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Antioxidant Effects and Fatty Acid Analysis of *Leucojum aestivum* Seed Coat Extracts

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Abstract

The aim of this study is to analyze the biochemical composition and antioxidant activity of *Leucojum aestivum* seed coats. In the crude extract the total phenolic content was determined to be 129.55 ± 1.94 mg gallic acid equivalent (GAE)/g, while the total flavonoid content was found to be 210.21 ± 7.91 mg quercetin equivalent (QE)/g. Additionally, the total flavonol, tannin, and proanthocyanidin contents were determined to be 9.63 ± 5.16 mg QE/g, 3.32 ± 0.14 mg GAE/g, and 129.89 ± 6.10 mg catechin equivalent (CAE)/g, respectively. Furthermore, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity showed a significant antioxidant potential with a concentration that results in 50% decrease in the initial DPPH concentration IC_{50} value of 214.97 ± 50.44 mg QE/g in the crude extract. GC/MS analysis revealed the presence of various biologically active compounds, including hexadecanoic acid, oleic acid, and stigmasterol. Moreover, galantamine and galathan were identified in the seed coat extracts. Fatty acid analysis indicated the presence of compounds such as linoleic acid, oleic acid, and palmitic acid. This comprehensive study provides a detailed examination of seed coats and highlights the presence of biologically active compounds such as galantamine. This finding represents a study conducted for the first time in the literature, as no similar research focusing on *L. aestivum* seed coats has been conducted previously. Therefore, the results of the study provide new insights into the potential biological and pharmacological effects of seed coats and offer significant findings that could serve as a basis for future research.

Keywords

antioxidant activity, phenolic compounds, galantamine, GC-MS analysis, *Leucojum aestivum*

1 Introduction

Leucojum aestivum is a bulbous perennial plant in the *Amaryllidaceae* family [1]. It naturally thrives in various regions of Türkiye, particularly in moist and semi-shaded habitats such as marshes, wetlands, and floodplains, ranging from sea level to elevated altitudes [2]. Commonly known as "summer snowflake", "loddon lily", and "snowflake lily" in various indigenous languages, it is referred to as "snowflake" in English [3]. *L. aestivum* is a medicinal and ornamental plant native to Türkiye, the Balkans, the Caucasus, and Southern Europe [2].

Amaryllidaceae alkaloids are exclusively isolated from the *Amaryllidaceae* plant family. Oil extracted from *Narcissus poeticus* has been used to treat uterine tumors [4]. Since the isolation of lycorine from *Lycoris radiata* in 1897 [5] and *Narcissus pseudonarcissus* in 1877 [6], the structures of numerous *Amaryllidaceae* alkaloids have been elucidated. Galantamine, widely found in *Amaryllidaceae* plants, has been approved by the United States Food and Drug Administration for the treatment of Alzheimer's disease [7]. The acetylcholinesterase inhibitor

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galantamine, still used in the treatment of Alzheimer's disease, inhibits acetylcholinesterase, which helps break down the neurotransmitter responsible for synaptic transmission [8]. Additionally, galantamine binds allosterically to nicotinic acetylcholine receptors in the central nervous system, modulating their conformation and increasing neurotransmitter release [9]. Its neuroprotective effect is evidenced by its ability to reduce oxidative stress, a common side effect in many human diseases including Alzheimer's, Parkinson's, Down syndrome, and cancer [10].

Understanding the chemistry and functional significance of seed coats, as well as their role in plant physiology, is crucial. However, the existing knowledge in this field mainly relies on histochemical studies involving staining processed or fresh plant material with pigments that interact at a molecular level with cell components and walls. The biochemical composition of seed coats significantly impacts seed germination and other physiological processes [11, 12]. Particularly, previous research on orchids has highlighted the importance of seed coat structures in germination and physiological effects [13].

