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Anatomical, Morphological, and Chemical Characterizations and Biological Activities of *Gagea taurica* Steven (Liliaceae): A New Record for the Turkish Flora

Hafize Yuca,[a] Songul Karakaya,*[b] Gülnur Ekşi,[c] Bilge Aydin,[d] Gamze Goger,[e] Mehmet Bona,[f] Enes Tekman,[g] Aydan Acar Şahin,[g] Oksana Sytar,[h] Nur Münevver Pinar,[g] and Zuhal Guvenalp[a]

Gagea genus, which is native to the Mediterranean and Black Sea regions, has attracted significant attention due to its biodiversity and potential health benefits. In this study, the biochemical composition and biological activities of methanol extracts from various parts of *G. taurica* were investigated, along with their anatomical and morphological characteristics. The best antimicrobial activity was found to be MeOH extracts of corm and leaf against several *Candida* strains with MIC = 640 μg/mL. The highest level of phenolics together with significant results of antioxidant activities were observed in flowers extracts. The α-amylase inhibition assay results showed that the highest inhibition percentage was observed with acarbose (59%), followed by leaf extract (43%). Leaf exhibited the most effective inhibitory activity in AChE inhibition assay, whereas flower demonstrated the most significant inhibitory activity in BChE inhibition assay. Hesperidin was found as 1621.0001 ng/ml value in flower extract and 283.9339 ng/ml value leaf.

Introduction

Richard Anthony Salisbury divided the seven species long placed in the *Ornithogalum* genus into the new *Gagea* genus because of their "difference in habit and fruiting". Famous for collecting rare European herbs and liberal in dispersing them, Hengrave Hall is named after Sir Thomas Gage of Suffolk. Although Salisbury's novel genus is based on species already described in the genus *Ornithogalum*, it did not take into account the primacy of earlier specific adjectives and created new ones; this was the first resource of taxonomic confusion in the genus. *Gagea* (Liliaceae) is a geophytic, perennial, usually Eurasian genus, with several species in North Africa, and contains somewhere between 70 and approx. 275 species connected with the opinion of varied authors.[1,2] It has been reported that bulb and leaves methanolic and ethanolic extracts of *G. fibrosa* indicated varying degrees of total phenolic content and antioxidant effect. Free radicals triggered by endogenous metabolism as well as various environmental chemicals play a role in a number of diseases like diabetes, tumors, brain dysfunction, inflammation, atherosclerosis, shock, ischemia, infertility, gastric mucosal injury, and cancer.[3] *G. taurica* is known as "bozkır yıldızı" in Turkey.[4]

Phenolic compounds are usually found in both edible and non-edible herbs and it has been reported that they possess various effects, containing antioxidant effects.[5,6] The signifi-

[a] *H. Yuca, Prof. Z. Guvenalp*
Department of Pharmacognosy,
Faculty of Pharmacy,
Ataturk University,
Erzurum, Turkey

[b] *Assoc. S. Karakaya*
Department of Pharmaceutical Botany,
Faculty of Pharmacy,
Ataturk University,
Erzurum, Turkey
E-mail: snglkarakaya@yahoo.com
songul.karakaya@atauni.edu.tr

[c] *Assoc. G. Ekşi*
Department of Pharmacognosy,
Faculty of Pharmacy,
Istanbul Medipol University,
İstanbul, Turkey

[d] *B. Aydin*
Department of Pharmacognosy,
Faculty of Pharmacy,
Erzincan Binali Yıldırım University,
Erzincan, Turkey

[e] *Assoc. G. Goger*
Department of Pharmacognosy,
Faculty of Pharmacy,
Afyonkarahisar Health Sciences University,
Afyon, Turkey

[f] *Assoc. M. Bona*
Department of Biology,
Faculty of Science,
Istanbul University,
Istanbul, Turkey

[g] *E. Tekman, A. A. Şahin, Prof. N. M. Pinar*
Department of Biology,
Faculty of Science,
Ankara University,
Ankara, Turkey

[h] *Prof. O. Sytar*
Institute of Plant and Environmental Sciences,
Faculty of Agrobiology and Food Resources,
Slovak University of Agriculture,
Nitra, Slovakia

cance of the antioxidant compounds of herbs is increasing because of the protection of health and maintenance from coronary heart disease and cancer, so its interests scientists, food manufacturers, and consumers.[7] Phytochemicals have received great attention principally because of their role in preventing illnesses that occur as a result of oxidative stress, which releases reactive oxygen species such as singlet oxygen and various radicals as a detrimental side effect of aerobic metabolism. The radicals are likely involved in a number of diseases, containing cardiovascular malfunctions, tumorigenesis, DNA damage, and tissue damage.[8] Some investigations propose that antioxidants can prevent the accumulation of these reactive oxygen species and may be useful in the therapy of the pathologies.[9] Biodiversity of *Gagea* genus is variable and not studied well, especially biochemical composition of species representatives together with morphological and anatomical description of different plant parts.

The infection processing often causes inflammation, and free radicals are released from phagocytes along with the inflammatory processing. Since several skin disorders, containing atopic dermatitis and acne vulgaris, are related to infection-induced inflammation, the existence of antimicrobial, antioxidant, and anti-inflammatory agents may express the efficacy of certain plant essential oils in the treatment of these syndromes.[10,11] For example, it was discovered potential of Tamanu (*Calophyllum inophyllum*) oil for atopic dermatitis treatment.[12] Therefore, special interest to discover more plant species with healthy benefits due huge plant biodiversity. To study their antimicrobial, antioxidant and anti-inflammatory effects are fine aims for natural product improvement. Also, in many parts of the world, aromatic plants still act important roles in main health care, especially in rural regions. Therefore, a realization of the biological effects of plants could supply numerous functional constituents and additives for medical, nutritional, and cosmetic products.[13]

Alzheimer's disease and diabetes are prevalent age-related illnesses. The primary pathology of Alzheimer's disease is the accumulation of misfolded proteins, leading to brain dysfunction. On the other hand, diabetes is characterized by disrupted insulin signaling, resulting in decreased glucose uptake and metabolic suppression of energy-consuming cells. Additionally, the liver may convert glucose to fat. Despite their differences, these diseases share similarities and may be viewed as fundamentally comparable disorders with distinct features, such as affected tissues, magnitude of specific traits, and time of onset.[14]

The aim of this research to assess the biochemical analysis, antimicrobial, antioxidant, α-amylase, α-glucosidase, acetylcholinesterase, and butyrylcholinesterase inhibitory activities of corm, leaf and flower methanol extracts, and anatomical [light (root, corm, leaf, scapus, pedicel, tepal, anther, filament, stigma, stylus, and ovary) and electron microscopes] and morphological properties of *G. taurica.*

Results

Morphology and Taxonomy

G. taurica has small (ca. 1 cm) bulbs with brownish tunics, often entwined with thickened roots. It has linear, canalicuate, glaucos, up to 3 mm broad, 8–12 cm long one basal leaf, and whorled, hairy cauline leaves. Leaves are usually exceeding flowers. Inflorescence is 2.5-16 cm long umbella with 1–3 flowers. Pedicels are errect and hairy, shorter than scape. Perianth segments are yellowish, lanceolate to ob-long-linear, 12–20 mm long, acuminate to acute with hyaline margin, and hairy at apex. Style and anthers are 1–1.5 cm long (Figure 1). This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

Microscopic Analysis

The anatomical structures of root, corm, leaf, scapus, flower (pedicel, tepal, anther, filament, stigma, stylus, ovary) of *G. taurica* were researched by ligt and electron microscopes.

Root anatomy: The outermost part of the root cross-section gets a 1–2 layers of the epider-mis. The cortex has 7–8 layers of parenchymatic cells. The cortex layer is thick from the center (Figure 2A). There are non-glandular trichomes on the root outer membrane (Figure 2B).

