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Research Article

Study of phytochemical compounds of *Plantago major* leaves grown in Kazakhstan

Aknur Turgumbayeva[1], Kairat Zhakipbekov[2], Zhanar Shimirova[3], Sholpan Akhelova[4], Akerke Amirkhanova[2], Moldir Koilybayeva[2], Gulnaz Seitimova[5], Daniyar Abdambayev[2]

1 *Al-Farabi Kazakh National University, Almaty, Kazakhstan*
2 *Asfendiyarov Kazakh National Medical University KazNMU, Almaty, Kazakhstan*
3 *South Kazakhstan Medical Academy, Shymkent, Kazakhstan*
4 *NJSC "Astana Medical University", Astana, Kazakhstan*
5 *Al-Farabi Kazakh National University, Faculty of Chemistry and Chemical Technology, Almaty, Kazakhstan*

Corresponding authors: Aknur Turgumbayeva (turgumbayeva.aknur@med-kaznu.com), Kairat Zhakipbekov (zhakipbekov.k@kaznmu.kz)

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Abstract

Leaves of *Plantago major* have been used for centuries to treat diseases relating to skin, digestive organs and blood circulation like wounds, inflammation and hypertension. *P. major* leaves contain biologically active substance and naturally compounds such as essential oils, minerals and amino acids beside polysaccharides, lipids, caffeic acid and its derivatives, iridoid glycosides, flavonoids and terpenoids. This work carried out an examination of *Plantago major* leaves in terms of phytochemical composition of methanol extract. The leaves contents in chosen nutrients, specifically amino acids and minerals, are moreover depicted. Gas Chromatography -Mass Spectrometry analysis identified the presence of 31 phytochemical compounds and a total amount of 20 amino acids (essential and nonessential) justifying its use as drugs and biological active supplements and its mineral content showed the presence of 7 essential chemical elements in *Plantago major* leaves. Nonetheless, additional research into this plant is needed because it has the potential to be used to development of new drugs and biological active supplements.

Keywords

Amino acids, chemical composition, endogenous plant, GC-MS, *Plantago major*

Introduction

The leaves of *P. major* have been used for centuries as a wound healing agent in Kazakhstan and in the treatment of a number of diseases, in addition to wound healing. These include diseases related to the skin, respiratory organs, digestive organs, reproduction, circulation, to relieve pain and against infections. Outwardly, plantain large has anti-inflammatory, antimicrobial, antipruritic and wound healing effects. The macerated leaves or the fresh juice of the plant are excellent quick-healing remedies for cuts, wounds, bruises, and earaches (Yernazarova et al. 2019).

Throughout a timeline of human evolution, people have sought out natural remedies to improve their well-being and treat disease. And concurring to mankind's involvement over the centuries, treatment with restorative plants is considered exceptionally secure as there's no or negligible side effects (Wal et al. 2021). The helpful benefits of restorative plants have been perceived around the world and much logical investigation has been done to

demonstrate their pharmacological activities. It has been suggested that the abundance of research was motivated by the increased pharmacopoeial, non-pharmacopoeial or synthetic drugs (Cragg et al. 2014). In reality, nearly half of the current pharmaceutical medications are determined from plants as previously reported (Newman and Cragg 2007). The ability of chemical compounds in particular plants to exhibit therapeutic effects is one of the key elements driving this trend. For occasion, therapeutic plants are wealthy supplies of phenolic compounds which act as a strong antioxidant (Jaberian et al. 2014).