The biochemical makeup of seed coats crucially influences nutrient storage, preservation, and release during and after germination. Additionally, seed coats act as vital barriers against environmental stresses and pathogens, affecting seed germination and subsequent plant development. Therefore, further exploration of seed coat biochemistry holds promise for enhancing productivity in plant cultivation and agricultural production.

This study aims to investigate the chemical composition and biological activity of *L. aestivum* seed coats. Extracts obtained from seed coats were analyzed for total phenolic, flavonoid, flavanol, tannin, and proanthocyanidin content, along with their antioxidant capacities using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Furthermore, the fatty acid composition of seed coats was assessed using gas chromatography-mass spectrometry (GC/MS) technique.

2 Material and methods

2.1 Chemical and reagents

For extraction, Sigma HPLC grade methyl alcohol was used. Merck HPLC purity grade hexane was used for the extraction of fatty acid components. 37% Merck fuming HCl was used for hydrolyzation. Flavonoid standards were of Sigma pure grade (quercetin, gallic acid, catechin). Deionized water was obtained using Millipore (Ultrapure Milli-Q) Water Purification Systems.

2.2 Collection of seed samples

In this study, the seeds of *L. aestivum*, commonly found in Samsun, Türkiye, were collected in July 2023. Mature capsules were opened in the laboratory, dried at room temperature for several days, and then stored at 4 °C in brown glass bottles. Subsequently, seed husks were separated from the embryos and prepared for analysis by grinding them into a powder using a blender (Fig. 1).

The seed coats of *L. aestivum* plant samples were first pulverized into fine powder. For the maceration method, 0.5 g of powder was extracted with 10 mL of 80% methanol at 35 °C for 24 h [14]. The schematic pathway for the source material and solvent extract starting from *L. aestivum* is shown in Fig. 2.

2.3 Phytochemical content analysis

2.3.1 Total phenolic content

In this study, equal volumes of the sample and diluted Folin-Ciocalteu reagent were mixed. After incubating at room temperature for 3 min, 1 mL of 2% sodium carbonate solution was added. The mixture was then left to incubate in the dark at room temperature for 1 h, followed by measuring the absorbance at 760 nm using a UV spectrophotometer. The total phenolic content was expressed as gallic acid equivalent (GAE) in milligrams per gram of dried extract (mg GAE/g extract). All measurements were performed in

Fig. 1 (a) *L. aestivum* flowering phase; (b) Transition to seed stage in *L. aestivum*; (c) Extraction of seeds from capsules; (d) Separation of seed coats

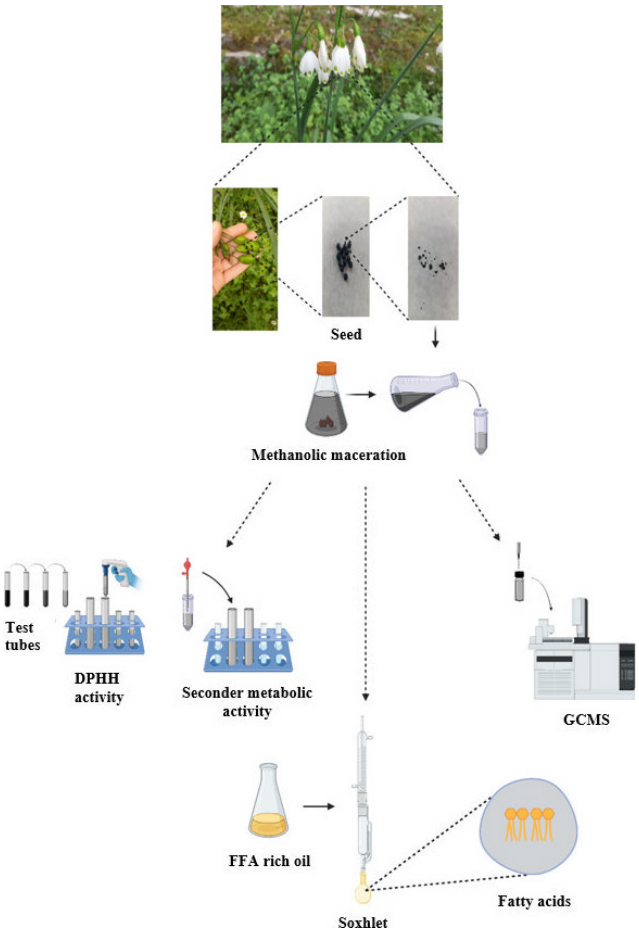


Fig. 2 The schematic pathway for the source material and solvent extract of *L. aestivum* is show

