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ANTIOXIDANT, ANTI-UREASE AND ANTI-ELASTASE ACTIVITIES OF *USNEA LONGISSIMA* ACH.

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Abstract

Lichens are complex associations composed of mycobiont and one or more algae or cyanobacteria which are living in symbiosis. Lichens are thought to possess therapeutic effects on many illnesses in worldwide. This study was performed to investigate antioxidant, anti-elastase and anti-urease activities of *Usnea longissima* Ach lichen. *U. longissima* was extracted with ethanol and ethyl acetate solvents. The antioxidant activities of the extracts of *U. longissima* were determined with methods such as 1,1-diphenyl-2-picrylhydrazyl **(**DPPH) radical scavenging activity, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging activity, copper reducing antioxidant capacity (CUPRAC), total antioxidant capacity, determination of total phenolic content and total flavonoid contents. The binding action of the DPPH and ABTS radicals, CUPRAC activity increases with concentration of the extracts. Ethanol extracts exhibited higher anti-urease and anti-elastase activity. Highest inhibition was found as 49.86% for elastase and 18.38% for urease. Results of the present study suggest that *U. longissima* extracts can be an alternative to synthetic antioxidant, anti-elastase and anti-urease agents.

Introduction

Oxidative stress is a process amongst protective systems and the generation of free radicals. The excess of reactive species might lead damages in cell lipids, proteins and DNA (Habib and Ibrahim 2011). They also cause many illnesses like neurodegenerative disorders, autoimmune pathologies, cataracts, atherosclerozis and digestive system disorders for example gastrointestinal inflammation (Assia *et al.* 2016).

Elastases are a group of serine proteases that have the capacity to cleave the elastin, which is abundant in the lung, arteries, skin, and ligaments. Anti-elastase action could be performed as a useful aim to protect against skin aging. The urease enzyme catalyzes the hydrolysis of urea into ammonia and carbon dioxide. Urease enzyme is associated with hepatic encephalopathy, hepatic coma urolithiasis, pyelonephritis, ammonia and urinary catheter encrustation (Sökmen *et al*. 2012). Lately, urease inhibitors have crucial role in the healing of the infectious diseases produced by urease producing bacteria (Tanoli *et al*. 2014)

Lichens contain unique and biologically active substances (Ramya and Thiyarajan 2017) which contribute in antimicrobial, antioxidant, antiviral, cytotoxic and enzyme inhibitory activities (Karagöz *et al.* 2009, Ramya and Thirunalasundari 2013). Different activities of lichen metabolites represent the therapeutic capacity of lichens that are attracted by pharmaceutical industries (Ramya and Thiyarajan 2017).

Usnea longissima is a fruticose lichen species which possess many therapeutic features. Early Chinese herbalists suggested *U. longissima* as an expectorant and a powder. *Usnea* species applied to cure external ulcers and is still utilized today for treating tuberculosis lymphadenitis. Former phytochemical research on *U. longissima*, which focused on the isolation

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of lichen acids, revealed analgesic, nematocidal, antipyretic, anticholesterol and antiinflammatory activities (Choudhary *et al.* 2005). *U. longissima* also plays a protective role in indomethacin-induced ulcers (Halıcı *et al*. 2005) and is used as plant growth inhibitor (Nishitoba *et al*. 1987).

The objectives of this research were to determine antioxidant, anti-elastase and anti-urease activities of the extracts of *U. longissima*.

Materials and Methods

Usnea longissima lichen was collected in 2014 from Dereli county (Giresun, Turkey) and identified by following Brodo *et al*. (2001). The specimen of *U. longissima* (Herbarium No: 6393) is kept at the herbarium of the Biology Department, Faculty of Science and Arts, Giresun University, Giresun, Turkey.

Powdered lichen (25 g) was extracted in Soxhlet apparatus for 7 hrs utilizing 250 ml, ethanol and ethyl acetate as solvents. The extracts acquired from ethyl acetate or ethanol were filtered through Whatman filter paper No. 1 and residues were evaporated (40°C) with rotary evaporator. The extraction yields of different extracts were determined (Kumar *et al.* 2012).

Antioxidant activity

Total phenolic contents of lichen extracts were identified accordingly with the procedure of Slinkard and Singleton (1977). The quantity of the total phenolic compounds was denoted as µg gallic acid equivalent (GAE)/g lichen.

Total flavonoid content of the extracts were performed by the method of Zhishen *et al.* (1999). The amount of total flavonoid compounds was calculated as µg cateschin (QE)/g lichen by using an equation that has been obtained from the rutin calibration curve.

Total antioxidant capacity of the extracts was estimated by phosphomolybdenum test (Prieto *et al.* 1999). Antioxidant capacity was clarified as µg ascorbic acid equivalent (AAE)/g lichen.

CUPRAC test was employed by the method of Özyürek *et al.* (2009). Butylated hydroxytoluene (BHT) was performed as standard antioxidant agent.

DPPH radical scavenging activity of *U. longissima* extracts was established by the method of Brand-Williams *et al.* (1995). ABTS radical scavenging assay was carried out by the method of Arnao *et al*. (2001). BHT and rutin used as standard antioxidant agents.