Plantago major is a perenial medicinal herb that belongs to the genus *Plantago* from the Plantaginaceae family. *Plantago major* structure contains a disposition of action of leaves radiating from the base of the stem, and bears several long with erect flower stalks It can become about 15 cm high, but the size varies a lot depending on the growth habitats. The leaves grow in rosettes, and they are ovate to elliptical with parallel venation. The leaves are glabrous and have an entire or irregularly dentate margin. The flowers are small, brownish-green on long non-ramified spikes (Samuelsen 2000). One individual plant produces more than 20,000 seeds during its lifetime. The plant is widely distributed in northern and central Asian and European countries, moreover has become widely naturalized elsewhere in the world (Jonsson et al. 1983). *P. major* is credited with such properties analgesic, cell damage prevention and slowing caused by radicals and unstable molecules, reverting pro-tumor inflammation and immune modulating, antiulcerogenic, antileukemic, antihypertensive activity, anti-inflammatory and wound healing due to the presence of bioactive compounds such as polysaccharides, fatty acids, variable phenolic structures, terpenoids, alkaloids, derivatives of caffeic acid, iridoid glycosides and plant organic acids (Bianco et al. 1984; Handjieva et al. 1991; Noro et al. 1991; Long et al. 1995; Ringbom et al. 1998; Taskova et al. 1999).

The leaves of *P. major* have been used for centuries as a wound healing agent in the territories of Kazakhstan and in the treatment of a number of diseases, in addition to wound healing (Yernazarova et al. 2019). These include diseases related to the skin, respiratory organs, digestive organs, reproduction, circulation, to relieve pain and against infections. Outwardly, plantain large has anti-inflammatory, antimicrobial, antipruritic and wound healing effects. The macerated leaves or the fresh juice of the plant are excellent quick-healing remedies for cuts, wounds, bruises, and earaches (Sultana et al. 2008; Arya et al. 2019). Plantain large is found throughout the country. It grows mainly in meadows, rarely in crops, very often in moist and waterlogged areas, along the banks of reservoirs, canals, irrigation and drainage systems, and also in mountainous areas (Tashpulatov et al. 2020).

Plantago major leaves are reported in standard documents as pharmacopoeial summaries of WHO, European Medicines Agency and European Union herbal monographs (European Pharmacopoeia 2014; Committee on Herbal Medicinal Products (HMPC) 2010). They have been described as a wealthy source of biologically crucial compounds like natural substances with variable phenolic structures, phenylpropanoids, isoprene polymers, polycarbohydrates, unsaturated fatty acids and essential micronutrient (ascorbic acid, representatives of carotenes, vitamin K) (Samuelsen et al. 1995; Samuelsen et al. 1996; Samuelsen 2000; Janković et al. 2012; Nazarizadeh et al. 2013; Gonçalves and Romano 2016). However, *Plantago major* leaves must be deeply studied in terms of the pharmacological, pharmacognostic and allelopathic traits as the remarkable medicinal properties of *P. major* are not only due to the high content of phenol derivatives, bioflavonoids and tannic acid derivatives. One of researches conducted for the purpose of anticancer effects based on ethanolic extract of *Plantago major* showed the greatest effect on tumor cell growth and this astonishing finding can not be the last in its beneficial list (McCutcheon 1995).

Presence of amino acids in nature can be either in the composition of peptide and protein chains as building blocks or in free form. Analysis of amino acid composition of plants plays a crucial role in the study of biological preparations. In nature, there are a total of 22 proteinogenic amino acids, with 20 genetically encoded protein amino acids serving as the building blocks of proteins and essential components of all biological systems. The majority of proteins in multicellular creatures are based on L-amino acids, which have a significant impact on human and animal nutrition and have significant therapeutic potential (Bercovici and Fuller 1995; Ambrogelly et al. 2007). The remaining nine amino acids, histidine, isoleucine, lysine, leucine, methionine, phenylalanine, threonine, tryptophan, and valine, are dubbed essential amino acids since only 11 of the 20 amino acids represented by the universal genetic code can be synthesized by humans. These are so-called because they must be ingested in order to keep cellular and physiological processes at their best. As a result, amino acid supplementation in the form of nutraceuticals may be advantageous to the human body. When consumed in concentrated form, it can have amazing effects on nutritional deficiency disorders and a variety of other diseases. Furthermore, additional *Plantago* species, such as *P. major*, *P. lanceolata*, and *P. medium*, are recognized to be valuable suppliers of amino acids and minerals, which may contribute to their use as a human food element (Guil-Guerrero 2001).