triplicate [15]. The results were expressed in milligrams of GAE per gram of crude extract (mg GAE/g crude extract), using the linear regression equation derived from the gallic acid calibration curve ($y = 0.134x + 0.1059$, $R^2 = 0.9962$).

2.3.2 Total flavonoid content

This method was assessed using the $AlCl_3$ method with minor adjustments, following the procedure by [16]. Extracts were mixed with distilled water, followed by $NaNO_2$ (5%) addition and a standing period. Subsequently, $AlCl_3$ (10%) was added, and the solution underwent incubation. NaOH (1 M) was then introduced, and the solution was left at room temperature. Absorbance was measured using a UV spectrophotometer, and total flavonoid content was quantified as quercetin equivalent (QE) per gram of dried extract (mg QE/g). All measurements were performed in triplicate. The total flavonoid content was calculated using the linear regression equation from the quercetin calibration curve ($y = 0.078x + 0.0222$, $R^2 = 0.9789$).

2.3.3 Total flavanols content

The total flavonoid content was determined using the aluminum chloride method [17]. Briefly, 1 mL of the extracts was mixed with of $AlCl_3$. Subsequently, 3 mL of sodium acetate solution was added. The mixture was then kept at room temperature in a dark environment for 30 min. After the incubation period, the absorbance of the sample was measured at 415 nm using quercetin as the standard. The total flavanols was expressed as quercetin equivalents (mg QE/g). Flavonol content was determined employing the linear regression equation derived from the quercetin calibration curve ($y = 0.0363x + 0.1767$, $R^2 = 0.9831$).

2.3.4 Total tannin content

Total tannin content was assessed using the Folin–Ciocalteu reagent as described in [18]. A calibration curve was prepared using different concentrations of gallic acid in methanol. Samples were mixed with diluted Folin–Ciocalteu reagent in water and aqueous sodium carbonate solution. After incubation in darkness at room temperature, absorbance was measured at 760 nm. Total tannin content was quantified as GAE per gram of dried extract (mg GAE/g). All measurements were performed in triplicate. Total tannin content was quantified using the linear regression equation of the gallic acid calibration curve ($y = 0.0103x + 0.0848$, $R^2 = 0.9942$).

2.3.5 Total proanthocyanin content

Total proanthocyanidins content was determined using the butanol-acid assay [19]. Diluted phenolic extract was mixed with *n*-butanol/HCl reagent, followed by the addition of ferric ammonium sulfate in HCl. After boiling and cooling, the absorbance of the solutions was measured at 550 nm. Total proanthocyanidins content was expressed as catechin equivalent (CAE) per gram of dried extract. All measurements were performed in triplicate. The total proanthocyanidin content was determined utilizing the linear regression equation from the catechin calibration curve ($y = 0.0038x + 0.3639$, $R^2 = 0.9978$).

2.4 DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

The DPPH assay was employed to evaluate the free radical scavenging potential of the extracts, with modifications based on [20]. Extracts at various concentrations were mixed with a methanol solution of DPPH radical, incubated in the dark at room temperature, and absorbance was measured at 517 nm using a UV spectrometer against a blank. Ascorbic acid served as a reference standard. The percent-

age of DPPH radical scavenging activity of the extract was calculated using the following formula.