Corm anatomy: The corm cross-sections anatomy were analysed with Sartur and chloral hydrate agents. The epidermis of corm cross-sections is composed of 1–2 layered rows of cells in a square shape, closely arranged. Under the epidermis layer, there are numerous thin-walled, starch-bearing parenchyma cells on the cortex and center (Figure 3A). Vascular bundles are larger and more many at the center. Its central surface has a larger area than the cortex (Figure 3B).

Leaf cross section anatomy: Below the upper and lower epidermis cells are a few rows of palisade parenchyma and spongy parenchyma. The cuticle layer is thick and the leaf is amphystomatic (has stomata on both the upper and below epidermis). There are glandular and non-glandular trcihomes on the epidermis (Figure 4A).

Leaf upper (B) and lower (C) superficial anatomy: The tissues of the upper and lower epidermis layers are similar. The shape of epidermis cells are isodiametric and long rectangular. The type of stomata are anomocytic. There are glandular and non-glandular trichomes on the epidermis (Figures 4B, 4C).

Scapus anatomy: The shape of scapus anatomy is cordat. The vascular bundles are located between the cortex and center, regularly. Its central surface has a larger area than the cortex. The cuticle layer is thick (Figure 5A).

Pedicel anatomy: The shape of the pedicel anatomy is the cylinder. The cells of the epidermis are composed of a single layer of square and a thin layer of cuticle. Vascular bundles are located in one circle and are bilateral, with the xylem under the

Figure 1. *Gagea taurica.* General morphology of plant (A–C), flower longituninal section (D), pistil (E), stamen (F) (Drawn by Gülnur Ekşi).

phloem. There are uni-cellular non-glandular trichomes on the epidermis layer (Figure 5B).

Tepal anatomy: It was found to have nectariferous tissue at the base of the tepal, consisting of 9–10 layers of secretory cells. The shape of the nectariferous tissue is round-helical. There are glandular trichomes on the epidermis layer (Figure 5C).

Anther and filament anatomies: Vascular bundles are very prominent and longitudinal in the filament. The shape of filament epidermis cells are isodiametric and long rectangular. There are pollens on both filament and anther. The endothecium is prominent (Figures 5D, 5E).

Stigma, stylus, and ovary anatomies: There are a lot of papillae on stigma and the shape of stylus is long rectangle. Vascular bundles are prominent on stylus (Figures 5F, 5G).

Secretory structures of corm, leaf, and flower samples of *Gagea taurica* was studied in detail using light and scanning electron microscopies. Both glandular and non-glandular types of trichomes had found on the epidermal tissues of *G. taurica.*

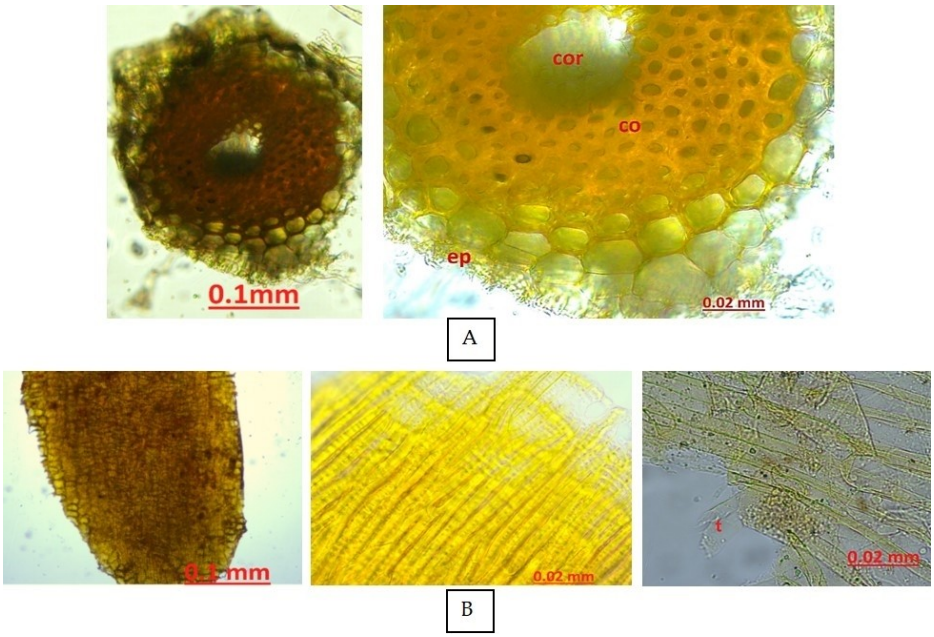

Figure 2. Cross section of root, ep: epidermis, co: cortex, cor: core (A); root outer membrane anatomy, t: glandular trichome (B).

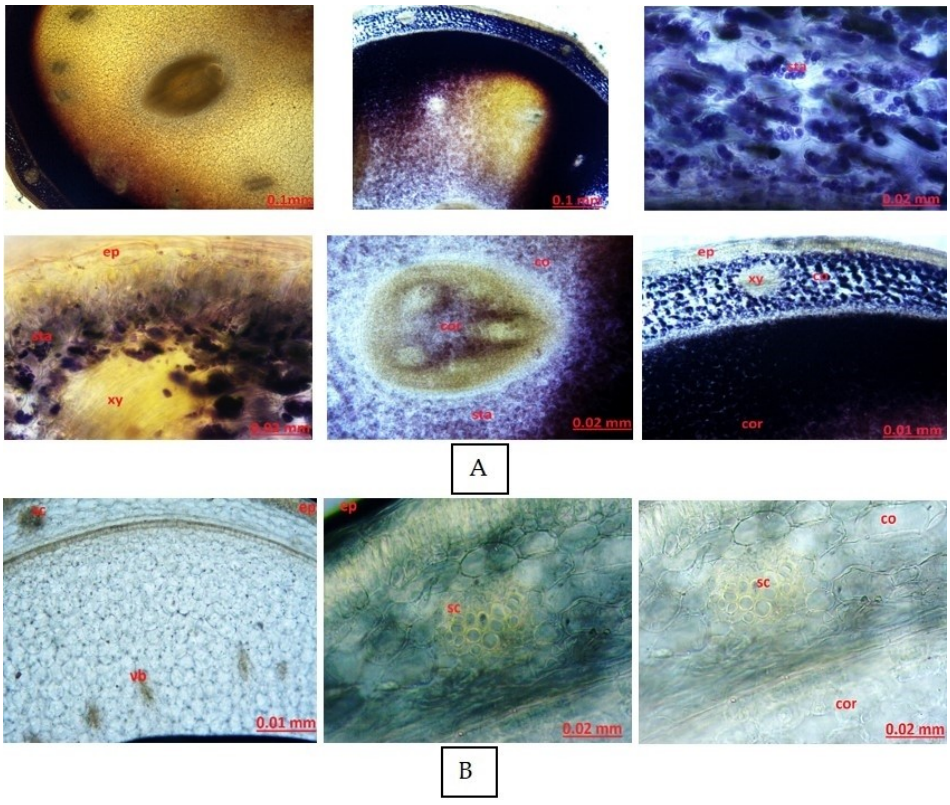

Figure 3. The corm cortex anatomy with Sartur agent, sta: starch, ep: epidermis, co: cortex, cor: core (A); the corm cortex anatomy with chloral hydrate agent, sc: sclerenchyma, vb: vascular bundle (B).