Thus, the objective of this study is to analyze the phytochemicals present by Gas Chromatography-Mass Spectrometry method and minerals by flame atomic absorption spectrometry in *P. major* leaves.

Materials and methods

Standards and reagents

For suitable and reliable analysis in mass spectrometry and high performance liquid chromatography analysis, water and methanol (≥99.9%) were purchased from

Sigma–Aldrich (St Louis, Missouri, USA). And standards (including lupeol, β-Sitosterol α-Amyrin, phytol) were acquired from Sigma–Aldrich, too. Filtration membranes (composed of cellulose nitrate and a small content of cellulose acetate, 0.45 μm) were purchased from Carl Roth, Germany.

Plant collection and sample preparation

Plantago major samples were collected in Almaty, Kazakhstan's mountainous region, in April 2020. For the further analysis, plants were divided into roots, leaves and flowers, oven dried for 3 days at 30 °C, milled and stored at 20 °C until use.

Phytochemical composition of the extracts

Sample volume was 0.5 μl, sample injection temperature at 280 °C. Separation was carried out using an SLB-5MS chromatographic capillary column 30 m long, with an inner diameter of 0.25 mm and a film thickness of 0.25 μm at a constant carrier gas (helium) (velocity of 1 ml/min). The chromatography temperature is programmed from 40 °C (hold 5 min) with a heating rate of 10 °C per 1 min to 280 °C (hold 15 min). Detection was carried out in the SCAN mode m/z 34-850. The Agilent MSD ChemStation software (version 1701EA) was used to control the gas chromatography system, record and process the obtained results and data. Data processing included determination of retention times, peak areas, as well as processing of spectral information obtained using a mass spectrometric detector. To interpret the obtained mass spectra, the Wiley 7th edition and NIST'02 libraries were used (the total number of spectra in the libraries is more than 550000).

Minerals

Methanol extract of *Plantago major* leaves were analysed for minerals (nickel – Ni, calcium – Ca, iron – Fe, magnesium – Mg, copper – Cu, manganese – Mn, lead – Pb cadmium – Cd, chromium – Cr, potassium – K and sodium – Na and zinc – Zn) by flame atomic emission spectrometry (novAA 350 – Analytik Jena, SW-version ASpect LS). Working standards of various concentrations were created from certified standard solutions; findings were corrected by subtracting a blank from the assessed metal concentrations for analytical quality assurance, and samples were analyzed in triplicate. Detection limits for mineral components were as follows: Cu 0.400 mg/L, Cd 0.130 mg/L, Cr 0.600 mg/L,Mn 0.050 mg/L, Ni 0.030 mg/L, Fe 0.170 mg/L, Mg 0.030 mg/L, Ca 1.300 mg/L, Pb 1.400 mg/L, Zn 0.090 mg/L. Results were expressed as mg/g dry weight.

Statistical analysis. The mean and standard deviation (sd) of the results were calculated, and each experiment was repeated at least three times..

