International Journal of Agriculture, Environment and Food Sciences

JAEFS

e-ISSN : 2618-5946

DOI: 10.31015/jaefs.2020.4.1

www.jaefs.com

Research Article

Int J Agric Environ Food Sci 4 (4): 394-399 (2020)

The antioxidant and antialzhemier activities of the *Diplotaenia turcica* with phytochemical analysis

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Abstract

Diplotaenia turcica is an endemic plant that grows in eastern Turkey. This herb is used in herbal cheese, in meals and in traditional therapies. In this study, we aimed to examine some of the biochemical activities of this plant. Liquid chromatography-mass spectrometry (LC-MS) analysis was conducted on hydro alcohol extract of aerial part of DT. This analysis was applied to determine the total phenolic and flavonoid content, antioxidant and anti-alzheimer activities. LC-MS analysis showed that malic acid and quinic acid were found to be major compounds. The key flavonoids detected were hesperidine and rutine. The end of examination, the total amount of phenolic compound of extract was measured as 27.54 µg PEs/mg. And the total flavonoid amount was measured as 7.31 µg KEs/mg. β-carotene-linoleic acid test, DPPH free radical scavenging method, and ABTS cation radical scavenging outcomes were determined as IC50 of 169.71 µg/mL, 164.42 and 68.74 µg/mL, respectively. Cholinesterase inhibition test which indicate of alzheimer's did not inhibit acetylcholinesterase (AChE), but butyrylcholinesterase (BCHE) enzyme inhibition was 76.57%. As a result, further studies are needed in order to use Diplotaenia turcica plant for treatment or support purposes in the health field.

Keywords: Antialzhemier, Antioxidant, Activities, *Diplotaenia turcica*

Introduction

Antioxidants reduce or eliminate the harmful effects of free radicals in metabolism. It is also used as a preservative in the food industry. The antioxidant properties of many plants that used as food are examined and their health effects are investigated (Meydan, 2019). In this respect, very little work has been done on *Diplotaenia turcica* plant. Earlier studies with the root part of *Diplotaenia turcica* plants was determined to be nontoxic and to have good antioxidant content (Özdek Yıldırım et al., 2020).

The most important feature of *Diplotaenia turcica* plant is that it can be used in herbs, in meals and in traditional treatments. *Diplotaenia turcica* plant is used for protection from the bites of snake and other poisonous animals, as well as the root part has been used by the public since ancient times as rheumatism, diabetes and blood pressure balancer (Kaval et al., 2014; Uce and Tunçtürk, 2014). *Diplotaenia turcica* is an endemic plant and a new plant species introduced to the world of science in 2011. It is known as "Siyabo" in the region (Özdek et al., 2018).

Oxidative stress is a condition in which the balance between oxidant formation and antioxidant defense is disturbed in favor of oxidants (Koçak et al., 2020). This balance shifts towards pro-oxidants due to either increased production of reactive oxygen species or a reduction in the amount of antioxidants (Yuksek et al., 2017).

The most sensitive molecule to reactive oxygen species is thought to be lipids, the main component of the cell membrane.

Cite this article as:
Ozdek, U. (2020). The antioxidant and antialzhemier activities of the Diplotaenia turcica with phytochemical analysis. Int. J. Agric. Environ. Food Sci., 4(4), 394-399
DOI: https://doi.org/10.31015/jaefs.2020.4.1
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Received: 24 July 2020 **Accepted:** 23 September 2020 **Published Online:** 11 November 2020
Year: 2020 **Volume:** 4 **Issue:** 4 (December) **Pages:** 394-399
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

A sufficient amount of a reactive agent in the living organism may initiate lipid peroxidation. The reactive agent forms a radical by breaking one of the hydrogens of the fatty acid. This radical, which comes to the forehead, breaks off the proton of one of the neighboring fatty acids and leads to the formation of a new radical. As a result of the ongoing reactions, the radical concentration in the medium increases and consequently the lipid peroxidation takes place (Nordberg and Arner, 2001).

The effect of proteins on free radical damage is mainly the formation of carbonyl groups in amino acids such as histidine, tyrosine, phenylalanine. As a result of fragmentation and cross-linking with protein oxidation, degradation of protein functions (catalysis, transport, receptor, etc.) and antigenic changes that can stimulate the immunity system can occur (Nordberg and Arner, 2001).