Non-glandular trichomes are uniseriate cells, bent and long (30–50 μm) on adaxial sides of the leaf; uniseriate, multicellular, unbranched, 8–15 μm long, sharp-pointed and erect on adaxial sides of the leaf; multiseriate, multicellular, unbranched, rounded at the tip, erect and 7–10 μm long on tepal; multiseriate cells and very long (80–100 μm) and bent on corm. We were observed very well secretory trichomes on adaxial and abaxial sides of the leaves, tepal and coat of corm. There are a lot of small capitate trichomes (0.7-1.5 μm). Small capitates trichomes consisted of a cylindrical head with an one-celled

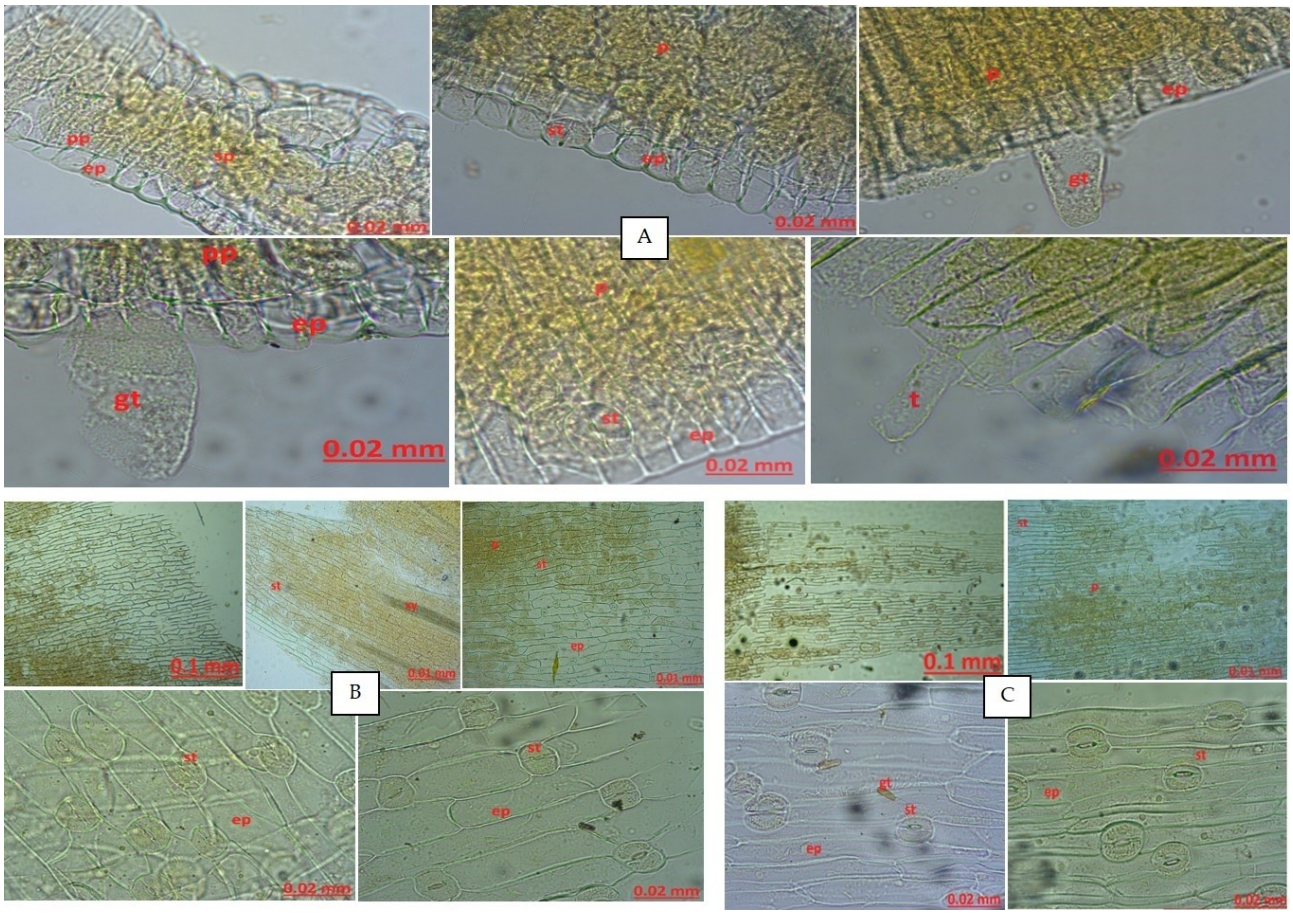

Figure 4. The leaf cross section anatomy; ep: epidermis, st: stomata, pp: palisede parenchyma, sp: spongy parenchyma, gt: glandular trichome, t: trichome (A); leaf upper superficial anatomy; xy: xylem, p: parenchyma (B); leaf lower superficial anatomy (C).

uniseriate stalk on adaxial and abaxial sides of the leaves and tepal. Surface of tepal was found short capitate trichomes made up of one basal cell, one or two cells forming the stalk and two secretory cells within the head which is covered with a cuticle layer. On coat of crom, the developed capitate trichomes are about 4–6 μm in height and 3–5 μm in diameter at the spherical head. There are a lot of papillae on adaxial and abaxial sides of the leaves. Corm cells exhibited variable sizes (3–15 μm) single and aggregated starch granules. Pollen grains are monosulcate, elliptic in equatorial longitudinal and transversal view, elliptic in polar view, large in size, polar axis 30–35 μm, longer equatorial diameter 50–65 μm, shape sub-prolate and heteropolar. Exine is tectate; ectexine (1.5 μm) is thicker than endexine (0.85 μm). Exine pattern ornamentation at both distal and proximal faces is perforate-foveolate. The sulcus extends from distal to proximal face and the edges of the sulci are irregular. The total length of the sulcus was 55–63 μm and reaches the proximal side of the pollen with acute ends (Figures 6–8).

Antimicrobial Activity Results

Antibacterial activity results

The antibacterial activity of the corm, leaf, and flower methanol extracts of *G. taurica* was defined as antimicrobial activity against *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, and *Bacillus subtilis* ATCC 19659. The extracts of *G. taurica* MIC values were observed between (1280-$>$2560 μg/mL) against Gram (+) and Gram (−) strains. The flower methanol extracts of *G. taurica* was the most active extract with a MIC = 1280 μg/mL against *Bacillus subtilis* ATCC 19659.

The minimum inhibitory concentrations were determined as MIC = 2560 μg/mL for corm and leaf methanol extracts of *G. taurica* against all tree bacteria strains. The effect of standard antibacterial clarithromycin and extracts on bacterial concentration is shown in the Table 1.

Anticandidal activity results

The anticandidal activity of the corm, leaf and flower methanol extracts of *G. taurica* were defined against *Candida albicans* ATCC 10231, *Candida krusei* ATCC 14243, and *Candida tropicalis*