Results and discussion

Phytoconstituents of *P. major*

Chromatographic analysis of methanolic extract of *Plantago major* obtained by GC-MS method enabled the identification of 30 compounds according to chromatograms (Fig. 1) which are presented in Table 1 in order based on their retention times and peak area percentage. The main constituents in methanol extract were lupeol 21.46%, benzimidazo[2,1-a]isoquinoline 17.41%, β-amyrin 9.88% and lup-20(29)-en-3-ol, acetate, (3β)- acid 9.46%, β-sitosterol 6.23% and α-amyrin 4.91%. Their chemical structures were described in Fig. 3. Triterpenes are significant structural components of plant membranes, and free triterpenes, like lupeol, serve to maintain phospholipid bilayers in plant cell membranes. Most triterpenes have 28 or 29 carbon atoms and one or two carbon-carbon double bonds, usually one in the sterol nucleus and occasionally another on the alkyl side chain. Triterpenes are naturally occurring compounds in humans' diets (Nazaruk and Borzym-Kluczyk 2015). It is not surprising that *Plantago major* is a widely used plant in traditional medicine and the presence of components such as lupeol in the composition of the methanol extract from *Plantago major* leaves increases its significance. One of the most current studies on active biomarkers presenting in *Plantago major* plant extract is critical for verifying its medicinal quality. And it was proved to have mutual antioxidative and antiviral properties of β-amyrin, β-sitosterol, lupeol and α-amyrin all together (Mohammad et al. 2018).

Triterpenes also have anti-cancer, antidiabetic, cardioprotective, hepatoprotective, anti-bacterial, anti-inflammatory, antioxidant, analgesic properties (Banerjee et al. 2019; Sharma et al. 2020).

Many other chemical constituents from *Plantago major* leaf extract were identified as acetoin 0.10%, 1,1-diethoxyethane 0.07%, propargyl alcohol 0.06%, 2-furanmethanol 0.11%, 4-cyclopentene-1,3-dione 0.04%, γ-Butyrolactone 0.11%, 2-hydroxycyclopent-2-en-1-one 0.16%, citraconic anhydride 0.18% (2,5-furandione, 3-methyl-), diglycerol 2.29%, toluene-2,6-diol 0.19% (1,3-benzenediol, 2-methyl-), 3,5-dihydroxy-6-methyl-2H-pyran-4(3H)-one 0.78%, 2,3-dihydrobenzofuran 0.15%, 5-hydroxymethylfurfural 0.20%, D-carvone 0.13%, isosorbide 0.20%, 2-methoxy-4-vinylphenol 0.31%, fitone (2-pentadecanone, 6,10,14-trimethyl-) 0.06% and phytol 0.30%. Their chemical structures were described in Fig. 2.

Fatty acids have also been isolated from the leaves of *Plantago major*. According to previous studies by using gas-liquid chromatography as well as permanganate oxidation and spectrophotometric techniques it was obvious the presence of some of organic fatty acids like lignoceric acid, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid mostly in seeds and leaves of *Plantago major* (Ahmed et al. 1968; Ahmad et al. 1980; Swiatek et al. 1980; Guil et al. 1997). Current study proved the presence

