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Research Article

Evaluation of potential anti-aging effects of *Achillea phrygia* Boiss. & Balansa (Asteraceae)

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Abstract: This study aims to determine the anti-aging effects of *Achillea phrygia*, an endemic plant, by evaluating its sun protection factor (SPF) level, antioxidant activity, total phenolic content, extracellular matrix-degrading enzymes (ECM) inhibition, genotoxic/anti-genotoxic, and cytotoxic activities. The SPF level was assessed using an *in vitro* quantitative method, while antioxidant capacity was determined through DPPH, β-carotene, and hydroxyl-radical (H_2O_2) scavenging assays. The total phenolic content was quantitatively conducted using the Folin Ciocalteu reagent. The inhibition of ECM-degrading enzymes was determined using matrix metalloproteinase-1 (MMP-1), hyaluronidase, and elastase enzymes. Genotoxic/anti-genotoxic properties were assessed using the AMES *Salmonella*/microsome assay, and cytotoxicity effects were assessed through the MTT assay. The results indicated that *A. phrygia* showed moderate SPF activity (SPF = 4.013) and exhibited IC_{50} values of 0.183 ± 0.03, 0.079 ± 0.51, and 1.18 ± 0.35 mg/mL for DPPH, β-carotene, and hydroxyl-radicals, respectively. The total phenolic content was measured to be 23.56 ± 1.42 mg GAE/g dry extract. Furthermore, the extract demonstrated inhibition of MMP-1 (47.98%) and elastase (39.2%) activities. Importantly, it did not induce DNA damage and showed antigenotoxic activity ranging from 10% to 65.6%. The cytotoxicity assay revealed an IC_{50} value of 42.41±4.05 µg/mL. These findings suggest that *A. phrygia* could be utilized as a cosmetic ingredient in skincare products due to its ability to protect against UV radiation, exhibit antioxidant properties, prevent extracellular matrix degradation, and inhibit DNA damage.

1. INTRODUCTION

Skin aging is a time-dependent, quite complicated, and natural process, and constitutes one of the important problems for both women and men. It is influenced by various internal and external factors, leading to structural and physiological changes in each layer of the skin. Internal aging progresses gradually, involving the production of reactive oxygen species (ROS) through oxidative metabolism (Lephart, 2016). ROS-induced oxidative stress is a key

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contributor to skin proteins, lipids, and DNA damage, contributing directly to skin aging (Pientaweeratch *et al*., 2016). Another significant aspect of internal skin aging is the decline in cellular replicative abilities and the increased degradation of extracellular matrix (ECM) components such as collagens, elastin, proteoglycans, and glycosaminoglycans. Disruption of the ECM results from increased expression of enzymes like matrix metalloproteinases (MMPs), which degrade the dermal ECM (Millis *et al*., 1992). This ECM damage leads to decreased elasticity, strength, and moisture retention in the skin. These processes can also be triggered by ROS.

Exposure to UV rays, also called photoaging, is the primary cause of external skin aging. UV radiation serves as a potent inducer of ROS formation in the skin (Masaki, 2010). Chronic exposure to low-grade UV radiation accounts for over 80% of skin aging. Repeated exposure to UV rays accelerates the degradation of ECM components and disrupts the synthesis of new components (Madan & Nanda, 2018).

Achillea L. is one of the significant genera of the Asteraceae family, which includes more than 115 species that grow in many parts of the world (Başer, 2016). The use of this genus in traditional medicine has a long-standing tradition in various countries and cultures. It has been utilized as a natural remedy for conditions such as bleeding, pain, burns, wounds, gastrointestinal disorders, skin infections, eczema, inflammation, hemorrhoids, and dysentery, as well as for its carminative, tonic, diuretic, and diaphoretic properties (Mohammadhosseini *et al*., 2017; Salehi *et al.*, 2020). *Achillea millefolium* L. is a widely distributed plant with extensive medicinal use worldwide. *A. millefolium* extract, also called Yarrow extract, is also one of the most well-known ingredients of cosmetic products (Aronson, 2016). This species alone is currently being used in 135 cosmetic products as active ingredients for personal care products and cosmetic formulations (Becker *et al.*, 2016). However, there is not enough research reported on extracts of other *Achillea* species for their potential use in skincare products. Recent studies suggest that other *Achillea* species may also yield safe compounds with beneficial biological properties, making them potential multifunctional ingredients for cosmetics.