DPPH scavenging activity (% inhibition) = $[(A_{control} - A_{sample}) / A_{control}] \times 100$, where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the reaction mixture with the extract. To determine the concentration of the extract required to cause a 50% decrease in the initial DPPH concentration, a concentration curve of the extract versus percentage inhibition was constructed, and the IC_{50} value was obtained through linear regression analysis. A lower IC_{50} value indicates higher antioxidant activity. All measurements were conducted in triplicate.

2.5 Extraction and analysis of the fatty acid

Fatty acid analysis was performed on *L. aestivum* samples following the protocol by [21]. Oil extraction was conducted using a manual Soxhlet apparatus. For GC analysis (Thermo Scientific Trace 1310), fatty acids were converted into methyl esters through derivatization. The extracted oil (0.25 g) was dissolved in 4 mL of heptane, and 0.4 mL of 2 M KOH in methanol was added. The mixture was vortexed for 2 min, followed by centrifugation at 5000 rpm for 5 min. The clear heptane phase was transferred to glass tubes for GC/MS analysis and injected into the instrument using an autosampler (Autosampler AI 1310).

The samples were analysed using a Thermo Scientific ISQ LT GC/MS gas chromatograph mass spectrometer, equipped with a Trace Gold TG-WaxMS capillary column (Thermo Scientific code: 26088–1540). The column temperature program started at 100 °C for 3 min, followed by an increase at a rate of 4 °C/min up to 240 °C. Helium gas was used as the carrier gas at a flow rate of 1 mL/min with a 1:20 split ratio. The MS unit (ISQ LT) operated in electron ionization mode (70 eV). Fatty acid identification was achieved by comparing their retention times with those of a standard fatty acid methyl ester (FAME) mixture consisting of 37 compounds. The FAME content was measured by gas chromatography following the European regulated procedure EN 14103:2020 [22]; Methyl heptadecanoate served as an internal standard [23].

$$\text{FAME yield}(\%) = \left(\sum A - A_{EI} / A_{EI}\right) \times \left(C_{EI} \times V_{EI} / m\right) \times 100 \quad (1)$$

In Eq. (1), $\sum A$ represents the total peak area of the methyl esters, A_{EI} is the peak area of the internal standard, C_{EI} (mg/mL) denotes the concentration of the internal standard solution, V_{EI} (mL) refers to the volume of the internal standard solution, and m (mg) is the mass of the sample. To examine the influence of external mass transfer resistance during the reaction, experiments were conducted at various stirring rates of 250, 500, 750, and 1000 rpm. The effect of external diffusion was considered negligible, as identical FAME yields were obtained at stirring rates of 500, 750, and 1000 rpm. Consequently, all experiments were performed at a stirring rate of 750 rpm. Internal diffusion limitations were deemed negligible due to the very small particle size of the catalyst (<120 mesh) used in slurry reactors.

2.6 Extraction and GC/MS analysis

Detailed analyses for the detection of galantamine content in *L. aestivum* seed coat samples are described below.

Firstly, *L. aestivum* seed coats were dried in an oven at 40 °C and then ground into powder using a blender. Subsequently, using the maceration method, 0.5 g of the powder was extracted with 10 mL of 80% methanol at 35 °C for 24 h. Optimization structures were analyzed following the GC/MS analysis method suggested by [14]. Briefly, the sample was centrifuged at 3500 rpm for 10 min, and the supernatant was used for GC/MS analysis. GC/MS analysis was performed using a SHIMADZU GCMS-QP2010 mass spectrometer equipped with an AOC-5000 auto injector. An Rxi-5MS column (30 m × 0.25 mm × 0.25 µm) was used for the analysis, with a scanning range between 30–450 Da. The analysis conditions were as follows: electron ionization system, ionization energy of 70 eV, helium gas at a constant flow rate of 1 mL/min, injection volume of 1.5 µL (with a 10:1 split ratio), injector temperature of 250 °C, and ion source temperature of 200 °C. The oven temperature was initially held constant at 70 °C for 10 min, then increased at a rate of 3 °C/min to 150 °C and maintained at this temperature for 5 min. Subsequently, the oven temperature was increased at a rate of 10 °C/min to 250 °C and held at this temperature for 5 min. The solvent delay was set from 0 to 2 min, with a total GC/MS run time of 56.67 min. Seed coat samples extracted with methanol using the liquid sampling method were diluted 100 times and placed in 1.5 mL vials. The NIST Standard Reference database was used for the analysis. This method successfully detected the presence of galantamine.