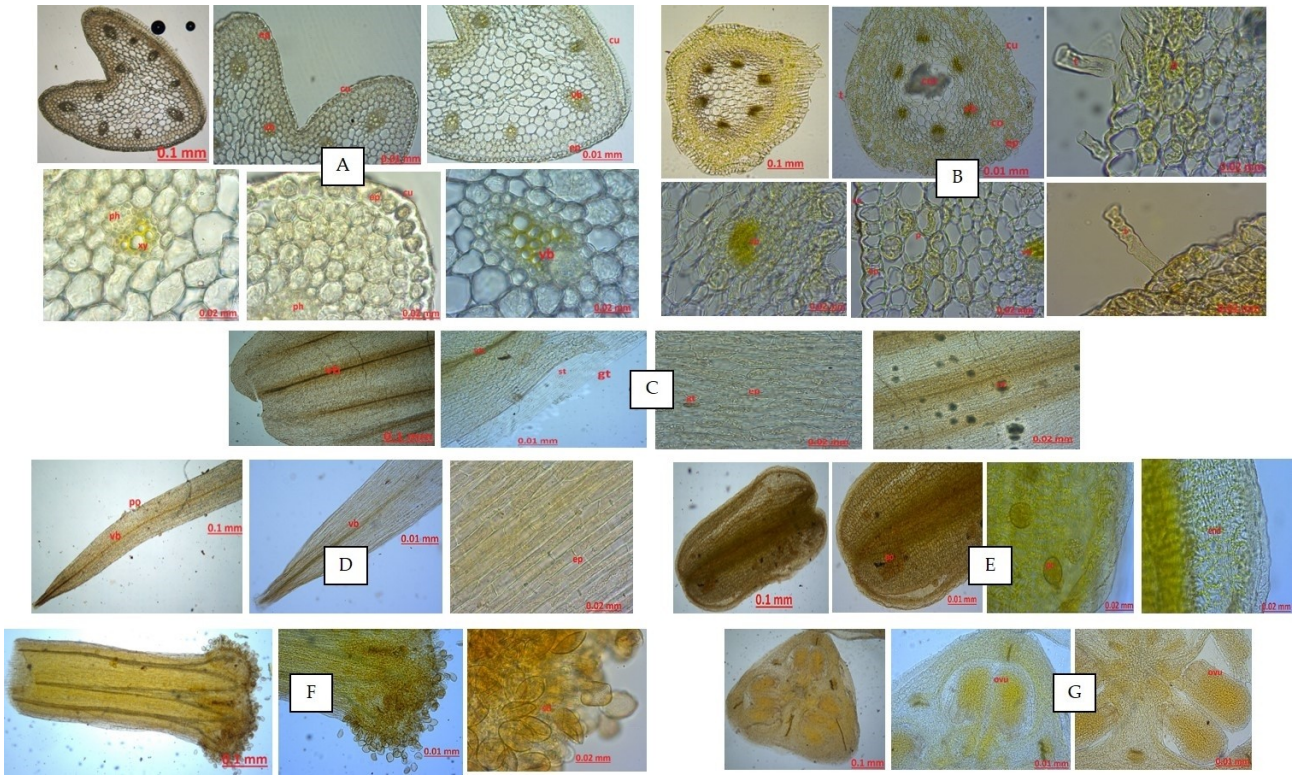

Figure 5. Scapus anatomy; cu: cuticula, ep: epidermis, vb: vascular bundle, ph: phloem, xy: xylem (A); pedicel anatomy; co: cortex, cor: core, p: parenchyma, t: trichome (B); tepal anatomy, st: stomata, gt: glandular trichome, nt: nectariferous tissue (C); anther (D) and filament (E) anatomies, po: pollen, end: endothecium; stigma and stylus (F) and ovary (G) anatomies, sti: stigma, ovu: ovule (E).

Table 1. Minimum inhibitory concentrations (MIC, μg/mL)

Extracts	*E. coli* *ATCC 8739*	*S. aureus* *ATCC 6538*	*B. subtilis* *ATCC 19659*	*C. albicans* ATCC 10231	*C. krusei* ATCC 14243	*C. tropicalis* ATCC 750
Corm	>2560	2560	2560	**640**	**640**	**640**
Leaf	2560	2560	2560	**640**	1280	1280
Flower	2560	>2560	1280	1280	1280	2560
Clarithromycin	32	2	0.125	–	–	
Terbinafine				4	8	8

(–): Not studied.

ATCC 750. MIC values of *G. taurica* extracts were observed as 640–2560 μg/mL in anticandidal activity. The corm methanol extract of *G. taurica* was found to be effective against all tree *Candida strains* with MIC = 640 μg/mL. Additionally, it was determined that the leaf methanol extracts of *G. taurica*, was found the most effective extracts with a MIC = 640 μg/mL against *Candida albicans* ATCC 10231. Flower extract of *G. taurica* was showed the same MIC = 1280 μg/mL against *Candida albicans* ATCC 1023 and *Candida krusei* ATCC 14243. The MIC values of standard terbinafine and extracts on *Candida* are shown in Table 1.

Antioxidant Activity

In our study, the polyphenolic compounds amount of methanolic extracts from *Gagea taurica* were detected by the test of total phenolic component (TPC). It was determined that the total phenolic component amount of the flower (F) extract has the highest value in terms of gallic acid equivalent (Table 2). Total phenolic compound test results for *G. taurica* [(F)51.3 > (L)47.3 > (C)41.8 μg GAE/mg extract]. Although the $ABTS^{\bullet+}$ radical scavenging capacities of the extracts were lower than the standard compounds, the results were considered significant in terms of antioxidant effect (Table 3). The extracts of *G. taurica*, flower extract (F) showed highest activity compared to corm (C) and leaf (L) extract. $ABTS^{\bullet+}$ radical scavenging activity results for *Gagea taurica* Steven and standards in percent

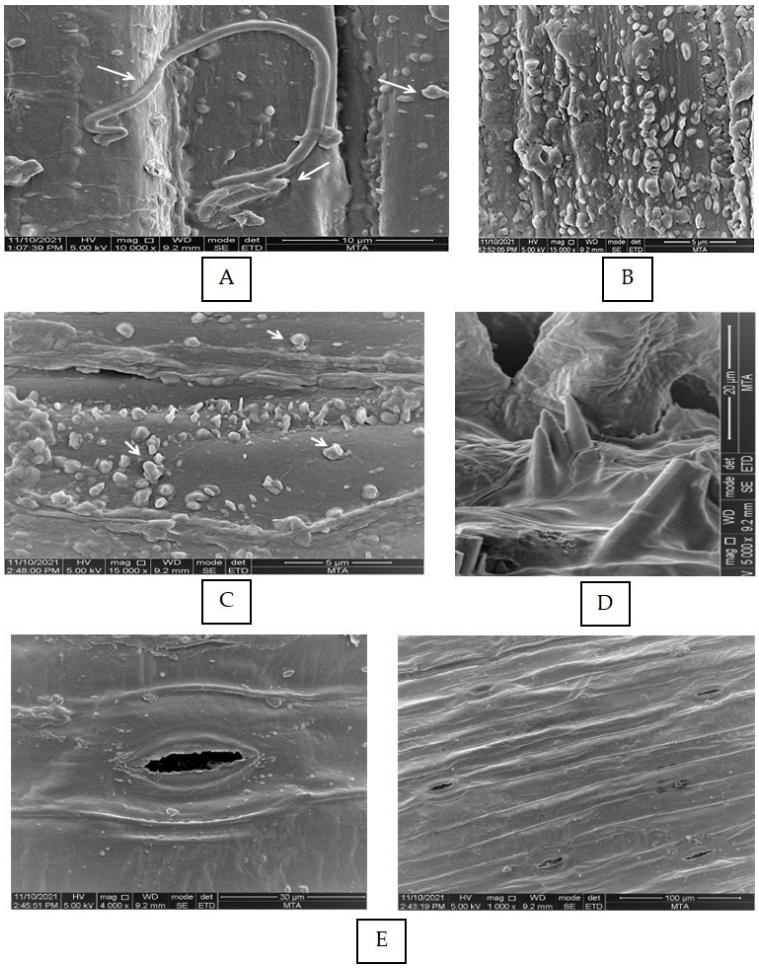

Figure 6. Leaf anatomy; non-glandular and glandular trichomes on leaf upper surface (A), papillas on leaf upper surface (B), glandular trichomes and papillae on lower leaf surface (C), non-glandular trichome on lower leaf surface (D), and stomatas on lower leaf surface (E).