Achillea phrygia Boiss & Balansa is a species that is endemic to Anatolia (Huber-Morath, 1975) and it is used in Western Anatolia as an appetizer, to aid digestion, to treat abdominal pain and nausea and wound healing (Deniz *et al.*, 2010). According to the studies performed on polyphenolic components, *A. phrygia* extracts obtained with different solvents were found to contain chlorogenic acid, rutin, luteolin, sinapic acid, ferulic acid, apigenin, quercetin, catechin, epicatechin, and kaempferol (Zengin *et al.*, 2017a). Furthermore, recent studies have demonstrated the enzyme inhibitory effects of *A. phrygia* extracts against tyrosinase, cholinesterases, amylase, lipase, and glucosidase (Zengin *et al*., 2017a) as well as their antioxidant activity (Zengin *et al*., 2017a; Doğan *et al*., 2022). Additionally, these plant extracts have shown anti-nociceptive properties (Küpeli *et al*., 2007) and potential anticancer activity (Doğan *et al*., 2022). The potential use of *A. phrygia* as an ingredient in skincare products has not been investigated. This study aims to determine the suitability of *A. phrygia* for cosmetic and dermatological applications by evaluating its sun protection factor, antioxidant activity, total phenol content, inhibition of ECM-degrading enzymes, and genotoxic/antigenotoxic and cytotoxic activity.

2. MATERIAL and METHODS

2.1. Plant Sample

The aerial parts of *Achillea phrygia* were harvested during flowering in August 2020 at Karaisalı, Çevlik village (altitude: 1650m) located at coordinates 37°22'16.2660"N and 35°4'3.6228"W in Adana, Türkiye. *A voucher specimen was* identified by Dr. Volkan Eroglu and deposited in Muğla Sıtkı Koçman University herbarium (ARB-A011). Air-dried aerial parts (35 g) were extracted using an ultrasonic-assisted extraction method using ethanol (350 mL) at

30 °C for 3 h and filtered through filter paper. Following filtration, the solvent was evaporated, and stored at 4 °C in a dark place until analysis.

2.2. Evaluation of the SPF

The *in vitro* photoprotective efficacy test was conducted using a UV-visible spectrophotometer (Saraf & Kaur, 2010). Hydroalcoholic dilutions (50% v/v) of *A. phrygia* (500 µg/mL) were prepared, and sample absorbance values were detected at 5 nm intervals within the mid-wave ultraviolet light range (290-320 nm). SPF values were estimated using the formula below:

$$\mathrm{SPF}_{\mathrm{spectrophotometric}} = \mathrm{CF} \times \sum_{290}^{320} \mathrm{EE}(\lambda) \times \mathrm{I}(\lambda)\ \mathrm{Abs}(\lambda)$$

where: CF is a correction factor (10), EE(λ) is an erythemogenic effect of wavelength radiation, I is the intensity of the sun, and Abs (λ) is the absorbance of the samples.

2.3. Antioxidant assays

The scavenging ability against DPPH radicals was assessed following a previously established method (Ebrahimabadi *et al*., 2010). DPPH solution (0.1 mM, 100 µL) in methanol was combined with the extract (100 µL) at various concentrations and incubated in darkness for 30 minutes. After incubation, abs were measured at 517 nm. α-tocopherol and ascorbic acid were used as references. The results were expressed as IC_{50} values.

Total antioxidant capacity was applied using the β-carotene/linoleic acid bleaching method as described by Rauter *et al.* (2012). β-carotene/linoleic acid solution was added to 250 µL of different concentrations of the extract or positive control solutions. The mixture tubes were then incubated at 50°C in the dark, and abs changes were recorded every 15 minutes at 470 nm. The results were expressed as IC_{50} values.

The hydroxyl-radical scavenging activity was investigated using the method described by Zhang *et al.* (2015). Different concentrations of the extract (250 µL) and salicylic acid (9 mmol/L) were added to a reaction mixture containing H_2O_2 (20 mmol/L), 500 µL of dH_2O and $FeSO_4$ (9 mmol/L). After 30 min incubation, the abs were evaluated at 510 nm. The *results are expressed* as IC_{50}.