2.7 Statistical analysis

All the data were subjected to statistical analysis using IBM SPSS Statistics 22 software [24].

3 Results and discussion

The obtained results were expressed as mg GAE/g crude extract using the linear regression equation of the gallic acid calibration curve. The total phenolic

content of the seed husk extract was determined to be 129.55 ± 1.94 mg GAE/g crude extract, total flavonoid content was 210.21 ± 7.91 mg QE/g crude extract, total flavonol content was 9.63 ± 5.16 mg QE/g crude extract, tannin content was 3.32 ± 0.14 mg GAE/g crude extract, and total proanthocyanidin content was 129.89 ± 6.10 mg CAE/g crude extract. The DPPH content of the seed husk extract was found to be 214.97 ± 50.44 mg QE/g crude extract. These results indicate that seed husks are rich in phenolic compounds, and the contents are presented in Table 1. Additionally, Table 1 demonstrates the DPPH radical scavenging activity of *L. aestivum* seed husks, showing high antioxidant activity. Phenolic compounds are significant due to their strong antioxidant properties. The capacity of polyphenolic compounds found in plants to neutralize free radicals is of great importance. The antioxidant activities present in plant extracts play a significant role in various diseases, including neurodegenerative diseases such as Parkinson's and Alzheimer's, as well as many other illnesses [25, 26].