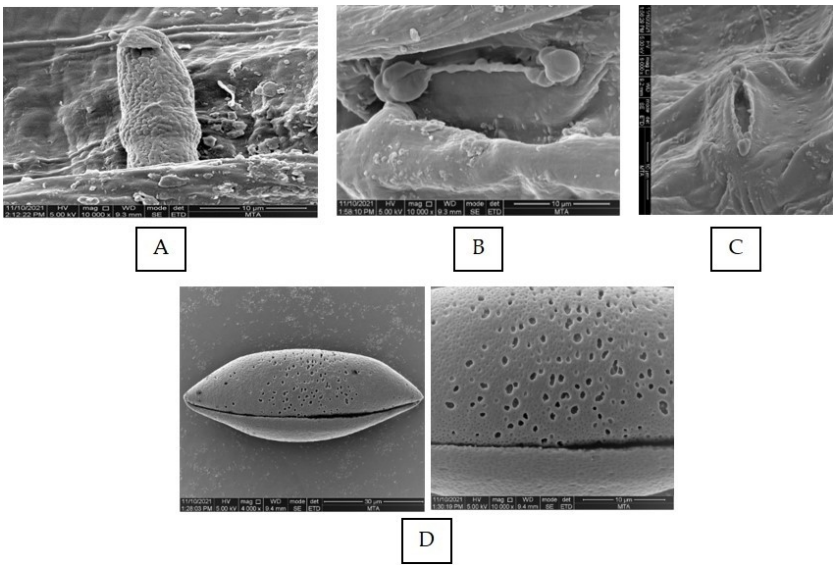

Figure 7. Tepal anatomy; non glandular trichomes on tepal (A), glandular trichomes on tepal (B), and stomatas on tepal (C); pollen anatomy (D).

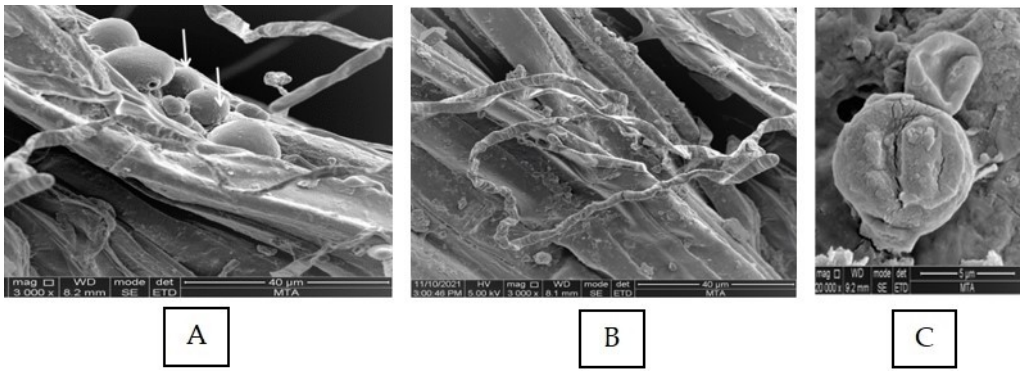

Figure 8. Corm anatomy; starches cells on corm (A), glandular trichomes on the inner surface of the corm (B), and glandular trichomes on corm tunic (C).

Table 2. Total phenolic component results of *Gagea taurica*.

Extracts	Total Phenolic Compound (μg GAE/mg extract)
Flower Extract (F)	51.385 ± 0.0017
Leaf Extract (L)	47.368 ± 0.0004
Corm Extract (C)	41.842 ± 0.0006

(–): Not studied.

Table 3. $ABTS^{\bullet+}$ radical scavenging activity results of *Gagea taurica*.

$ABTS^{\bullet+}$ Radical Scavenging Activity		
	% Inhibition (25 μg/mL)	% Inhibition (32.5 μg/mL)
Flower Extract (F)	23.76 ± 0.0046	29.64 ± 0.0066
Leaf Extract (L)	20.11 ± 0.0148	26.50 ± 0.0117
Corm Extract (C)	9.140 ± 0.0044	12.13 ± 0.0038
Trolox	96.55 ± 0.0025	97.32 ± 0.0049
α-Tocopherol	66.73 ± 0.0019	87.62 ± 0.0025

Table 4. $DPPH^{\bullet}$ radical scavenging activity results of *Gagea taurica*.

$DPPH^{\bullet}$ Radical Scavenging Activity		
	% Inhibition (400 μg/mL)	% Inhibition (1000 μg/mL)
Flower Extract (F)	21.26 ± 0.0545	71.13 ± 0.0150
Leaf Extract (L)	3.690 ± 0.0708	50.82 ± 0.0063
Corm Extract (C)	0.940 ± 0.1078	28.86 ± 0.0692
	% Inhibition (10 μg/mL)	% Inhibition (32.5 μg/mL)
Trolox	22.79 ± 0.0882	72.59 ± 0.2435
α-Tocopherol	17.39 ± 0.0052	38.49 ± 0.1106

inhibition [(TR)97.3 > (TK)87.6 > (F)29.6 > (L)26.5 > (C)12.1 %; at 32.5 μg/ml)]. $DPPH^{\bullet}$ scavenging activity was not found within the limits determined for *G. taurica* (Table 4).

α-Amylase and α-glucosidase inhibitory activities

All extracts had not showed any inhibitory activity against both α-glucosidase and α-amylase enzymes when compared with standard drug acarbose at any concentration. According to α-glucosidase inhibition assay, all the values of inhibition (%) were found as below zero. In the α-amylase inhibition assay, the inhibitory activity percentages were ranked as follows at a concentration of 5 mg/mL: acarbose (59 %) > leaf extract (43 %) > corm extract (39 %) > flower extract (37 %). The results are given in Table 5.

Acetylcholinesterase and butyrylcholinesterase inhibitory activities

None of the extracts exhibited any inhibitory activity against either AChE or BChE enzymes at any concentration, as compared to the standard drug donepezil. The leaf extract exhibited the most effective inhibitory activity in the AChE inhibition assay, whereas the flower extract demonstrated the most significant inhibitory activity in the BChE inhibition assay. It can be referred to Table 6 for detailed results.

Quantitative analysis of secondary metabolites

The LC/MS/MS technique was employed to quantitatively analyze 35 different phenolic compounds in three methanolic extracts of *Gagea taurica*. These compounds include quinic acid, fumaric acid, gallic acid, pyrogallol, keracyanin chloride, cyanidin-3-*O*-glucoside, chlorogenic acid, catechin, peonidin-3-*O*-glucoside, 4-OH-benzoic acid, epicatechin, epigallocatechin gallate, caffeic acid, vanillic acid, syringic acid, vitexin, naringin, ellagic acid, hesperidin, *p*-coumaric acid, sinapic acid, taxifolin, ferulic acid, rosmarinic acid, vanillin, myricetin, resveratrol, luteolin, quercetin, apigenin, naringenin, isorhamnetin, chrysin, galangin, and curcumin (Table 7).

7 compounds (Keracyanin chloride, cyanidin-3-*O*-glucoside, vanillic acid, hesperidin, *p*-coumaric acid, vanillin, and quercetin) were observed in the samples. Hesperidin, cyanidin-3-*O*-gluoco-

Table 5. The results of α-glucosidase inhibitory activity of *Gagea taurica*.

Concentration (μg/mL)	α-Glucosidase Inhibition (%)			
	Leaf	Flower	Corm	Acarbose
5000	−13.79 ± 3.53	−6.43 ± 6.98	−8.83 ± 1.66	62.88 ± 1.03
2500	−10.08 ± 5.72	−11.87 ± 1.91	−11.86 ± 0.43	49.05 ± 0.96
1000	−10.10 ± 1.05	−10.67 ± 2.79	−11.55 ± 2.13	31.58 ± 1.20
750	−11.04 ± 0.56	−10.93 ± 1.70	−12.55 ± 0.74	20.81 ± 2.35
500	−10.92 ± 0.36	−11.55 ± 0.95	−13.11 ± 0.41	16.39 ± 0.55
250	−4.91 ± 4.02	−10.19 ± 1.03	−11.88 ± 0.71	7.91 ± 2.04
100	−6.87 ± 6.61	−8.81 ± 2.96	−10.48 ± 2.23	6.51 ± 1.05
50	−8.20 ± 0.19	−6.22 ± 5.04	−10.91 ± 1.00	3.76 ± 2.40

Table 6. The results of AChE and BChE inhibitory activity of *Gagea taurica*.