2.4. Total phenolic content

In brief, a 50 *µ*L extract was incubated with 25 *µ*l Folin–Ciocalteu *reagent for 3 min.* Subsequently, Na_2CO_3 was added to the mixture, followed by an additional 2-hour incubation period. Abs readings were taken at 760 nm (Singleton *et al.*, 1999). The outcomes were expressed as mg GAE/g dry weight using the standard curve.

2.5. Enzyme Inhibitory Activity

2.5.1. MMP-1 inhibition

The reaction mixture consisted of *Clostridium histolyticum* collagenase (MMP-1), tricine buffer (pH 7.5), and the extract solution. After 20 min, N-(3-[2- Furyl]-acryloyl)-Leu-Gly-Pro-Ala was mixed with the test solution and abs were recorded at 2-min intervals on a plate reader at 335 nm (Barrantes & Guinea, 2003).

2.5.2. Elastase inhibition

For elastase inhibition, 25 µL of elastase and 25 µL of Tris–HCl buffer were mixed with 50 µL of the sample and pre-incubated for 20 minutes in the dark at 25°C. Subsequently, the mixture was inoculated with 125 µL of N-Succinyl-Ala-Ala-Ala-p-nitroanilide and again incubated under the same conditions. Abs were recorded at 410 nm on a plate reader (Lee *et al.*, 1999).

2.5.3. Hyaluronidase inhibition

Regarding hyaluronidase inhibition, the extract's effect on bovine hyaluronidase was determined by pre-incubating the hyaluronidase at 37°C for 30 minutes. Then, 100 µL of CaCl2 and sodium hyaluronate were added to the test mixture and incubated for 40 minutes. Following this, the mixture was supplemented with sodium borate and NaOH, heated in hot water, cooled, and mixed with p-dimethyl amino benzaldehyde. The resulting mixture was then incubated away from light at 37°C for 20 min, and abs was detected at 585 nm (Lee *et al.*, 1999). The enzyme inhibition abilities were determined using the following equations:

$$\text{Inhibition (\%)} = [(\beta - \alpha) - (\delta - \gamma)] / (\beta - \alpha) \times 100$$

Where β is the abs without the sample, α is the abs without the sample and enzyme, δ is the abs with the sample, and γ is the abs with the sample without the enzyme.

2.6. Genotoxicity and Antigenotoxicity Assay

The genotoxic/antigenotoxic effects of the extract were assessed using the *Salmonella typhimurium*/microsome test with *S. typhimurium*TA98 and *S. typhimurium*TA100 strains. Before testing, the original mutations of the test strains were verified for test reliability, and the spontaneous mutation rates as well as the cytotoxic doses of the sample extract were determined (Maron & Ames 1983).

For the genotoxicity test, sodium-phosphate buffer (pH 7.4), fresh overnight bacterial culture, and test compounds (ranging from 0.1 µg to 1 mg per plate) were combined in test tubes containing top agar supplemented with histidine/biotin. The mixture was then spread onto plates containing minimal glucose agar (MGA) and incubated at 37°C for 48 hours. The number of His+ colonies was subsequently counted. Positive controls included 4-nitro-o-phenylenediamine and sodium azide.

In the anti-genotoxicity test, fresh overnight bacterial culture, sodium-phosphate buffer, the test substance, and mutagens were mixed in test tubes containing top agar supplemented with histidine/biotin. The resulting mixture was spread onto plates containing MGA and incubated at 37°C for 2–3 days. The number of His+ colonies in the positive control was considered as 100%, and any decrease in the number of revertant colonies obtained from the extracts in the presence of mutagen was evaluated as anti-mutagenicity

2.7. Cell Culture

NIH-3T3 mouse fibroblast cells were cultured in DMEM medium supplemented with 10% fetal bovine serum and antibiotics (100 µg/mL streptomycin and 100 IU/mL penicillin) under conditions of 5% CO2 at 37°C in a humidified atmosphere.