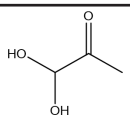
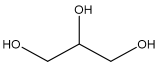
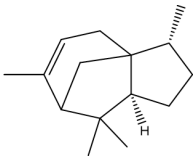
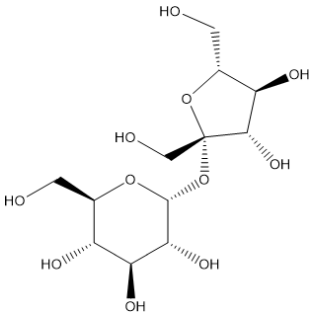
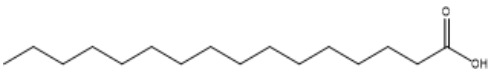
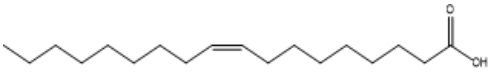
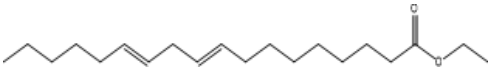
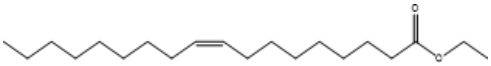
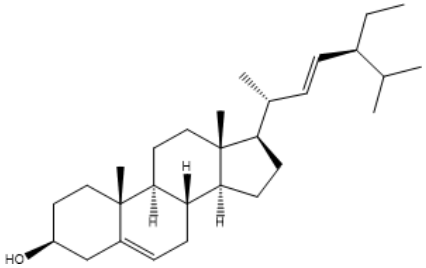
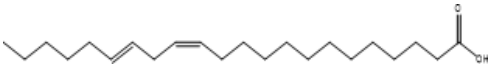
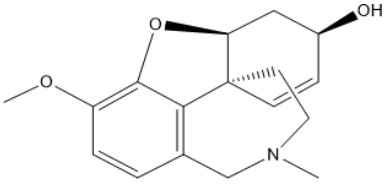
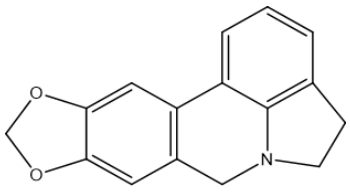
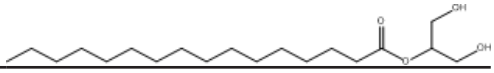
In a study conducted by Al-Faris et al., the methanolic extracts of *L. aestivum* flowers and leaves were evaluated for their DPPH antioxidant activity and were found to exhibit superior antioxidant activity compared to methanolic extracts from bulbs. This study reported *L. aestivum* as an important source of antioxidants [27]. Another study by Demir et al. [3] focused on determining the total phenolic content of *L. aestivum* bulbs and leaves. The findings indicated that the phenolic content in bulbs ranged from 2.51 to 30.24 mg GAE/g, while in leaves, it ranged from 10.47 to 21.03 mg GAE/g. Additionally, the flavonoid content in bulbs ranged from 3.10 to 70.70 mg QE/g, whereas in leaves, it ranged from 71.49 to 170.85 mg QE/g [3]. In an *in vitro* culture study conducted by Ates et al. [28], the total phenolic content of *L. aestivum* bulbs and leaves was reported to be 19.02 ± 0.08 GAE/g and 11.46 ± 0.03 GAE/g, respectively. The total flavonoid content of methanol extracts from *L. aestivum* bulbs and leaves was found to be 8.22 ± 0.02 mg QE/g and 41.50 ± 0.11 mg QE/g, respectively. Additionally, the IC_{50} value for methanol extracts of *L. aestivum* bulbs and leaves was reported to be >20 mg/mL *in vitro* culture [28]. In a study by Hundur et al. [29], it was observed that the total phenolic content was higher in *L. aestivum* bulbs (58.92 mg GAE/g) and leaves (53.93 mg GAE/g). Furthermore, the flavonoid content in bulbs was higher than that in leaves (85 mg QE/g compared to 68.33 mg QE/g). Moreover, the antioxidant activity of *L. aestivum* bulbs and leaves was reported to be 317 µg/mL and 345 µg/mL, respectively, indicating a strong antioxidant potential [29]. In a study by Nikolova et al., the IC_{50} value for the methanol extract of naturally grown *L. aestivum* leaves was reported to be >200 µg/mL [30]. Resetár et al. found antioxidant activity in naturally grown *L. aestivum* bulbs and leaves [31]. In a study by Abdallah et al. [32], the total polyphenol content of *Narcissus tazetta* flower ethanol extracts were reported to be 64.14 mg GAE/g DW, and the flavonoid content was reported to be 70.79 mg CE/g DW. Additionally, the total antioxidant activity was determined to be 134.57 mg GAE/g DW [32]. In a study by Eröz Poyraz et al., the total phenolic content of methanol:chloroform (v:v) extracts of *Narcissus* sp. carlton bulbs, belonging to the *Amaryllidaceae*, ranged from 20.78 ± 0.25 to 57.97 ± 0.49 mg GAE/g extract [33]. Boshra et al. [34] reported that the total phenolic content of total ethanol extract from *N. pseudonarcissus* bulbs was 1.29 ± 0.005 mg GAE/g crude extract, and the total flavonoid content was 1.19 ± 0.04 mg QE/g crude extract. The total phenolic content of the petroleum ether fraction from *N. pseudonarcissus* bulbs was 0.03 ± 0.01 mg GAE/g crude extract, and the total flavonoid content was 0.31 ± 0.01 mg QE/g crude extract. The total phenolic content of the aqueous fraction from *N. pseudonarcissus* bulbs was 0.96 ± 0.008 mg GAE/g crude extract, and the total flavonoid content was 0.11 ± 0.00 mg QE/g crude extract [34].