Extracts	AChE Inhibition (%) (5 μg/mL)	BChE Inhibition (%) (500 μg/mL)
Leaf	3.83 ± 1.82	6.00 ± 4.51
Flower	1.58 ± 1.40	15.12 ± 0.85
Corm	-0.64 ± 3.54	-2.93 ± 9.33
Donepezil	97.36 ± 5.30	97.96 ± 6.70

side, and keracyanin chloride (cyanidin 3-*O*-rutinoside) were found the highest amount in extracts. Hesperidin was found as 1621.0001 ng/ml value in flower extract and 283.9339 ng/ml value leaf extract. Vanillic acid and *p*-coumaric acid were only found in corm extract. In addition, the amounts of hesperidin was found to be high in the leaf and flower extracts with the highest antidiabetic, anticholinesterase, antimicrobial, and antioxidant activities.

Discussion

Morphology and Taxonomy

Gagea was firstly assessed by Rix (1984)[15] in Flora of Turkey with 25 species including *G. taurica*. However, Tekşen and Karaman Erkul[16] reported that *G. taurica* is widely found in the North Caucasia and Crimea, does not occur in Turkey and the

Table 7. Quantitative analysis of 35 different phenolic compounds via LC/MS/MS in methanol extracts.

No.	Compound	Samples	Final Conc	Unit
1	Cyanidin-3-*O*-rutinoside (Keracyanin Chloride)	Flower	51.2573	ng/ml
		Leaf	15.8312	ng/ml
		Corm	0.3208	ng/ml
2	Cyanidin-3-*O*-glucoside	Flower	66.5908	ng/ml
		Leaf	21.6602	ng/ml
		Corm	< LOD	ng/ml
3	Vanillic Acid	Flower	< LOD	ng/ml
		Leaf	< LOD	ng/ml
		Corm	74.1939	ng/ml
4	Hesperidin	Flower	1621.0001	ng/ml
		Leaf	283.9339	ng/ml
		Corm	< LOD	ng/ml
5	*p*-Coumaric Acid	Flower	< LOD	ng/ml
		Leaf	< LOD	ng/ml
		Corm	3.4970	ng/ml
6	Vanillin	Flower	20.9817	ng/ml
		Leaf	7.8714	ng/ml
		Corm	44.7633	ng/ml
7	Quercetin	Flower	0.4344	ng/ml
		Leaf	0.0759	ng/ml
		Corm	0.0002	ng/ml

samples formerly identified as *G. taurica* are actually to refer to *G. commutata* or *G. alexeenkoana*. According to authors *G. taurica* differentiated from *G. alexeenkoana* with the strongly sclerified roots always enclose the bulb, the basal leaf is retroflexed at the tip, cauline leaves are hairy, tepals are hairy on the back as in our specimens. Tekşen and Karaman Erkul[16] and Zarrei et al. (2007)[1] were both indicated that in *G. commutata* tepals are narrowly long acuminate and pedicels are c. 4 times longer than tepals. On the contrary, in our specimen pedicels are shorter than lanceolate tepals. Thus, according these findings we are identified our samples as *G. taurica* (Figure 1). It is represented in here as a new record for the Turkish flora and the species number is increased 32.

Microscopic Analysis

Anatomical characteristics change as organs improve and care was taken to specimen mature basal leaves, pedicels and tepals. It has been found the same anatomical data in another investigations such as Novikov (2021)[17] and Zarrei et al. (2010).[18] The tepals of *Gagea* sp. is characterized by nectariferous tissue. There were various types of nectariferous tissue from *G. lutea*, *G. pusilla*, *G. reticulata*, *G. fragifera*, and *G. serotine*[17] The leaves of *G. alexeenkoana*, *G. anonyma*, *G. bergii*, *G. caroli-kochii*, *G. robusta*, *G. reticulata*, *G. setifolia*, *G. afghanica*, *G. bulbifera*, *G. dschungarica*, *G. lutea*, *G. kunawurensis*, *G. tenera*, *G. chomutovae*, *G. confusa*, *G. fragifera*, *G. gageoides*, *G. iranica*, and *G. villosa* were found as amphistomatic.[18] The results of light and electron microscopes were found similar.

Antimicrobial activity

In conclusion, there is a report that total parts of *G. stipitata* Merckl. ex Bunge extract did not show any antimicrobial activity against *Escherichia coli* ATCC 10536, *Klebsiella pneumoniae* ATCC 10031, *Salmonella typhi* PTCC 1185, *Bacillus subtilis* ATCC 6633, *Morganella morganii* PTCC 1078, and *Candida albicans* ATCC 10231. The extract was found significant effect against *Pseudomonas aeruginosa* ATCC 4027, and *Staphylococcus aureus* ATCC 29737 using the cylinder plate assay method. Zones of inhibition were expressed in comparison with gentamycine and clotrimazole.[19] To the best of knowledge, antimicrobial activities of the the corm, leaf and flower methanol extracts of *Gagea taurica* Steven has not been investigated. So this is the first study on antimicrobial activity for plant extracts.

Antioxidant Activity

Free radicals accumulate in cells over time and cause many diseases as they damage DNA, lipids and proteins. Free radicals can be taken into the body due to environmental factors, or they can occur in the body as a product of endogenous metabolism. Disruption of the oxidant/antioxidant balance in the body in favor of oxidants causes many diseases such as cardiovascular diseases, nervous system diseases, gastric and mucosal diseases, infertility, diabetes, inflammation and cancer.[3] Antioxidants are very important because they destroy free radicals. Studies have shown that some medicinal plants are effective against damage caused by free radicals, thanks to the active compounds they contain.[20] These antioxidant components produced by the plant for the purpose of self-defense and vitality, polyphenols, carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, tocotrienols etc. can be counted. Antioxidants are vital substances that have the ability to protect the body against oxidative stress caused by free radicals.[21]

In a study examining the antioxidant effect of ethanolic and methanolic extracts prepared from the leaves and bulbs of *G. fibrosa*, it was determined that the ethanolic extract prepared from the leaves had the lowest antioxidant capacity. When the methanol extracts were evaluated in themselves, it was determined that the leaf extract had higher antioxidant capacity (methanolic extract of leaf 72.54 % > ethanolic extract of leaf 33.23 %).[3]

In another study, the antioxidant capacity of water, acetone and methanol extracts prepared from the underground parts and fresh material of the *G. bohemica* plant were compared and it was determined that the acetone extract prepared from the underground parts had the lowest IC_{50} value (0.652 μg/ml; IC_{50}).[22]

α-Amylase and α-glucosidase inhibitory activities

According to literature, it is the first evaluation of antidiabetic activity of this species and genus. In contrast to our study, popular plants from Liliaceae family, the *Allium* genus (onion, garlic) has just displayed antidiabetic activity. The ether extract (0.25 g/kg body weight, orally) of bulbus of *Allium cepa* significantly reduced blood sugar level both in normal and alloxan-induced diabetic rabbits. In another study, juice of *Allium sativum* bulbus was found as effective in controlling the hyperglycemic effect when compared to tolbutamide.[23,24]