Antioxidants are molecules that generally inhibit the formation of free radicals, or sweep up existing radicals, and which generally have phenolic function in their structure (Kähkönen et al., 1999). Under normal physiological conditions, cells are protected by antioxidant defense systems against oxidative damage caused by free radical products and molecules such as peroxides. Antioxidants have a complex structure and act in two types of mechanisms. These are defined as direct antioxidants and indirect antioxidants. Direct antioxidants (such as glutathione, phenolic compounds, tocopherols, ascorbic acid, and carotenoids) take part in physiological, biochemical, or cellular processes to inactivate free radicals or prevent chemical reactions initiated by free radicals (Rice-Evans et al., 1997). Indirect antioxidants do not play a role in preventing free radical or redox reactions. They strengthen the antioxidant capacity of the cell. This is because a group of enzymes (glutathione transferase, quinone reductase, epoxide hydrolase) in the human body cause detoxification of electrophilic species (Papetti et al., 2006).

Antioxidants are divided into two groups as enzymatic antioxidants and non-enzymatic antioxidants. Enzymatic antioxidants Glutathione peroxidase, Glutathione-S-transferase, glutathione reductase, superoxide dismutase, peroxidase and catalase. Non-enzymatic antioxidants include glutathione, flavonoids, ascorbate (Vit.C), β-Carotene (Vit.A), α-Tocopherol (Vit.E), urea, bilirubin, melatonin, ceruloplasmin, transferin, ferritin, lactoferrin, albumin and lipoic acid (Scandalios, 2002).

In this study, phytochemical profile, determination of total phenolic and flavonoid contents, antioxidant and anticholinesterase activities of aerial part of *Diplotaenia turcica* were investigated.

Materials and Methods

Plant material

The aerial part of *Diplotaenia turcica* was collected from Hakkari in June. Identification of the plant was carried out by Mehmet Fırat (Herbarium no: *32858 VANF)*, Department of Biology of the Faculty of Education Van Yüzüncü Yıl University.

Preparation of the extract

The aerial part of plant was dried up and powdered by using an electrical mill. A 100 g powdered sample was added to 1000 ml of 96% ethanol. Initially, 96% ethanol was utilized and after 24 hours of time period, the solution was filtered. A mixture of 70% ethanol 30% water was added to the pulp obtained after filtration. After 24 hours, the solution was filtered and then both filtered solutions were mixed together and then evaporated repeatedly by rotary evaporator at 50 °C and 70 rpm. 5.7% w/w dry extract were obtained from Concentrated extract by lyophilizing and stored at -20 °C (Özdek Seçkin et al., 2020).

LC–MS/MS analysis

LC–MS/MS analyses of the phenolic compounds were performed by using a Nexera model Shimadzu UHPLC coupled to a tandem MS instrument. MS detection was performed using Shimadzu LC–MS 8040 model triple quadrupole mass spectrometer equipped with an ESI source operating in both positive and negative ionization modes. The liquid chromatograph was equipped with LC-30AD binary pumps, DGU-20A3R degasser, CTO-10ASvp column oven and SIL-30AC autosampler. The chromatographic separation was performed on a C18 reversed phase Inertsil ODS-4 (100 mm × 2.1 mm, 2 µm) analytical column. The column temperature was fixed at 35 °C. The elution gradient consisted of mobile phase A (water, 10 mM ammonium formate and 0.1% formic acid) and mobile phase B (acetonitrile). The gradient program with the following proportions of solvent B was applied t (min), 0-10 minutes %B (5-20), 10-22 minutes (20), 22-36 minutes (20-50), 36-40 minutes (95), 40-5- minutes (5). The solvent flow rate was maintained at 0.25 mL/min and injection volume was settled as 4 µL. Subsequent to several combinations of trials, a gradient of acetonitrile and water (10 mM ammonium formate and 0.1% formic acid) system was concluded to be the best mobile phase solution. For rich ionization and the separation of the molecules, the mentioned mobile phase was proved to be the best of all. ESI source was chosen instead of APCI (Atmospheric Pressure Chemical Ionization) and APPI (Atmospheric Pressure Photoionization) sources as the phenolic compounds were small and relatively polar molecules. Tandem mass spectrometry was used for the current study since this system is commonly used for its fragmented ion stability. The working conditions were as follows interface temperature; 350 °C, DL temperature; 250 °C, heat block temperature; 400 °C, nebulizing gas flow (Nitrogen); 3 L/min and drying gas flow (Nitrogen); 15 L/min.

Determination of total phenolic and flavonoid content

Phenolic content was determined using the Folin-Ciocalteu colorimetric method (Slinkard and Singleton, 1977) with some modifications. Phenolic amounts were expressed as micrograms pyrocatecol (µg PEs/mg extract) per milligram of sample, and were calculated according to the following equations.