Various bioactive compounds were identified in the methanol extract of *L. aestivum* seed husks. The retention time (RT), concentration (% area), and chemical structure of 14 bioactive phytochemical compounds in the extract are presented in Table 2. The main components of the seed husk were identified as hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (11.03%), oleic acid (8.71%), and stigmasterol (6.29%). Additionally, galantamine (0.86%) and galathan, 1,2,3,12,15,16-hexadehydro-9,10-[methylenebis(oxy)] (5.06%) were found in the

Table 1 Antioxidant activity and phenolic content of seed coats

Plant name	DPPH (IC_{50} mg/mL)	Total flavonol compound (mg QE/g extract)	Total flavonoid compound (mg QE/g extract)	Total phenolic compound (mg GAE/g extract)	Total proanthocyanidin content (mg CAE/g extract)	Total tannin content (mg GAE/g extract)
L. aestivum-seed coat	214.97 ± 50.44	9.63 ± 5.16	210.21 ± 7.91	129.55 ± 1.94	129.89 ± 6.10	33.25 ± 1.43

Table 2 GC/MS analysis results and molecular structures of seed coat

No.	RT (min)	Name of the compunds	Area %	Probability %	Structure
1	3.271	Dihydroxyacetone	3.33	85	
2	5.196	Glycerin	1.51	90	
3	27.043	1-Pentadecene	1.31	80	
4	30.454	1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro	1.75	65	
5	31.453	Sucrose	4.40	97	
6	48.021	n-Hexadecanoic acid	2.24	90	
7	50.333	Oleic acid	8.71	88	
8	50.534	9,12-Octadecadienoic acid, ethyl ester	1.72	84	
9	50.603	Ethyl oleate	1.97	89	
10	51.850	Stigmasterol	6.29	87	
11	52.594	cis-13,16-Docasadienoic acid	1.90	78	
12	53.148	Galantamin	0.86	74	
13	54.404	Galathan, 1,2,3,12,15,16-hexadehydro-9,10-[methylenebis(oxy)]-	5.06	62	
14	54.746	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	11.03	93	

seed husk. The most abundant fatty acids in the seed husk were determined to be total linoleic acid (24.11%), oleic acid (18.29%), cis-13,16-docosadienoic acid (10.04%), and palmitic acid (8.85%). These findings provide an understanding of the bioactive composition of *L. aestivum* seed husk extract. These results, along with the GC/MS analysis of seed husks and the molecular structures, are presented in Tables 2 and 3, which include the results of seed husk fatty acid composition.

L. aestivum, a species under threat from the *Amaryllidaceae*, produces alkaloids with biological effects, among which galantamine has been identified as the most important. Today, galantamine is widely used in the treatment of Alzheimer's disease, poliomyelitis, and other neurological disorders as it is recognized as an acetylcholinesterase inhibitor [9].

In a study by Berkov et al., alkaloid fractions obtained from *L. aestivum in vitro* cultures were analyzed, and galantamine and N-formylnorgalantamine were detected through GC/MS analysis [35]. Similarly, Pavlov et al. analyzed alkaloid fractions from young fruits of *L. aestivum* and reported the detection of galantamine through GC/MS analysis [36]. Methanolic extracts of *L. aestivum* bulbs and leaves were analyzed using HPLC. The content of galantamine in bulbs ranged from 6.89 to 25.37 mg/g throughout the year, whereas in leaves, it ranged from 1.82 to 8.09 mg/g [2].

The study by Zhang et al. highlighted the efficacy of galantamine, administered at doses of 24 mg and 32 mg, in alleviating symptoms associated with mild and moderate Alzheimer's disease [37]. Additionally, linoleic acid isolated from the fruit of *Holigarna caustica* was reported by Panda et al. to exhibit nematocidal properties [38]. Linoleic, oleic, and docosahexaenoic acids have been recognized as essential components in the human diet due to their important roles [39]. Furthermore, oleic acid, identified in the seed extract of *Raphia taedigera*, was found by Awonyemi et al. to possess antifungal, anti-inflammatory, antioxidant, and antibacterial activities [40]. Moreover, linoleic acid was reported by Mizushina et al. to exhibit anti-inhibitory activity on DNA polymerase β [41]. Linoleic acid has also been shown to have topical anti-inflammatory activity in mouse models induced with tissue plasminogen activator (TPA) [42], while palmitic acid was found to exhibit anti-inflammatory effects among the identified compounds [43].