AchE and BChE inhibitory activities

According to our literature research, this study is the first investigation of anticholinesterase activity on the genus *Gagea*. In a study, to explore the mechanism underlying *Asparagus adscendens* Roxb. (Liliaceae) nootropic effects, it was investigated the impact on the activities of acetylcholinesterase in the cortex and hippocampus of mice. After 15 days of pretreatment with extract at doses of 50, 100, and 200 mg/kg (i.p.), it was observed a significant reduction in working memory errors, reference memory errors, and retrieval latency in the radial arm maze, as well as a decrease in step-down latency in the passive shock avoidance paradigm. Additionally, the extract administration led to a significant decrease in acetylcholinesterase activity and oxidative stress parameters in the cortex and hippocampus of mice.[25] In another study, it was investigated

the in vitro enzyme inhibition activities of the crude methanolic extract and various fractions of *Colchicum luteum* Baker (Liliaceae), including chloroform, ethyl acetate, butanol, and aqueous, against acetylcholinesterase and butyrylcholinesterase enzymes. The results showed that the crude methanolic extract and its fractions exhibited low to significant activity (32–75 %) was observed against butyrylcholinesterase. However, the crude methanolic extract and its various fractions demonstrated low activity (29–61 %) against acetylcholinesterase as in our study.[26]

Quantitative analysis of secondary metabolites

In vitro and *in vivo* experiments were conducted to test the inhibitory activity of cyanidin-3-rutinoside on intestinal α-glucosidase. The IC_{50} values of cyanidin-3-rutinoside for intestinal maltase and sucrase were determined to be 2.323 ± 14.8 and 250.2 ± 8.1 μM, respectively. Furthermore, treatment of normal rats with acarbose and cyanidin-3-rutinoside led to a greater reduction in postprandial plasma glucose levels compared to acarbose alone. These findings suggest that cyanidin-3-rutinoside inhibits α-glucosidase and may be useful as a potential inhibitor for the prevention and treatment of DM.[27] The study found that phenolic ligands bind strongly to the hairpin turn of Aβ1-40 and Aβ1-42 monomers and also identified binding sites in the tau fibril protein structures. New lead compounds, including cyanidin-3-*O*-rutinoside, luteolin-4′-*O*-rutinoside, luteolin-7,4-*O*-diglucoside, oleuricine A, isorhoifolin, and luteolin-7-*O*-rutinoside, were predicted, which could lead to the development and evaluation of novel compounds with potential efficacy against Alzheimer's disease.[28] It has been reported that hesperidin had useful effects on serum TNF-α, IL-6, and hs-CRP in the supplemented group. Obtaining results assists the anti-inflammatory effects of hesperidin in DM.[29] The study aimed to investigate the effects of hesperidin on retinal and plasma abnormalities in streptozotocin-induced diabetic rats, which may be related to increased production of advanced glycosylation end products and elevated aldose reductase activity, leading to oxidative stress and inflammation. The administration of hesperidin resulted in a notable augmentation in the thickness of the retina, while simultaneously decreasing levels of blood glucose and inflammatory biomarkers including TNF-α, ICAM-1, VEGF, and IL-1β. In addition, hesperidin treatment was found to considerably decrease plasma malondialdehyde (MDA) levels and increase superoxide dismutase (SOD) activity in diabetic rats.[30] Oral administration of hesperidin along with $AlCl_3$ injection for a period of 60 days resulted in a significant reversal of Al concentration, AChE activity, and Aβ synthesis-related molecules in the various brain regions examined. The detrimental effects of aluminum exposure on spontaneous locomotor and exploratory activities, as well as learning and memory impairments, were significantly attenuated by the administration of hesperidin. Furthermore, histopathological analyses of the hippocampus and cortex of rat brains demonstrated that hesperidin (at a dosage of 100 mg/kg) was effective in reducing the toxicity of $AlCl_3$ and preserving the normal histoarchitecture pattern of these brain regions. Based on these findings, it can be concluded that hesperidin may be a promising therapeutic agent for the treatment of memory loss induced by aluminum intoxication, through its ability to attenuate AChE activity and amyloidogenic pathways.[31] Bioflavonoids have emerged as promising neuroprotective agents for the treatment of various neurological disorders, including Alzheimer's disease (AD). In a study, three-month-old APPswe/PS1dE9 transgenic mice were randomly assigned to receive either vehicle, two different doses of hesperidin (50 or 100 mg/kg per day), or Aricept (2.5 mg/kg per day). After 16 weeks of treatment, it was observed that although there was no significant change in Aβ deposition in the hesperidin-treated (100 mg/kg per day) group, the administration of hesperidin (100 mg/kg per day) resulted in a reduction of learning and memory deficits, improvement in locomotor activity, and an increase in anti-oxidative defense and mitochondrial complex I–IV enzyme activities. Additionally, phosphorylation of glycogen synthase kinase-3β (GSK-3β) was significantly increased in the hesperidin-treated (100 mg/kg per day) group. These results suggest that the improvement in cognitive function in the APPswe/PS1dE9 transgenic mouse model of AD by hesperidin may be attributed to a reduction in mitochondrial dysfunction through the inhibition of GSK-3β activity, coupled with an increase in anti-oxidative defense.[32]

Conclusions

The investigation demonstrates the first study of the *in vitro* antimicrobial, antioxidant, antidiabetic activities, detailed anatomical and morphological properties of *Gagea taurica* Steven (Liliaceae). In particularly, flower extract showed the best antioxidant, α-glucosidase inhibitory, and antimicrobial effects. The anatomical and morphological structures of the root, corm, leaf, scapus, pedicel, tepal, anther, filament, stigma, stylus, and ovary of *G. taurica* were analyzed by light and electron microscopes. Hesperidin was found the highest level in all extract, so we could say it may be responsible for the activities. As a conclusion, we could conclude that *G. taurica* may be used as an antimicrobial, antidiabetic, antioxidant, and anti-alzheimer agent.

Experimental Section

Plant material

G. taurica was collected from Erzurum (Abdurrahman Gazi Türbesi), 1910 m at 1 May 2019 and identified by the authors Mehmet Bona. A voucher specimen has been conserved at Atatürk University, Biodiversity Application and Research Center with the number AUEF 1369.

Extraction

Dried corms (7 g), leaves (12 g), and flowers (10 g) were comminuted and macerated with methanol at room temperature via a mechanical blender at 200 rpm. The corm, leaf, and flower extracts

were infiltrated and evaporated by a rotating evaporator. The yields of corm, leaf and flower extracts were found as 1.03 g, 1.92 g, and 1.45 g, respectively. The extract yields for the corm, leaf, and flower extracts were determined as 14.71 %, 16 %, and 14.5 %, respectively.

Microscopic Analysis

For anatomical studies, sections were be made manually from the root, corm, leaf, flower (tepal, ovary, anther, filament, stigma) of *G. taurica* in 70 % alcohol, and the sections were be prepared with Sartur, chloral hydrate and distillate water reagents. Images prepared with these reagents were registered with a Zeiss 51425 camera connected to a light microscope (Zeiss 415500-1800-000). In the scanning electron microscopy (SEM) research, corm, leaf, and flower parts were connected to aluminium stubs and covered within gold for 4 min in a sputter-coater. Morphological investigations were made in a Jeol JSM 6490LV scanning electron microscope at the Turkish Petroleum International Company (TPAO) Research Center SEM laboratory, Ankara.