The elastase inhibitory potential of the lichen extracts was examined utilizing N-succinyl-Ala-Ala-Ala-p-nitroanilide (STANA) as a substrate (Moon *et al.* 2010). Urease inhibitory activity was determined according to Van Slyke and Archibald (1944). Ursolic acid was used as standard urease inhibitor agent.

DPPH radical scavenging activity, ABTS radical scavenging activity, anti-urease and anti-elastase activities were calculated using the following equation:

$\%\ \text{inhibition} = (A_0 - A_1 / A_0) \times 100$

A_0 is the absorbance of the control

A_1 is the absorbance of the sample

For all antioxidant, anti-elastase and anti-urease tests, three replicates of each extract were used and the values are expressed as Mean ± Sd.

Results and Discussion

Extraction yield of *Usnea longissima* lichen was determined as 12.44% in ethanol solvent and 4.44% for ethyl acetate solvent.

DPPH binding activity is the most frequently exploited method in antioxidant activity evaluation. It is demonstrated that phenolic compounds usually display remarkable scavenging effects towards the DPPH free radical.

ABTS assay is practible for both hydrophilic and lipophilic antioxidants. Both DPPH and ABTS methods are substrate-free. Popularity of these tests might be arised from simplicity and speed of analysis (Kazazic *et al.* 2016).

The mixed impact of phenolic compounds, flavonoids and other reducing agents in the plant extracts are well expressed by total antioxidant capacity. The posphomolybdenum method is based on the reduction of molybdenum, Mo (VI) to Mo (V) by the affect of antioxidant constituents and the creation of a green phosphate (Hossain *et al.* 2017).

CUPRAC activity, DPPH and ABTS radicals scavenging activities of the extracts of *U. longissima* were presented in Table 1. Ethanol extract of *U. longissima* had higher CUPRAC activity, DPPH and ABTS radicals scavenging activities compared with ethyl acetate extract of *U. longissima*. CUPRAC activity, DPPH and ABTS radicals scavenging activities are less affective than the commercial available synthetic like BHT and rutin. It can be said that the scavenging effects on the DPPH and ABTS radicals, CUPRAC activity strongly dependent on the extract or standard concentration.

Table 1. Radical scavenging and CUPRAC activities of the lichen extracts.

Lichen extracts and standards	Conc. (µg/ml)	DPPH radical scavenging activity (%)	Conc. (µg/ml)	ABTS radical scavenging activity (%)	Conc. (µg/ml)	CUPRAC activity (nm)
Ethanol extract of *Usnea longissima*	1000	14.60 ± 0.017	250	17.08 ± 0.004	100	0.243 ± 0.019
	2000	24.56 ± 0.015	500	35.31 ± 0.012	200	0.387 ± 0.007
	3000	26.38 ± 0.016	750	51.31 ± 0.011	300	0.546 ± 0.009
	4000	33.83 ± 0.015	1000	63.77 ± 0.040	400	0.678 ± 0.036
	5000	37.56 ± 0.008	2000	67.78 ± 0.003		
			3000	71.68 ± 0.011		
			4000	73.31 ± 0.007		
Ethyl acetate extract of *U. longissima*	1000	9.01 ± 0.018	250	NA	100	0.244 ± 0.005
	2000	11.66 ± 0.017	500	NA	200	0.346 ± 0.027
	3000	20.33 ± 0.022	750	NA	300	0.452 ± 0.009
	4000	26.31 ± 0.036	1000	NA	400	0.534 ± 0.041
	5000	33.30 ± 0.03	2000	21.20 ± 0.004		
			3000	42.19 ± 0.005		
			4000	54.92 ± 0.010		
BHT	250	72.76 ± 0.011	250	92.56 ± 0.005	100	0.667 ± 0.014
	500	73.49 ± 0.001	500	94.04 ± 0.009	200	0.702 ± 0.004
	750	77.87 ± 0.006	750	96.24 ± 0.015	300	0.714 ± 0.026
	1000	87.45 ± 0.012	1000	95.68 ± 0.006	400	0.858 ± 0.046
Rutin	250	51.69 ± 0.025	250	92.36 ± 0.007	-	-
	500	61.82 ± 0.043	500	94.40 ± 0.010	-	-
	750	75.17 ± 0.021	750	94.60 ± 0.008	-	-
	1000	88.57 ± 0.010	1000	95.56 ± 0.004	-	-

NA: No activity.

As seen in Table 2, high amounts of total phenolic and flavonoid contents were observed in the ethanol extract of *U. longissima* followed by ethyl acetate extract of *U. longissima.* Similarly, ethanol extract had higher total anioxidant capacity as compared with ethyl acetate extract.

Many studies have been conducted about antioxidant activity of *U. longissima* lichen. For example, Atalay *et al.* (2011) revealed that different fractions which obtained from *U. longissima* had lipid peroxidation inhibition activity and DPPH radical scavenging activity at different levels. Likewise, DPPH radical scavenging activity of ethanol and ethyl acetate extracts of *U. longissima* were determined.