Absorbance = 0.0351 pyrocatechol (µg) + 0.0466 (R^2: 0.9952)

Flavonoid content was determined according to the aluminum chloride method (Moreno et al., 2000) with some modifications. Quantities of flavonoid content in the extract were expressed as quercetin equivalents (QEs) in micrograms per milligram of sample (µg QEs/mg extract) and were calculated according to the following equations.

Absorbance = 0.0353 quercetin (µg) + 0.0477 (R^2: 0.9914)

Antioxidant activities

Antioxidant activities of *Diplotaenia turcica* plant were investigated using β-carotene-linoleic acid test (total antioxidant activity test) (Kosanić et al., 2012), DPPH free radical scavenging method (Kosanić Ranković, Dašić, 2012), ABTS cation radical scavenging method (Re et al., 1999) and copper II ion reducing method (CUPRAC) (Apak et al., 2004).

Enzyme inhibitory activities

A spectrophotometric method was used to demonstrate acetyl- and butyryl-cholinesterase inhibitor activity by the method developed by Ellman et al. (Boğa et al., 2011).

Statistical analysis

The mean of 3 parallel measurements obtained from the results of antioxidant and anticholinesterase activities assays were taken as ± SD (n=3). Significant differences between means were determined by student's-t test, p values <0.05 were regarded as significant.

Results and Discussion

It is known that about 13000 plant species are used worldwide for therapeutic purposes (Pattanayak et al., 2010). Plants protect the cells against natural oxidation reactions due to the antioxidant substances they contain (Kähkönen Hopia Vuorela Rauha Pihlaja Kujala, Heinonen, 1999).

DT is an endemic plant species growing in eastern Turkey Van-Bitlis-Hakkari (Özdek Yıldırım, Değer, 2020).

Phenolic compounds carry an aromatic hydroxyl nucleus. There are about 8000 different compounds in nature. Phenolic compounds found in plants are free radical terminators and are known as important antioxidants. Flavonoids are important phenolics. Flavonoids have more than 4000 species found in the roots, flowers and leaves of plants. (Ertaş et al., 2014). According to the LC-MS analysis results, hesperidin (27.71µg/g extract), p-coumaric acid (31.18 µg/g extract), gallic acid (238.86 µg/g extract), caffeic acid (7.68 µg/g extract), quinic acid (3505.57 µg/g extract), 4-OH-benzoic acid (40.83 µg/g extract), tr-ferulic acid (69.38 µg/g extract), chlorogenic acid (1011.51 µg/g extract), protocatechuic acid (27.26 µg/g extract), malic acid (15641.23 µg/g extract) and rutin (89.02 µg/g extract) molecules were detected (Table 1).

Table 1. Analytical parameters and results of LC-MS/MS analysis of the aerial part of *Diplotenia turcica* extract

No	Analytes	RT[a]	M-H$^+$ (m/z)[b]	Linearity Range (µg/L)	LOD/LOQ (µg/L)[c]	U[d]	Quantification (µg analyte / g extract)[e] *Diplotaenia turcica*
1	Hesperidin	20.118	610.90	25-1000	3.4/4.2	0.0262	27.71±0.007
2	p-Coumaric acid	15.675	162.95	25-1000	7.3/9.1	0.0516	31.18±0.016
3	Gallic acid	4.427	168.85	250-10000	95.5/106.9	0.0282	238.86±0.067
4	Caffeic acid	12.182	178.95	25-1000	18.4/22.4	0.0354	7.68±0.003
5	Quinic acid	1.27	190.95	250-10000	75.8/79.4	0.0082	3505.57±0.287
6	4-OH-benzoic acid	10.1	136.95	250-10000	33.2/38.1	0.0289	40.83±0.012
7	tr-Ferulic acid	17.113	192.95	250-10000	36.6/42.0	0.0494	69.38±0.034
8	Chlorogenic acid	10.189	353.15	25-1000	6.2/8.1	0.0069	1011.51±0.070
9	Protocatechuic acid	7.16	152.95	100-5000	28.2/31.4	0.0411	27.26±0.011
10	Malic acid	1.45	133.00	250-10000	55.3/67.5	0.0113	15641.23±1.767
11	Rutin	17.486	609.05	25-1000	5.5/6.5	0.0159	89.02±0.014

aRT: Retention time, bM-H+(m/z): Molecular ions of the standard compounds (mass to charge ratio), eLOD/LOQ (µg/L): Limit of detection/ Limit of quantification, dU (%): Percent relative uncertainty at 95% confidence level (k=2), eValues in µg/g (w/w) of plant extract

The total amount of phenolic compounds (27.54 µg PEs/ mg extract) and total flavonoid amounts of DT were found to be low (7.31 µg QEs/mg extract) (Table 2). In our study, β-carotene-linoleic acid test, DPPH free radical scavenging and ABTS cation radical scavenging activities results were determined as 169.71 µg/mL, 164.42 µg/mL and 68.74 µg/mL in terms of IC_{50}, respectively (Table 3).