2.7.1. Cytotoxicity assay

To initiate the experiment, fibroblast cells were initially seeded into individual wells of 96-well plates at a density of $1x10^4$ cells per well. Subsequently, the cells were exposed to the test extract and allowed to preincubate for 24 hours. Following this incubation period, the culture medium was carefully aspirated, and the wells were gently washed with phosphate-buffered saline (PBS). Next, 200 µL of MTT solution was added to each well, followed by the addition of 200 µL of DMSO to dissolve the resulting MTT crystals. The plates were then placed in an incubator for 20-30 minutes to facilitate the reaction. After incubation, the absorbance of each well was measured at 540 nm using a spectrophotometer (Mosmann, 1983).

2.8. Statistical Analysis

Readings for all SPF, enzyme inhibition tests, total phenol, and antioxidant assays were taken in triplicate. Genotoxicity and anti-genotoxicity experiments were conducted in 3 repetitions, 2 in parallel. The results were averaged with their standard deviations. A one-way ANOVA followed by Tukey's test was used to investigate statistical significance. The statistical significance was indicated by $p < 0.001$.

3. RESULTS

To assess the SPF activity of the *A. phrygia* extract, the UV absorbance method was employed. Sample absorbance values were measured between 290 and 320 nm at 5 nm intervals. The extract demonstrated moderate protection activity, with an SPF value of 4.013 (see Table 1).

Table 1. Spectrophotometrically calculated sun protection factor value of *A. phrygia*

Wavelength (nm)	EE(λ) x I(λ) (normalized)	*A. phrygia*	
		Absorbance	SPF
290	0.0150	0.437	0.065
295	0.0817	0.429	0.350
300	0.2874	0.413	1.201
305	0.3278	0.395	1.295
310	0.1864	0.389	0.725
315	0.0837	0.372	0.311
320	0.0180	0.369	0.066
Total	=1.000		4.013

EE: Erythemal efficiency spectrum;
I: Solar simulator intensity spectrum.

The antioxidant activity of the sample was evaluated through three different methods and compared with α-tocopherol and ascorbic acid (see Table 2). The methods employed were the DPPH assay, β-carotene-linoleic acid bleaching test, and H_2O_2 scavenging test, yielding IC50 values of 0.183 ± 0.03, 0.079 ± 0.51, and 1.18 ± 0.35 mg/mL, respectively. Ascorbic acid showed IC_{50} values of 0.035 ± 0.17, 0.021 ± 0.7, and 0.35 ± 0.041 µg/mL for the same tests. Additionally, the total phenolic content of the extract was measured as 23.56 ± 1.42 mg GAE/g dry extract (see Table 2).

Table 2. Antioxidant activity and total phenol content of the *A. phrygia*.

Samples	Test Systems			
	DPPH scavenging activity[a] IC_{50} (mg/mL)	β-carotene–linoleic acid test activity [a] IC_{50}(mg/mL)	Hydroxyl-radical scavenging activity[a] IC_{50} (mg/mL)	Total phenol[a] (mg GAE/g extract
A. phrygia	0.183 ± 0.03	0.079 ± 0.51	1.18 ± 0.35	23.56 ± 1.42
α-tocopherol[b]	0.015 ± 0.21	–	–	–
Ascorbic acid[b]	0.035 ± 0.17	0.021 ± 0.7	0.35 ± 0.041	–

[a]IC_{50} values are presented as the means ± standard deviation from three parallel measurements (p< 0.001).
[b] Reference compounds

The anti-aging potential of *A. phrygia* was assessed based on its inhibition activities against MMP-1, elastase, and hyaluronidase. The extract demonstrated inhibition of MMP-1 (47.98%) and elastase (39.2%), while exhibiting a weaker inhibitory effect on hyaluronidase (16%) (see Table 3). EGCG exhibited MMP-1 and elastase inhibitory activities of 20.36% and 25.3%, respectively. At a concentration of 100 µg/mL, tannic acid exhibited a 62.2% inhibition of hyaluronidase activity.

Table 3. Extracellular matrix-degrading enzyme inhibition of the *A. phrygia*.