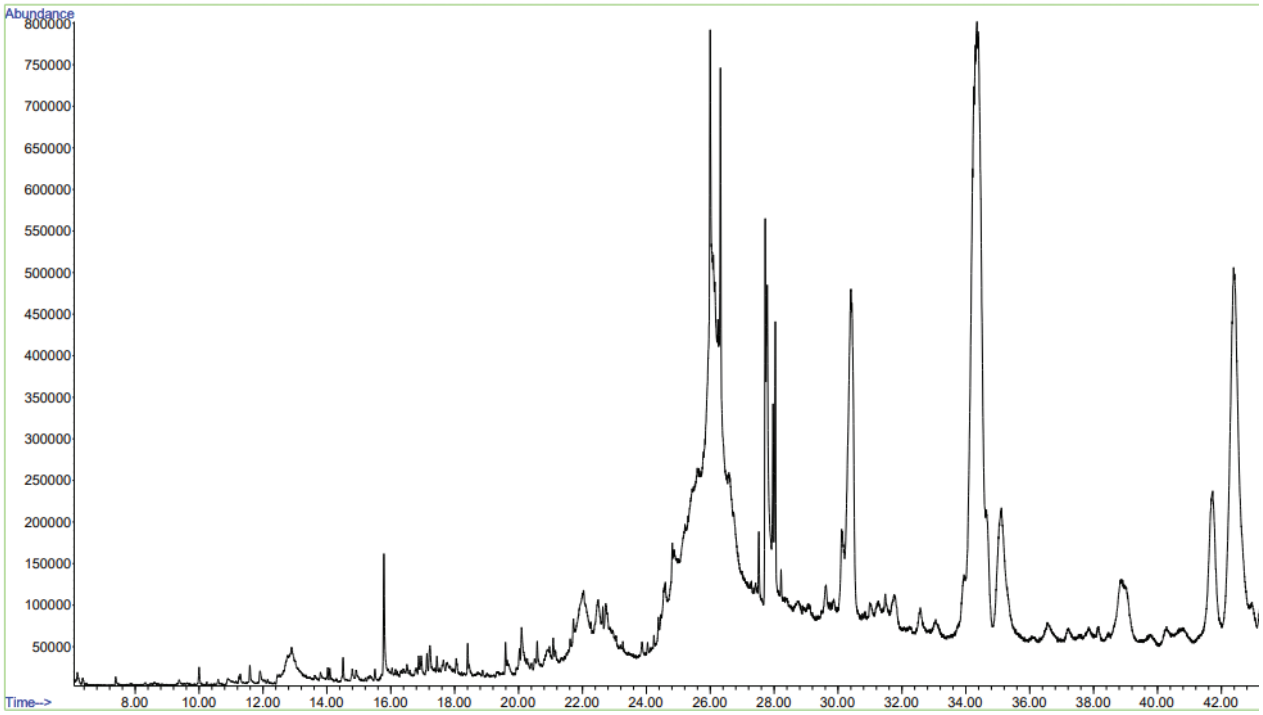

Figure 1. Chromatogram of the methanolic extract of *Plantago major* leaves.

Table 1. Chemical composition of methanol extract of *Plantago major* leaves.

S.No	Retention time, min	Compounds	Concentration, %	MW, g·mol^{-1}	MF
1	6.19	Acetoin	0.10	88.11	$C_4H_8O_2$
2	6.36	1,1-diethoxyethane	0.07	118.18	$C_6H_{14}O_2$
3	7.39	Propargyl alcohol	0.06	56.06	C_3H_4O
4	10.00	2-Furanmethanol	0.11	98.10	$C_5H_6O_2$
5	10.60	4-Cyclopentene-1,3-dione	0.04	96.08	$C_5H_4O_2$
6	11.29	γ-Butyrolactone	0.11	86.09	$C_4H_6O_2$
7	11.59	2-Hydroxycyclopent-2-en-1-one	0.16	98.10	$C_5H_6O_2$
8	11.91	2,5-Furandione, 3-methyl-	0.18	112.08	$C_5H_4O_3$
9	12.90	Diglycerol	2.29	166.17	$C_6H_{14}O_5$
10	14.51	1,3-Benzenediol, 2-methyl-	0.19	124.14	$C_7H_8O_2$
11	15.79	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	0.78	144.12	$C_6H_8O_4$
12	16.87	2,3-dihydrobenzofuran	0.15	120.15	C_8H_8O
13	16.95	5-Hydroxymethylfurfural	0.20	126.11	$C_6H_6O_3$
14	17.44	D-Carvone	0.13	150.22	$C_{10}H_{14}O$
15	18.05	Isosorbide	0.20	146.14	$C_6H_{10}O_4$
16	18.41	2-Methoxy-4-vinylphenol	0.31	150.17	$C_9H_{10}O_2$
17	24.81	2-Pentadecanone, 6,10,14-trimethyl-	0.06	268.48	$C_{18}H_{36}O$
18	26.00	Hexadecanoic acid (Palmitic acid)	12.68	256.42	$C_{16}H_{32}O_2$
19	26.32	Hexadecanoic acid, ethyl ester	4.01	284.50	$C_{18}H_{36}O_2$
20	27.52	Phytol	0.30	296.54	$C_{20}H_{40}O$
21	27.72	9,12-Octadecadienoic acid (Z,Z)-	1.97	280.40	$C_{18}H_{32}O_2$
22	27.78	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	3.87	278.40	$C_{18}H_{32}O_2$
23	27.97	Propyl 9,12-octadecadienoate	1.16	322.50	$C_{21}H_{38}O_2$
24	28.04	Ethyl 9,12,15-octadecatrienoate	1.51	306.50	$C_{20}H_{34}O_2$
25	30.40	β-Amyrin	9.88	426.70	$C_{30}H_{50}O$
26	34.35	Benzimidazo[2,1-a]isoquinoline	17.41	218.25	$C_{15}H_{10}N_2$
27	34.39	Lup-20(29)-en-3-ol, acetate, (3β)-	9.46	468.75	$C_{32}H_{52}O_2$
28	35.11	Lupeol	20.45	426.70	$C_{30}H_{50}O$
29	38.86	β-Sitosterol	6.23	414,71	$C_{29}H_{50}O$
30	41.72	α-Amyrin	4.91	426.70	$C_{30}H_{50}O$
31	42,39	Carveol	1.01	152.23	$C_{10}H_{16}O$