Table 2. Total phenolic and flavonoid amounts of extract of Diplotaenia turcica aerial part (DT)[a]

	Phenolic content (µg PEs/mg extract)[b]	Flavonoid content (µgQEs/mg extract)[c]
DT	27.54 ± 1.87	7.31 ± 0.60

a: Values expressed are means ± standard deviation of three parallel measurements (p<0.05)
b: PEs, pyrocatechol equivalents (y=0.0351x + 0.046 R^2=0.9952)
c: QEs, quercetin equivalents (y=0.0353x + 0.0477 R^2=0.9914)

Table 3. Antioxidant activities results of *Diplotaenia turcica* aerial part extract (DT), BHT, BHA and α-TOC [a]

	IC_{50} values (µg/mL)		
Samples	β-Carotene-Linoleic acid	DPPH Free Radical	ABTS Cation Radical
DT	169.71±2.56	164.42±3.02	68.74±0.94
BHA	1.5±0.01	7.88±0.20	17.59±0.10
α-TOC	2.1±0.10	16.30±0.79	9.74±0.42
BHT	1.3±0.03	58.86±0.50	13.25±0.27

a: Values expressed are means ± standard deviation of three parallel measurements (p<0.05)

In the study, β-carotene-linoleic acid test, DPPH free radical scavenging and ABTS cation radical scavenging activities results can be said to be moderate active. On the contrary, in the CUPRAC results were found to be low in all concentrations compared to the same standards (Table 4).

Table 4. CUPRAC results of *Diplotaenia turcica* aerial part extract (DT), BHT, BHA and α-TOC [a]

	10 µg/mL	25 µg/mL	50 µg/mL	100µg/mL
DT	0.106±0.015	0.153±0.006	0.216±0.023	0.384±0.042
BHT	0.605±0.086	1.344±0.035	2.256±0.042	3.987±0.007
BHA	0.205±0.014	0.365±0.027	0.616±0.029	1.171±0.110
α-TOC	0.305±0.023	0.746±0.057	1.528±0.068	2.551±0.066

a: Values expressed are means ± standard deviation of three parallel measurements (p<0.05)

In antioxidant activity studies, it is stated that it is necessary to use different methods because the reaction conditions such as pH, temperature, working sensitivity and solvent affect the results (Frankel et al., 1994; Koleva et al., 2002). These different results may be due to them.

The *Diplotaenia turcica* extract used in the study was prepared using ethanol-water as a non-toxic solvent. The vegetal samples exhibit structural differences in their content and therefore different solvents may be used for each sample in extraction methods (Boğa Hacıbekiroğlu, Kolak, 2011). In other studies, the most suitable solvent for the plant can be selected by working with different solvents. Thus, accurate and high results can be obtained about the antioxidant capacity of plants.

In recent years, Alzheimer's disease has increased significantly. Researching new and useful strategies for the treatment of Alzheimer's disease is one of the most important issues. The enzyme inhibition method has been one of the research subjects. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes are enzymes that play an important role in Alzheimer's disease. It is known that this disease increases as acetylcholine deficiency increases. Thus, Alzheimer's disease has been associated with these enzymes. Therefore, inhibition of acetylcholinesterase and butyrylcholinesterase enzymes is thought to have a positive effect on the course of the disease (Raskind et al., 2004).

In the study, the effect of DT on inhibition of BChE enzyme was 76.57% as inhibition, while the inhibition value of galantamine used as standard was 84.3. It was observed that DT did not have AChE enzyme inhibition effect (Table 5).

Table 5. Anticholinesterase activity results of *Diplotaenia turcica* aerial part extract (DT) [a]

Samples	AChE(%inhibition)	BChE (%inhibition)
DT	N.A.	76.57±0.67
Galantamine[b]	84.04±1.13	84.30±0.99

Values expressed are means ± standard deviation of three parallel measurements (p<0.05).
[a] 200µg/mL
[b] Standard substance.
N.A.: Not active.

Conclusion

The present study shows that the total phenolic content is more than flavonoid content of the *Diplotaenia turcica* aerail part extract and has moderate antioxidant properties and strong anti-butyrylcholinesterase activity. Phytochemical studies are required to characterize the active components of the *Diplotaenia turcica*. To better understand the antioxidant and anticholinesterase potential, more laboratory and clinical trials of the active compounds found in the *Diplotaenia turcica* plant extract are required.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

I would like to thank Mr. Mehmet Fırat for taxonomic examination of the plant and Dr. Mehmet Boğa for his support.

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