		Inhibition (%)		
Samples	Concentrations	MMP-1	Elastase	Hyaluronidase
A. Phrygia	1000 µg/mL	47.98 ± 0.17[a]	39.2 ± 0.35 [a]	16 ± 0.23 [a]
EGCG	100 µg/mL	20.36 ± 0.58 [a]	25.3 ± 0.42 [a]	–
Tannic acid	100 µg/mL	–	–	62.2 ± 0.72 [a]

[a] Standard error meaning,
Significance compared to control at p<0.001

To assess the genotoxic potential of *A. phrygia* (ranging from 0.1 µg/plate to 1000 µg/plate), the Ames *Salmonella*/microsome test was conducted. None of the tested doses of the extract induced an increase in the number of revertant colonies compared to the control (data not shown). Subsequently, the same doses were evaluated to determine the antigenotoxic effect of *A. phrygia*. Notably, it exhibited a strong anti-mutagenic effect (44.5%) against 4-NPD mutagenicity, while demonstrating moderate anti-mutagenic effects at concentrations of 100 µg/plate and 10 µg/plate. Furthermore, against NaN_3 mutagenicity, a strong inhibition effect (ranging from 65.6% to 46.6%) was observed for TA100 using all concentrations (see Table 4).

Table 4. Anti-genotoxicity of the *A. phrygia*.

Samples	Concentration	TA98		TA100	
		Number of colonies	Mutagenicity inhibition (%)	Number of colonies	Mutagenicity inhibition (%)
Negative control		20 ± 4.1[a]		150 ± 6.6[a]	
4-NPD	3.0 µg/plate	654 ± 21.03			
NaN_3	8.0 µg/plate			888 41.7	
A. phrygia	1000 µg/plate	372 ± 11.5[b]	44.5	455 ± 10.0	65.6
	100 µg/plate	425 ± 18.4	36.2	506 ± 11.1	60.0
	10 µg/plate	410 ± 15.3	38.5	591 ± 23.8	50.3
	1 µg/plate	556 ± 21.8	15.5	575 ± 19.1	52.0
	0.1 µg/plate	590 ± 20.3	10	624 ± 21.0	46.6

[a] Values expressed are means ± standard deviation.
[b] $p < 0.001$

The cytotoxic analysis of the extract was conducted on 3T3 fibroblast cells. Fibroblasts were exposed to various extract concentrations (ranging from 15.625 µg/mL to 500 µg/mL) for 24 hours. The results revealed that the extract did not exert a significant negative effect on cell viability. The IC_{50} value for 3T3 cells was determined to be 42.41 µg/mL.

4. DISCUSSION and CONCLUSION

According to recommendations from the US Food and Drug Administration (FDA), sunscreen formulations should ideally have an SPF value exceeding 2 to be considered effective (FDA, 2013). Consequently, many natural compounds are investigated to ascertain if they meet these criteria and can be categorized as 'green sunscreens' (Cefali *et al*., 2016). In this study, the SPF level was evaluated by an *in vitro* quantitative method, and the SPF value of the 500 µg/mL concentration of *A. phrygia* was determined as 4.013 (see Table 1). This value falls within the 4–6 category on the SPF scale (Jaradat *et al*., 2018), indicating moderate protection. In another study, *A. millefolium* (14.04%) and *A. biebersteinii* Afan (11.67%) extracts exhibited notable UV protective properties at a concentration of 5%, while they demonstrated the lowest SPF values at a concentration of 1.25% (*A. millefolium* (1.90%) and *A. biebersteinii* (1.85%)). (Gaweł-Bęben *et al*., 2020).

If the skin is consistently exposed to the sun, its ability to repair damage diminishes. UV radiation can penetrate deep layers of the skin, breaking down collagen and elastin fibers, and reducing the synthesis of pro-collagen. Additionally, UV exposure can generate reactive oxygen and nitrogen species, disrupting cell metabolism and functions, and triggering genetic changes (Krutmann, 2001). Natural ingredients have been explored as potential sources of sunscreen due to their antioxidant activities and ability to prevent UV ray absorption (Cefali *et al*., 2016; Fibrich & Lall, 2018; Jaradat *et al*., 2018). To our knowledge, the SPF protective activity of *A. phrygia* was examined for the first time in this study. The SPF effect of *A. phrygia* may be attributed to active components such as apigenin, quercetin, rutin, or luteolin. These

components have chemical groups and conjugated bonds capable of absorbing UV radiation (Choquenet *et al*., 2008; Wölfle *et al*., 2012; Saewan & Jimtaisong, 2013).

