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IN VITRO DETERMINATION OF THE SKIN ANTI-AGING POTENTIAL OF *CENTAUREA URVILLEI* DC. SUBSP. *ARMATA* WAGENITZ

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Abstract

This study investigated the potential of the ethanolic extract of *Centaurea urvillei* DC. subsp. *armata* WAGENITZ for use in dermatological treatments. This evaluation involved the assessment of its sun protection factor (SPF), inhibitory effects on extracellular matrix-degrading enzymes, antioxidant activity, as well as genotoxic and antigenotoxic properties. The results demonstrated that the extract exhibited a significant SPF value (11.755) and inhibited collagenase, elastase, and hyaluronidase enzymes. Additionally, the extract revealed significant antioxidant activity, as evidenced by its prominent DPPH (IC_{50} = 2.35 mg/ml) and β-carotene/linoleic acid (IC_{50} = 0.802 mg/ml) inhibitory effects. Furthermore, genotoxicity tests revealed no harmful effects, while antigenotoxicity assessments indicated its potential to protect against DNA damage. These findings underscore the potential of *C. urvillei* subsp. *armata* as a promising bioactive plant for natural skin anti-aging cosmetic formulations.

Introduction

The genus *Centaurea* L., a member of the Asteraceae family, is a highly diverse and economically significant group, comprising over 500 species primarily distributed across the Mediterranean region and West Asia (Koca *et al.*, 2009). Turkey is considered the main center of diversity for *Centaurea* species, hosting around 220 taxa, approximately 60% of which are endemic, making it one of the country's most endemic-rich genera (Davis 1965, Salachna *et al.* 2021). The use of *Centaurea* species in traditional medicine has been documented for their applications in treating inflammatory conditions, promoting wound healing, and addressing dermatological concerns (Koca *et al.* 2009, Salachna *et al.* 2021). Phytochemically, it contains a diverse range of bioactive compounds, including flavonoids, sesquiterpenes, and phenolic compounds, which contribute to their anti-inflammatory, antidiabetic, antimicrobial, and antioxidant activities (Koca *et al.* 2009, Salachna *et al.* 2021, Yırtıcı *et al.* 2022, Kahraman *et al.* 2025). Despite these promising bioactivities, the genus remains underexplored in terms of its full therapeutic applications. Given their diverse bioactive profile, *Centaurea* species may hold potential for dermatological and skincare applications, particularly in anti-aging formulations (Malinowska and Kiewlicz 2017).

Centaurea urvillei subsp. *armata*, a Turkish endemic perennial species, is indigenous to the Middle, Eastern Anatolian, and Mediterranean regions of Turkey (Wagenitz 1975). To our knowledge, there are no studies yet that have assessed the sun protection factor (SPF) or the inhibitory effects of this plant on elastase, hyaluronidase, and collagenase. Additionally, its genotoxic and antigenotoxic properties have not been explored. The goal of this study is to

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determined the potential of *C. urvillei* subsp. *armata* in promoting anti-aging processes. The present study evaluated the SPF properties of the plant, its inhibitory effects on elastase, collagenase, and hyaluronidase, along with its antioxidant and genotoxic/antigenotoxic activities.

Materials and Methods

Centaurea urvillei subsp. *armata* samples were collected during the flowering period from wild populations in Turkey, Adana (N 37.4°, W 35.1°). The plant was taxonomically identified (Voucher Specimen: ARB-A027) by Dr. Murat Ekici from the Biology Department at Gazi University, Turkey. The plant material was air-dried and extracted using an ultrasonic-assisted ethanol extraction method (45 g/500 ml) at 30°C for 30 min. The extract was then filtered, evaporated in a rotary evaporator to remove all traces of ethanol, and the final product was stored at 4°C in the dark for further analysis.

Sun protection factor (SPF) was determined using a UV-Visible spectrophotometer by measuring the absorbance of 50% hydroalcoholic dilutions (100 and 250 µg/ml) of the extract in the 290–320 nm range. The values of the SPF were calculated on the basis of the erythema effect, sun intensity, and sample absorbance (Saraf and Kaur 2010).

The DPPH scavenging ability was determined by mixing the sample with a DPPH solution and measuring the resulting change in color at 517 nm after 30 min (Ebrahimabadi *et al.*, 2010). The total antioxidant capacity was measured using the β-carotene/linoleic acid method, which involved evaluating the samples at 510 nm after an incubation period (Rauter *et al.*, 2012). Finally, the total phenolic content (TPC) was quantified by using the Folin–Ciocâlteu reagent and presented as mg of gallic acid equivalents per gram of dry weight of extract (mg GAE/g extract) (Singleton *et al.* 1999).

