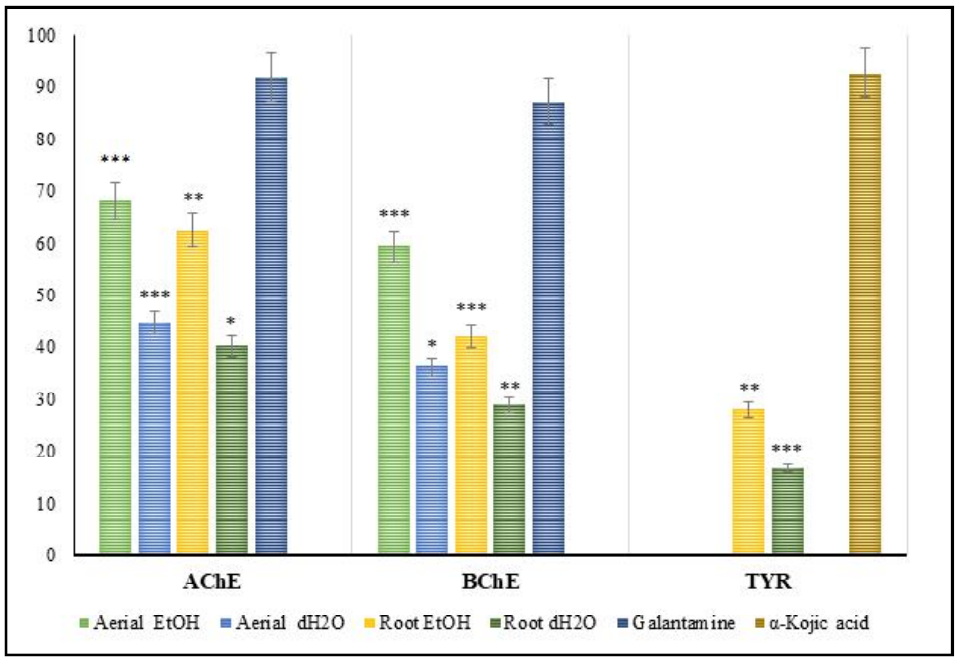

Figure 1: Enzyme inhibition capacities of *A. neurocarpus* extracts against AChE, BChE and TYR.
[The values were presented as inhibition (%) ± standard deviation] [*p value of < 0.05; **p value of < 0.01; ***p value of < 0.001]

Table 4: Neuroprotective potentials of *A. neurocarpus* extracts against AChE, BChE and TYR at 800 µg ml^{-1}

Plant part	Extract type	% Inhibition ± SD[a]		
		AChE	BChE	TYR
Aerial	EtOH	68.14 ± 0.03***	59.44 ± 1.02***	__[d]
	dH_2O	44.68 ± 0.12***	36.15 ± 0.06*	__[d]
Root	EtOH	62.50 ± 0.60**	42.10 ± 0.79***	28.03 ± 0.10**
	dH_2O	40.26 ± 1.14*	28.94 ± 0.58**	16.84 ± 0.08***
Galantamine[b]		91.86 ± 0.72	87.14 ± 0.35	__
α-Kojic acid[c]		__	__	92.75 ± 1.60

[a]The values were given as inhibition (%) ± standard deviation (n=3).
[b] Galantamine is a commercial standard for AChE and BChE enzymes
[c] á-Kojic acid is a commercial standard for TYR enzyme.
[d] No inhibitory activity.
*p value of < 0.05; **p value of < 0.01; ***p value of < 0.001

As consistent with the total polyphenolic quantities of the extracts, enzyme inhibitory potentials of the tested extracts on cholinesterase enzymes were resulted in the superiority of the EtOH extracts from the aerial part (68.14 ± 0.03%) inhibition on AChE, and (59.44 ± 1.02%) inhibition on BChE, respectively. In the contrary the cholinesterase enzyme inhibition, the extracts obtained from the aerial parts of the plant were found inactive against TYR enzyme, while root- dH_2O extracts of the plant were exerted 16.84 ± 0.08%, the lowest TYR enzyme inhibition at 800 µg ml^{-1}(Table 4).