Antimicrobial activity (MIC, μg/mL)

Candida albicans ATCC 10231, *Candida krusei* ATCC 14243, *Candida tropicalis* ATCC 750, *Staphylococcus aureus* ATCC 6538, *Esherichia coli* ATCC 8739, and *Bacillus subtilis* ATCC 19659 were purchased from Microbiologics (San Diego, CA). The corm, leaf and flower methanol extracts of *G. taurica* with a final concentration range (2500 to 78.12 μg/mL) were diluted 2-fold initially. The standard drugs clarithromycin and terbinafine (64–0.125 μg/mL) were prepared within water and dimethyl sulfoxide (DMSO). Antimicrobial activity was determined utilising a slight modification of microdilution methods for aerobic microorganisms (M-7-A7) and fungi (M-27-A3) published by the Clinical Laboratory Standards Institute (CLSI).[33,34] The antimicrobial activity of methanol extracts obtained from the leaves, flowers and corms of the *Gagea taurica* were tested with respect to the method described in the reference Karakaya et al. (2022).[35]

Antioxidant and Free Radical Scavenging Activity

Determination of Total Phenolic Component

The determination of total phenolics of the methanolic extracts gained from different parts of the *G. taurica* was detected utilising the method developed by Folin and Denis and modified by Singleton[36,37] and was carried out some modifications. The details of determination of total phenolics of the samples was described in the reference Karakaya et al. (2022).[35] The test was conducted three times.

DPPH• scavenging activity

The DPPH• scavenging activities of methanol extracts obtained from the leaves, flowers and corms of the *Gagea taurica* were tested with respect to the Blois method[38] with slight modifications. The details of DPPH radical scavenging activities of methanol extracts was described in the reference Karakaya et al. (2022).[35] The test was conducted three times. The percentage inhibition levels of the reference standards were assessed at specific doses against DPPH·, determining the appropriate concentration range for the samples under investigation. The antioxidant properties of the samples, subjected to a serial dilution within the concentration span of 10–150 μg/mL, were gauged against a DPPH· solution. All measurements were taken at 517 nm, referencing an ethanol blank. Each analysis was iterated three times. The DPPH· scavenging efficacy was computed using the subsequent formula:

$$\% \text{ Inhibition} = [(\text{Acontrol} - \text{ASample})/\text{Acontrol}] \times 100$$

ABTS•+ scavenging activity

The ABTS•+ scavenging activities of methanol extracts obtained from the leaves, flowers and corms of the *Gagea taurica* were tested with respect to the Re method[39] with slight modifications. The details of ABTS radical scavenging activities of methanol extracts was described in the reference Karakaya et al. (2022).[35] The test was conducted three times. The antioxidant potentials of the specimens, formulated via sequential dilution within the concentration span of 10—150 μg/mL, were evaluated against an ABTS·+ solution. The measurements were taken at 734 nm, relative to a blank containing phosphate buffer. The ability to counteract ABTS·+ was determined using the subsequent formula:

$$\% \text{ Inhibition} = [(\text{Acontrol} - \text{ASample})/\text{Acontrol}] \times 100$$

α-Amylase and α-glucosidase inhibitory activities

α-*Glucosidase inhibitory assay*

The α-glucosidase inhibitory effect was established with respect to Bachhawat et al. (2011) with some small changes as defined in advance Yuca et al. (2021).[40,41] The details of the utilised method were described in the reference Karakaya et al. (2022).[35] The test was conducted three times. In a 96-well plate, a mixture of samples (20 μL), enzyme solution, and potassium phosphate buffer (50 μL, 50 mM, pH 6.9) was combined and incubated at 37 °C for 5 min. The reaction commenced by introducing the substrate, *p*-nitrophenyl-α-D-glucopyranoside, into each well. Subsequently, the mixtures were subjected to a 37 °C incubation for 30 min. To halt the reaction, 0.1 M sodium carbonate (50 μL) was introduced to each well. As a positive control, acarbose was utilized. Quantification of *p*-nitrophenol transpired through a 96-well microplate reader at 405 nm. The inhibition percentage was determined utilizing the subsequent formula:

$$\text{Inhibition } (\%) = (1 - \Delta\text{A405sample}/\Delta\text{A405control}) \times 100$$

α-Amylase inhibitory assay

The α-amylase inhibitory effect was established with respect to Nampoothiri et al. (2011)[42] with several small changes as characterised in advance Yuca et al. (2021).[41] The details of the utilised method were described in the reference Karakaya et al. (2022).[35] The test was conducted three times. In a 24-well microplate, a blend of samples (100 μL) and 1 % starch solution (100 μL) within a 20 mM sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride) underwent incubation at 25 °C for 10 min. Following this incubation, 100 μL of α-amylase solution (0.5 mg/mL) was introduced to each well, and the mixtures were subsequently incubated at 25 °C for an additional 10 min. The reaction was terminated by the introduction of a dinitrosalicylic acid color reagent (200 μL). The microplate was then heated in a boiling water bath for 5 min and allowed to cool down to 25 °C. Post-cooling, 50 μL from each well was extracted and transferred to a 96-well microplate. The reaction

mixture was diluted by supplementing it with 200 μL of distilled water, and the absorbance was gauged at 540 nm. Acarbose was employed as a positive control. The percentage inhibition was deduced employing the subsequent equation:

$$\text{Inhibition } (\%) = (1-\Delta A540\text{sample}/\Delta A540\text{control}) \times 100$$

AChE and BChE inhibitory activities

The acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities were performed based on the method of Ingkaninan et al. (2000) with slight modifications.[43] 125 μL of 5,5′-dithiobis-(2-nitrobenzoic acid) (3 mM, DTNB, Ellman's Reagent), 25 μL of the substrate (15 mM, acetylthiocholine iodide for AChE and butyrylthiocholine iodide for BChE), 50 μL of Tris-HCl buffer (50 mM, pH 8), and 25 μL of the sample were mixed in a 96-well plate. Finally, 25 μL of the enzyme (AChE and BChE) were added to the mixture and the reaction was incubated for 10 min for AChE inhibition assay and 15 min for BChE inhibition assay. The reaction was then measured spectrophotometrically at 405 nm. Donepezil was used as the positive control. Each assay was performed three times. The percentage inhibition was calculated using the following equation:

$$\text{Inhibition } (\%) = (1-\Delta A_{405\text{sample}}/\Delta A_{405\text{control}}) \times 100$$

Quantitative analysis of secondary metabolites

Quantitative analysis of secondary metabolites in the three methanolic extracts was established with the Agilent 6460 Triple Quadrupole Liquid Chromatography-Tandem Mass/Mass Spectrometer System (LC/MS/MS), a system created by combining chromatography and spectroscopy systems at Eastern Anatolia Atatürk University High Technology Application and Research Center (DAYTAM). Separation of analytes Agilent Poroshell 120 EC–C18 (4.6×100 mm, 3.5 μm) column equipped with the Agilent 1260 HPLC system was utilised. HPLC systems were operated in positive ion mode with electrospray ionization (ESI). Firstly, the protonated product ion $[M+H]^+$ was established for determination and quantification by working in the reference scanning mode of each compound prepared at specific concentrations. The dual carrier phase utilised for this occurs in carrier phase A (0.5% formic acid and water), and carrier phase B (0.5% formic acid-acetonitrile). The volume of the injected sample was 5.0 μl and the analysis was conducted using the MRM (Multiple Reaction Monitoring) modes.

Statistical analysis

Entire tests were carried out in triplicate for in vitro antidiabetic and antioxidant assays. Kruskal-Wallis test was utilised to establish statistical expressiveness. The data were analyzed utilizing SPSS (IBM SPSS Statistics 20, IBM Corporation, Armonk, NY, USA) at the expressiveness level of $P=0.05$. The IC_{50} value data for specimens are presented as averages ± standard deviation.

Author Contributions

H. Yuca and S. Karakaya collected plant material for investigations. M. Bona identified the plant. H. Yuca, S. Karakaya, G. Ekşi, E. Tekman performed anatomical and morphological analyses. H. Yuca, Z. Güvenalp, B. Aydın, G. Göger, E. Tekman performed biological activities. A. Acar Şahin and N. M. Pınar performed SEM analysis. H. Yuca, S. Karakaya, O. Sytar wrote the draft manuscript. All the authors revised and edited the manuscript. All authors approved the published version of the manuscript.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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