The scavenging activity of free radicals DPPH, H_2O_2, and the total antioxidant effect of *A. phrygia* were determined and are presented in Table 2. The test results were compared with α-tocopherol and ascorbic acid. In the DPPH test, the extract exhibited antioxidant potency with an IC_{50} value of 0.183 ± 0.03 mg/mL. Considering that raw plant extracts contain various ingredients, the synergistic effect between these components may contribute to the antioxidant effect. Subsequently, the activity of *A. phrygia* was analyzed using the β-carotene method and compared to ascorbic acid. The total antioxidant activities of *A. phrygia* and ascorbic acid were determined to have IC_{50} values of 0.079 ± 0.51 mg/mL and 0.021 ± 0.7 mg/mL, respectively. The hydroxyl radical is known to be one of the most powerful oxidants, capable of attacking organic molecules and causing mutagenesis, cytotoxicity, carcinogenesis, and other diseases (Sun *et al*. 2020). The IC_{50} inhibitory concentration of *A. phrygia* was measured as 1.18 ± 0.35 mg/mL, while for ascorbic acid, it was 0.35 ± 0.041 mg/mL.

Several studies have been carried out to explore the antioxidant properties of various *Achillea* species. However, only two studies have been identified concerning the antioxidant activity of extracts from *A. phrygia* (Zengin *et al*., 2017a; Doğan *et al*., 2022). Zengin *et al*. (2017a) focused on *A. phrygia*, assessing the antioxidant capacities of its methanol, water, and ethyl acetate extracts, with the methanol extract displaying the highest DPPH scavenging activity and the water extract showing the highest ABTS radical scavenging ability. Another study by Doğan *et al*. (2022) evaluated eight sub-extracts derived from *A. phrygia*, revealing varying antioxidant activities with DPPH IC_{50} values ranging from 0.399 to 1.399 mg/mL, CUPRAC values ranging from 0.422 mM to 2.149 mM, and FRAP values ranging between 28.050 to 40.984 mM.

Antioxidants play a crucial role in alleviating skin aging by neutralizing ROS that have already formed. They also serve to prevent photodamage and offer protection against skin cancer. Among the ingredients utilized in cosmetics, antioxidants hold significant importance. While sunscreens provide essential UV protection, they alone cannot offer complete defense against UV damage. Therefore, antioxidants are often incorporated into the formulation of sun protection products to prevent and mitigate skin wrinkles induced by UV radiation. The primary claim regarding antioxidants revolves around their anti-aging effect, which involves reducing wrinkles by combatting free radicals generated by sunlight exposure (Ramos-e-Silva *et al*., 2013).

The phenolic contents were examined using the Folin-Ciocalteu assay, yielding a value of 23.56 ± 1.42 mg GAE/g extract, as detailed in Table 2. Previous studies have highlighted *Achillea* species as rich sources of phytochemical compounds (Agar *et al*., 2015; Zengin *et al*., 2017b; Afshari *et al*. 2018; Barda *et al*., 2021). For example, Zengin *et al*. (2017a) examined various *A. phrygia* extracts and determined that the highest total phenolic content was in the methanol (41.13 mg GAE/g extract)> water (35.53 mg GAE/g extract)>ethyl acetate (23.62 mg GAE/g extract) extracts, respectively.

In vitro enzyme inhibitory activity experiments have revealed that *A. phrygia* exhibits significant inhibitory effects on elastase and MMP-1 enzymes, with percentages of 39.2% and 47.98% observed at a concentration of 1 mg/mL, respectively (Table 3). Collagen, hyaluronic acid, and elastin, which are key components of the ECM, play crucial roles in maintaining skin moisture, elasticity, and structural integrity. Therefore, depletion or damage to these components can lead to skin wrinkling and other signs of aging (Madan & Nanda, 2018). The increased activation of ECM-degrading enzymes contributes to the degradation and irregularity of these components, which is a primary cause of skin aging. There exists a delicate balance between the degradation and synthesis of these components in young and healthy skin, a balance that diminishes with age (Fibrich & Lall, 2018). Additionally, UVB exposure disrupts

biological processes that support the expression of extracellular matrix components (Madan & Nanda, 2018).