of palmitic acid (12.68%) as the main component among other organic fatty acids (9,12-Octadecadienoic acid (Z,Z)- (1.97%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (3.87)), and their structures were described in Fig. 2. Additionally, it was identified as another group of phytochemicals, which are fatty acid esters like hexadecanoic acid, ethyl ester (4.01%), propyl 9,12-octadecadienoate (1.16%) and ethyl 9,12,15-octadecatrienoate (1.51%). Plant based fats

and their derivatives are not only involved in vital physiological processes in the body, but also they are indispensable for a number of important biological functions, for example, pancreatic β-Cell, Enteroendocrine and metabolic functions, including growth and development and they can be applicable in different fields (Grundmann et al. 2021). For instance, short half-lives, poor absorption, low selectivity, quick degradation, and resistance development plague many pharmacological compounds. Lipophilic prodrugs, when designed and developed properly, can provide various advantages in overcoming these obstacles. The use of fatty acids as a developmental level of high prodrugs to treat the nervous system and enhance immunity appears promising (Francisco et al. 2020). As a result, fatty acid-based prodrugs can increase the biological properties of parent drugs, and the most common bonds employed to generate fatty acid-drug conjugates are ester and amide bonds (Fattahi et al. 2020).

In this study carveol (1.01%) was firstly identified from leaves of *Plantago major*. In one of previous studies, the presence of carveol was described in *Plantago* asiatica and some *Plantago* species but not in *Plantago major* (Chung et al. 2008). Another pharmacological study reported antitumor, antimicrobial, neuroprotective, vasorelaxant, antioxidant and anti-inflammatory activity of carveol (Serafim et al. 2021). Its structure was described in Fig. 3.

Previous studies of the phytochemical composition of other *Plantago* species revealed similar components to those found in *Plantago major*, such as phenol, benzofuran,