The enzyme inhibitory activities for collagenase (Barrantes and Guinea 2003), elastase, and hyaluronidase (Lee *et al.* 1999) were determined spectrophotometrically. Tannic acid and EGCG were utilized as the standard control compounds, while 5% (v/v) DMSO was employed as the negative control. The inhibition percentage was calculated following the method described by Boran *et al.* (2018).

The genotoxicity and antigenotoxicity of the extract were determined using the *Salmonella typhimurium*/microsome assay with TA98 and TA100 strains. The mutagenic potential was determined by counting His^+ colonies after incubation. To assess antigenotoxicity, the extract was pre-incubated with mutagens (4-NPD for TA98 and sodium azide (NaN_3) for TA100), and the revertant colonies were then compared to the positive control to evaluate its anti-mutagenic effects (Maron and Ames, 1983). The inhibition percentage was calculated following the method described by Boran *et al.* (2018).

The results were averaged and expressed with standard deviations. Statistical significance was assessed using one-way ANOVA, followed by Tukey's test, with a significance threshold set at $p < 0.005$.

Results and Discussion

The SPF of *C. urvillei* subsp. *armata* extract was measured using the UV absorbance method, with measurements recorded at 5 nm intervals from 290 to 320 nm. The extract demonstrated minimal protection at 100 µg/ml (SPF of 2.8) and maximum protection at 250 µg/ml (SPF of 11.755), suggesting that higher concentrations provide enhanced UV protection. Despite the paucity of research in this area, with only one study on the SPF of *Centaurea* species is available, Bensaid *et al.* (2024) reported an SPF of 40.20 for *C. acaulis* at 2 mg/ml. The lower SPF values

observed for *C. urvillei* subsp. *armata* could be due to differences in chemical composition, concentration, or the types of active compounds present in the extract.

The ethanol extract of *C. urvillei* subsp. *armata* demonstrated significant antioxidant activity, with IC_{50} values of 2.35 mg/ml in the DPPH• assay and 0.802 mg/ml in the β-carotene/linoleic acid assay (Table 1). The findings demonstrate the ability of the extract to neutralise free radicals and inhibit lipid peroxidation.

Table 1. Antioxidant activity and total phenol content of *Centaurea urvillei* subsp. *armata.*

Samples	Test Systems		
	DPPH scavenging activity[a] IC_{50} (mg/ml)	β-carotene/linoleic acid test activity[a] IC_{50} (mg/ml)	Total phenol[a] (mg GAE/g extract)
C. urvillei subsp. *armata*	2.35 ± 0.41	0.802 ± 0.81	26.85 ± 0.01
α-tocopherol	0.020 ± 0.10		
Ascorbic acid	0.031 ± 0.27	0.034 ± 0.27	

[a]The IC_{50} values are expressed as means ± standard deviation based on three parallel measurements ($p < 0.05$).

In comparison with previous studies, the antioxidant capacity observed in this study is moderate. As reported by Shoeb *et al*. (2007), a much weaker DPPH• scavenging effect (IC_{50}: 51.6 mg/ml) was observed for the same subspecies. In contrast, Taştan *et al*. (2022) found a significantly stronger effect for *C. urvillei* subsp. *hayekiana* (IC_{50}: 17.18 µg/ml). As reported by Kahraman *et al*. (2025), the DPPH scavenging rates ranged from 28.3 to 63.4% at a concentration of 1 mg/ml, with the performance of methanol extracts demonstrating the highest efficacy across a series of DPPH, ABTS, and CUPRAC assays. In addition, Ayaz *et al*. (2017) evaluated *C. urvillei* subsp. *stepposa* methanol extracts from two different regions and reported DPPH values of 144.6 and 118.17 µmol TE/100 g dw, FRAP values of 86.32 and 54.81 µmol TE/100 g dw, and CUPRAC values of 94.38 and 61.94 µmol TE/100 g dw. As posited by Zengin *et al*. (2011), the *C. urvillei* subsp. *hayekiana* methanol extract demonstrated moderate activity, exhibiting a total antioxidant capacity of 39.70 mg AE/g and a free radical scavenging capacity of 137.06 µg/ml. These variations can be attributed to differences in subspecies, extraction solvents, methods, and ecological conditions.

The total phenolic content (TPC) of the ethanolic extract in this study was measured as 26.85 mg GAE/g. This is lower than the values reported by Kahraman *et al*. (2025), who found TPCs of 71.0–90.1 mg GAE/g for hydroalcoholic and methanol extracts, and also lower than the values reported for *C. urvillei* subsp. *stepposa* (761.25 and 597.14 mg GAE/100 g dw) by Yırtıcı *et al*. (2022). Zengin *et al*. (2011) found a TPC of 17.12 mg GAE/g for *C. urvillei* subsp. *hayekiana*, suggesting that while the phenolic content in this study is not among the highest, it falls within the reported range for the genus.