Table 2. Total phenolic content, total flavonoid content and total antioxidant capacity of the extracts.

Lichen	Total phenolic content (µg GAE/g lichen)	Total flavonoid content (µg QE/g lichen)	Total antioxidant capacity (µg AAE/g lichen)
Ethanol extract of *Usnea longissima*	3.55 ± 0.008	20.85 ± 0.005	4.63 ± 0.020
Ethyl acetate extract of *U. longissima*	1.183 ± 0.005	6.03 ± 0.016	1.44 ± 0.004

Odabaşoğlu *et al.* (2004) investigated antioxidant capacity, total phenolic content and reducing power of methanol and water extracts of *U. longissima* which was collected from Giresun. It was concluded that methanol extract had antioxidant activity. In aggrement with this study, antioxidant activity in extracts of *U. longissima* was also found.

Sinha (2013) found that total phenolic content was 115 ± 0.58 mg GAE/g dry lichen and the total flavonoid content was 1.625 ± 0.001 mg rutin equivalent/g dry lichen of ethanol extract of *U. longissima* which was collected from India. Also, ethanol extract showed high DPPH radical scavenging activity. Higher activity in ethanol extract of *U. longissima* was observed in the present study.

Verma *et al.* (2017) also revealed DPPH scavenging activity of ethanol extract of *U. longissima* lichen. IC_{50} value of DPPH assay for ascorbic acid and *U. longissima* extract was found as 340.33 ± 33 µg/ml and 2.63 ± 0.06 µg/ml, respectively. Verma *et al.* (2017) found higher DPPH scavenging activity than standard (ascorbic acid). In the present study, DPPH radical scavenging activity of ethanolic extract of *U. longissima* was 37.86% at 5 mg/ml concentration and the extract exhibited weak activity when compared with standard antioxidant. This difference may be related with collecting lichen sample from different locations.

Owing to structural diversity, the antioxidant characteristics vary among the plants. Different antioxidant activity results can be linked to climate, plant species, testing methods and solvents used by the explorers (Kazazic *et al.* 2016).

Skin firmness and elasticity are caused by elastin. Slowing the metabolism of the connective tissue proteins and increasing elastase activity breaks down elastin. An alternative to hinder loss of elasticity is to utilize active ingredients which have ability to inhibit these enzyme (Mathen *et al.* 2014).

Urease is the major protein component of *Helicobacter pylori*. Urease hydrolyzes urea, dismissing ammonia, which neutralizes acids of stomach and thus facilitates colonization of *H. pylori*. Moreover, urease serves as a virulence factor in human and animal illnesses of gastrointestinal and urinary tracts (Ghous *et al.* 2010).

Anti-elastase and anti-urease activities of the extracts are presented in Table 3. Ethanol and ethyl acetate extracts of *U. longissima* were found to be active between 0.001 and 0.000001 µg/ml concentations. Ethanol extracts exhibited higher anti-urease and anti-elastase activities. Highest inhibition was found as 49.86% for elastase and 18.38% for urease.

To the best of authors knowledge, this is the first report related to anti-elastase and anti-urease activities of *U. longissima* lichen.

Table 3. Anti-elastase and anti-urease activities of the extracts and standards.

Lichen	Concentration (µg/ml)	Anti-urease activity (%)	Concentration (µg/ml)	Anti-elastase activity (%)
Ethanol extract of *Usnea longissima*	0.000001	10.42 ± 1.85	0.000001	26.34 ± 1.44
	0.00001	13.86 ± 0.39	0.00001	29.64 ± 0.26
	0.0001	15.89 ± 0.96	0.0001	35.93 ± 1.17
	0.001	18.38 ± 1.59	0.001	40.39 ± 0.43
			0.01	49.86 ± 1.03
Ethyl acetate extract of *U. longissima*	0.000001	6.76 ± 0.54	0.000001	14.42 ± 0.67
	0.00001	10.73 ± 0.55	0.00001	15.15 ± 0.021
	0.0001	12.99 ± 0.82	0.0001	25.69 ± 0.29
	0.001	14.66 ± 0.70	0.001	30.99 ± 0.67
			0.01	32.55 ± 0.48
Thiourea	0.000001	28.71 ± 1.36	-	-
	0.00001	32.24 ± 0.92		
	0.0001	36.25 ± 0.04		
	0.001	38.65 ± 1.37		
Ursolic acid	-	-	0.000001	18.45 ± 1.10
			0.00001	23.25 ± 1.20
			0.0001	24.12 ± 0.60
			0.001	27.61 ± 2.02
			0.01	30.02 ± 1.19

All these surveys show the encouraging potential of *U. longissima* for the discovery of brand anti-elastase and anti-urease agents. As the plant derived extracts are safe and have no toxicity when compared with synthetic antioxidants such as BHT, these extracts could be exploited as antioxidant. *U. longissima* lichen can be utilized also in pharmacy and cosmetic industries because of their superior anti-elastase, anti-urease and antioxidant activities.

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