4. Discussion

Some neurodegenerative mechanisms have been reported to cause pathogenesis of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), prion diseases, *etc*. Of which, AD and PD are most common types of neurological disorders that lead to loss of memory and behaviour skills in elderly people (Sekeroglu *et al.*, 2012; Gezici and Sekeroglu, 2019b). According to statistical data, there has been a great increase in AD and PD incidence worldwide (Harris, 2019). Nowadays, finding effective and safely therapy approaches are required to manage the high incidence of these diseases. From this point, the inhibition of key enzymes linked to the pathogenesis of the AD and PD diseases, used medicinal plant and plant-derived compounds are suggested as one of the most effective strategies. For example, inhibition of acetylcholinesterase (AChE), involved in the acetylcholine hydrolysis, is the most accepted treatment strategy in AD. On the other hand, butyrylcholinesterase (BChE) also has been considered to be involved in the pathogenesis of AD.

Furthermore, AD and PD can be seen at the same, as reported by previous researches. Therefore, the inhibition of tyhrosinase (TYR) alongside cholinesterases (ChE) is of great importance for the treatment of these diseases as multitarget drug approaches (Sekeroglu *et al*., 2012; Roleira *et al*., 2015; Liu *et al*., 2018; Gezici and Sekeroglu, 2019b). In our ongoing researches on finding natural enzyme inhibitors, neuroprotective potentials of AN extracts through AChE, BChE and TYR enzyme inhibition were aimed to screen, combined with total polyphenolic contents, free radical scavenging activities and ion reducing power capacities in the presented work.

However, phenolic and flavonoid contents of AN extracts were analysed for the first time in the presented research. Previous researches about the phytochemical compositions of different *Astragalus* species reported to have their rich phenolic and flavonoid contents in free and glycosidic forms. In regards of chemical compositions of *Astragalus* species, *A. membranaceus* and *A. mongholicus* (*A. membranaceus* var. *mongholicus*) are the best known species that isolated and purified the polysaccharides which could be also responsible for their high antioxidant activity. In fact, rich polyphenolic contents contribute to the radical scavenging activity and reducing power capacities of the extracts, as reported for different plants by previous studies (Tang and Eisenbrand, 1992; Rios and Waterman, 1997; Senol *et al*., 2018; Gezici *et al*., 2017; Sekeroglu *et al*., 2017).

According to the antioxidant results, AN extracts were found to have great antioxidant potentials, which were consistent with previous research conducted to investigate antioxidant potentials of different *Astragalus* species such as *A. membranaceus, A. mongholicus,* and *A. lentiginosus* (Li, 1991; Shimizu *et al*., 1991; Kang *et al*., 1993). These may be due to the fact that rich secondary metabolites components especially certain flavonoids, saponins, polysaccharides of the *Astragalus* species, as described previously (Rios and Waterman, 1997; Block and Mead, 2003). On this basis, *A. neurocarpus* with both aerial and root part could offer significant benefit for management of the oxidative-stress related diseases.

Although, previous studies focused on revealing the chemical compositions of some *Astragalus* species, a few researches have been performed to screen neuroprotective potentials of the extracts and bioactive compounds isolated from *Astragalus* species (Rios and Waterman, 1997; Chan *et al*., 2009; Gulcemal *et al*., 2013; Ionkova *et al*., 2014). On the other hand, this research is the first work that screened neuroprotective potentials of the extracts obtained from the different part of *A. neurocarpus.* In the light of the findings of these results, a significant correlation was not determined between the antioxidant capacities and neuroprotective potentials of the extracts.

5. Conclusion

In the presented work, enzyme inhibitory potentials of different parts of AN were analysed through inhibition of the enzymes associated with pathogenesis of neurodegenerative diseases. The results revealed from the work that the extracts of AN have considerable amount of antioxidant activity as well as anti-cholinesterase and anti-tyrosinase activity. There has been suggestion that the plant species, especially root part of its would have considerable as a potential source in the treatment of Alzheimer's and Parkinson diseases for their inhibitory activity on the AChE, BChE, and TYR enzymes, and also for its rich antioxidant capacity. This is the first research conducted to evaluate neuroprotective potentials of the extracts of AN, as well as polyphenolic content and antioxidant activity. The author suggest that further *in vivo* studies should be performed to ascertain neuroprotective potentials of the plant.

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Conflict of interest

The authors declare that no conflict of interest exists in the course of conducting this research. Both the authors had final decision regarding the manuscript and the decision to submit the findings for publication.

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