Table 3 Results of fatty acid composition in *L. aestivum* seed coats

No.	Retention time (min)	Name of the fatty acid	Area %
1	24.50	Palmitic acid	8.85
2	28.25	Stearic acid	6.15
3	28.74	Oleic acid	18.29
4	29.81	Linoleic acid	24.11
5	30.04	Linolelaidic acid	1.15
6	30.99	α-Linolenic acid	1.13
7	31.37	Arachidic acid methyl ester	1.54
8	34.26	Behenic acid	0.66
9	34.86	Erucic acid	0.85
10	35.98	Trichosanoic acid	4.04
11	35.99	cis-13, 16 Docosadienoic acid	10.04
12	37.68	Nervonic acid	0.74

Galantamine, an alkaloid, is obtained from various plant species such as *Galanthus* spp, *Leucojum* spp, and *Narcissus* spp, from bulbs and above-ground parts [44]. Its isolation was first reported in 1952 from *Galanthus woronowii* in the Caucasus region. Subsequently, it was isolated from *Galanthus nivalis* in Bulgaria in 1957 [45]. Extracts of *G. nivalis* have been traditionally used in folk medicine around Eastern Europe and the Mediterranean. In the early 1960s and 1970s, galantamine, under the trademark Nivalin, was used in the treatment of neurological diseases such as poliomyelitis, myasthenia gravis, and myopathy in Eastern European countries [45, 46].

In a study by Hasan et al., the bioactive compounds of *Narcissus tazetta* were determined through HPLC analysis, revealing salicylic acid, sinapic acid, anilic acid, caffeic acid, chlorogenic acid, p-coumaric acid, and galanthamine [47]. Another study identified galantamine, sanguinine, narwedine, demethylmaritidine, anhydrolycorine, O-methylnorbelladine, pancratinin C, lycorine, 9-O-methylpseudolycorine, pseudolycorine, 1-O-acetyl-3-O-methylnarcissidine, 11-hydroxygalantamine, narcissidine, 9-O-demethyl-2alpha-hydroxyhomolycorine, ismine, tazetine, lycorenine, lycorine, masonine, 3-epimacronine, 1,2-dihydroclidanidine, assoanine, hippeastrine, and 4,5-ethylene-8,9-dimethoxy-6-phenanthrendione [48–50].

In our study, galantamine stood out among the bioactive compounds in *L. aestivum* seed husk extract. When compared to *Narcissus* spp. species, it was observed that galantamine, a bioactive compound present in these plant species, was also found in *L. aestivum*, indicating similar contents among these plant species. Our research examined the phytochemical and antioxidant effects of *L. aestivum* seed husks, confirming the presence of galantamine through GC/MS analysis. While there are numerous studies related to *L. aestivum* in the literature, studies specifically focusing on seed husks are lacking. The richness of *L. aestivum* seed husks in phenolic compounds and their

significant antioxidant activity suggest potential benefits not only in the field of health but also in the development of more efficient seed germination techniques in agriculture, and positive effects on plant cultivation. Furthermore, understanding the biochemical properties of seed husks can contribute significantly to the preservation of plant genetic resources and breeding studies.

4 Conclusions

This study marks the first comprehensive analysis of the biochemical composition of *Leucojum aestivum* seed coats, uncovering not only the presence of significant levels of fatty acids but also the inclusion of galantamine within this structure. This discovery underscores the vital role of seed coats in plant physiology and nutrient storage. Moreover, the identification of biologically active compounds such as galantamine in seed coats indicates the potential of this plant component as a source for developing plant-derived medications. These findings offer promising prospects for further research in both plant cultivation practices and pharmacological investigations. Overall, this study paves the way for new avenues of exploration in understanding the biochemical makeup of seed coats and their potential applications in medicine and agriculture.

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