It is noteworthy that phenolic compounds play a pivotal role in the enhancement of antioxidant activity. This observation is corroborated by the moderate TPC, which is consistent with the antioxidant performance observed. Collectively, these findings lend support to the potential of *C. urvillei* subsp. *armata* as a moderate antioxidant agent, which may be beneficial in applications aimed at reducing oxidative stress-related skin damage.

To the best of our knowledge, this is the first study to investigate the extracellular matrix-degrading enzyme inhibition potential of *Centaurea urvillei* subsp. *armata*, which exhibited 17.14, 16.61, and 10.89% inhibition of collagenase, elastase, and hyaluronidase, respectively, at 1 mg/ml, respectively (Table 2). These enzymes play key roles in the degradation of collagen, elastin, and hyaluronic acid, all of which contribute to wrinkle formation and are involved in skin aging, a process that accelerates wrinkle formation, as previously demonstrated (Fibrich and Lall 2018).

Table 2. Extracellular matrix-degrading enzyme inhibition of *Centaurea urvillei* subsp. *armata.*

		Inhibition (%) ± S.E.M.		
Samples	Concentrations	Collagenase	Elastase	Hyaluronidase
C. urvillei subsp. *armata*	1 mg/ml	17.14 ± 0.22[a]	16.61 ± 0.11[a]	10.89 ± 0.01[a]
Tannic acid	100 µg/ml			30.8 ± 0.13 [a]
EGCG	100 µg/ml	20.4 ± 0.03 [a]	41 ± 0.01 [a]	

[a] The data are presented as means ± standard deviation of three parallel measurements ($p < 0.05$).

The Ames test, performed using *S. typhimurium* strains TA98 and TA100, demonstrated that the extract did not induce base substitution mutations in the TA100 strain or frameshift mutations in the TA98 strain. The results of this study indicate that the extract does not demonstrate genotoxic potential under the conditions that were examined.

Furthermore, the extract demonstrated moderate antimutagenic properties, exhibiting inhibition rates ranging from 21.81 to 29.45% against 4-NPD-induced mutagenicity and from 26.43 to 35.84% against NaN_3-induced mutagenicity, at concentrations ranging from 0.01 to 1 mg/plate (Table 3). These findings are consistent with the role of antigenotoxic compounds in reducing DNA damage, which is crucial for preventing aging and photocarcinogenesis.

Table 3. Antigenotoxic activity of *Centaurea urvillei* subsp. *Armata.*

		Number of revertant colonies			
Samples	Concentration	TA98		TA100	
		Mean ± S. error	Inhibition (%)	Mean ± S. error	Inhibition (%)
Negative control		19 ± 1.1[a]		104 ± 12.6[a]	
4-NPD*	3.0 µg/plate	294 ± 19.26			
NaN_3*	8.0 µg/plate			1337 ± 37.74	
C. urvillei subsp. *armata*	1.0 mg/plate	213 ± 29.3[b]	29.45	895 ± 35.5	35.84
	0.1 mg/plate	234 ± 34.2	21.81	907 ± 32.1	26.43
	0.01 mg/plate	224 ± 31.7	25.45	1008 ± 26.8	26.68
	0.001 mg/plate	260 ± 21.3	12.36	1008 ± 32.31	26.68

[a] The data are presented as means ± standard deviation from triplicate plates in two separate experiments ($p < 0.05$). *4-NPD served as a positive control for the TA98 strain, while NaN_3 was used as a positive control for the TA100 strain.

To date, the genotoxic and antigenotoxic effects of *C. urvillei* have not been investigated, and only one study addressing these effects exists for the genus *Centaurea*. Uysal *et al*. (2016) conducted a study to evaluate the genotoxic and antigenotoxic effects of *C. pterocaula* extracts

(ethyl acetate, methanol, and aqueous) using the Ames test. The extracts did not demonstrate mutagenic properties in the *S. typhimurium* TA98 and TA100 strains. The aqueous extract exhibited the most pronounced antimutagenic activity against both 4-NPD and NaN_3, with inhibition rates reaching up to 80%. The extracts of methanol and ethyl acetate exhibited variable degrees of activity, ranging from moderate to strong, depending on the concentration employed and the specific mutagen evaluated.

Centaurea urvillei subsp. *armata* exhibits strong potential as a natural ingredient for skin protection and anti-aging applications. The extract offers UV protection, antioxidant activity, and enzyme inhibition associated with skin aging, along with antigenotoxic effects, making it a promising candidate for incorporation into cosmetic formulations.

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