Inhibition of elastase and collagenase activities can prevent the loss of skin structural integrity or sagging of the skin. In a study involving *A. clypeolata* Sibth. & Sm., a methanolic extract from this species exhibited inhibitory effects against hyaluronidase, collagenase, and elastase enzymes (Barak *et al*., 2023). Similarly, aqueous and ethanolic extracts of *A. sintenisii* Hub.-Mor demonstrated concentration-dependent inhibition of hyaluronidase activity (Anlas *et al*., 2023). Additionally, ethanolic extracts of *A. sintenisii* exhibited dose-dependent inhibitory effects against collagenase, hyaluronidase, and elastase enzymes (Eruygur *et al*., 2023). The enzyme inhibition observed in *A. phrygia* may be attributed to its phenolic and flavonoid compounds. For example, in a study, apigenin inhibited MMP-1 induction in human dermal fibroblasts (Lee *et al*., 2007). Similarly, in another study, it displayed inhibition of collagenase activity. Quercetin, another active ingredient, exhibited elastase and collagenase inhibition activities (Lim & Kim, 2007). Furthermore, luteolin has been shown to inhibit MMP-1 expression and hyaluronidase activity (Wölfle *et al*., 2012).

According to genotoxicity test results, *A. phrygia* can be considered genotoxically safe. Anti-aging facial moisturizers containing a synthetic chemical product can be highly genotoxic (Alnuqaydan & Sanderson, 2016). Genotoxicity assessment of compounds with cosmetic potential is a fundamental need at an early stage of any cosmetic formulation (Meena & Mohandass, 2019). In the study, none of the tested doses of the extract had genotoxic effects. Additionally, *A. teretifolia* Willd. aqueous extract showed no mutagenic effects on *S. typhimurium* strains TA98 and TA100 in the Ames test (Akyil *et al*., 2012).

The extract showed a strong anti-mutagenic effect (44.5%) against the 4-NPD mutagenicity at 1000 µg/plate concentration, demonstrating strong inhibition effects (ranging from 65.6% to 46.6%) against NaN_3 mutagenicity across all concentrations (Table 4). The extract showed better activity in preventing NaN3-induced nucleotide substitution mutations compared to 4-NPD-induced frameshift mutations. When DNA damage, whether endogenous or exogenous, is not adequately repaired, mutations can occur. In somatic cells of multicellular organisms, these mutations may contribute to aging. Compounds with anti-genotoxic properties have the potential to prevent DNA damage by reducing mutagenic effects. As far as current knowledge goes, there is a lack of studies specifically investigating the antimutagenic effects of *A. phrygia*. However, research suggests that various *Achillea* species, such as *A. millefolium*, might harbor antimutagenic properties (Düsman *et al*., 2013; Hussein *et al*., 2019).

According to cytotoxic activity results of *A. phrygia*, demonstrated no significant negative impact on cell viability in 3T3 fibroblasts, with an IC_{50} value of 42.41±4.05 µg/mL. In a study, it was found that the methanolic extract from *A. wilhelmsii* C. Koch. did not induce negative effects on fibroblast viability even at concentrations ranging from 10 to 200 µg/mL. Additionally, this extract notably stimulated fibroblast proliferation starting from a concentration of 25 µg/mL (Anlaş *et al*., 2022). Similarly, within the concentration range of 50-200 µg/mL, both the ethanolic and aqueous extracts of *A. sintenisii* did not exhibit a significant decrease in fibroblast viability compared to untreated cells (Anlaş *et al*., 2023). Furthermore, Agar *et al*. (2015) observed potent proliferative effects of *A. kotschyi* Boiss. on mouse fibroblasts over a concentration range of 2.5–20 µg/mL.

A. phrygia shows promise in delaying skin aging by potentially reducing the signs of aging through various mechanisms. Its SPF property, inhibition of MMP-1 and elastase enzymes, free radical scavenging activity, and antigenotoxic effect suggest its ability to counteract skin aging. Moreover, it demonstrated genotoxic and cytotoxic safety at tested concentrations. These findings suggest that *A. phrygia* could be utilized as an anti-aging agent in cosmetic products for skincare purposes.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Rukiye Boran Gülen: Investigation, methodology, resources, visualization, writing—review and editing, **Nurdan Saraç:** methodology, validation, review and editing, **Aysel Uğur:** supervision, validation, review and editing

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