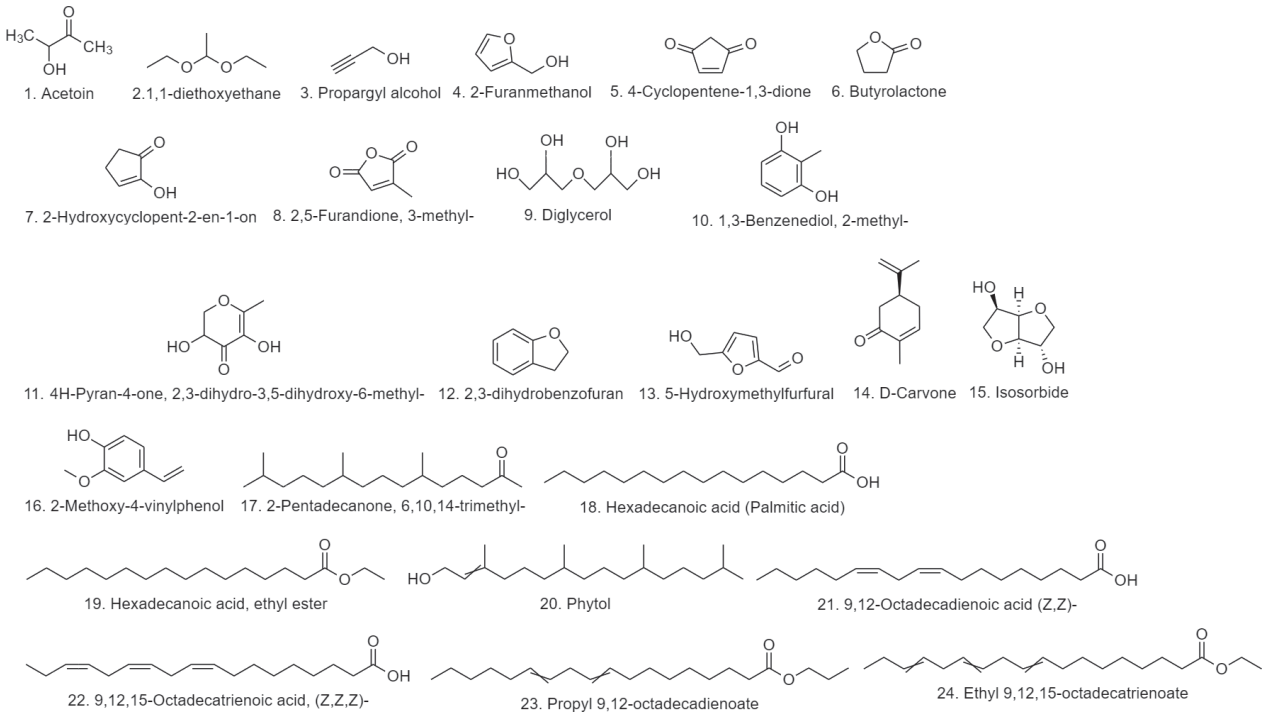

Figure 2. Chemical structure of identified phytochemicals present in methanolic extract of *Plantago major*.

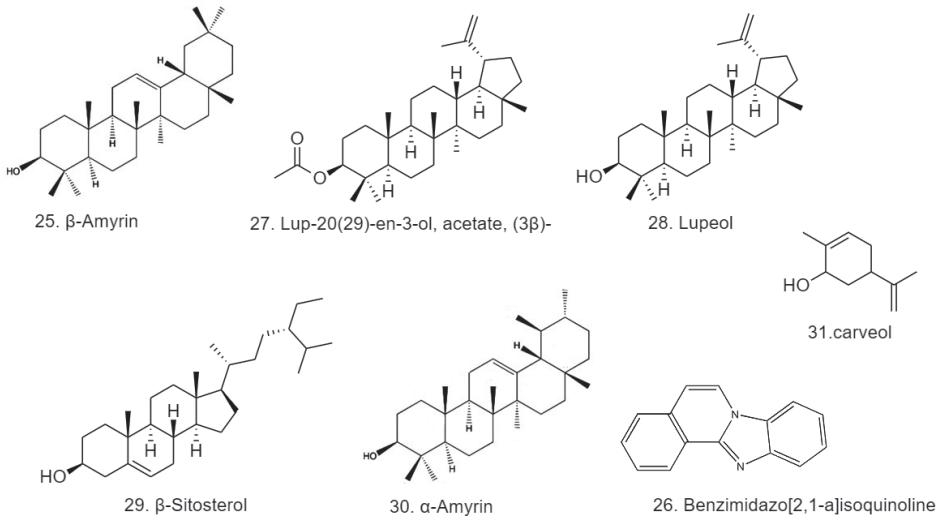

Figure 3. Chemical structures of the main phytochemicals present in *Plantago major* leaves.

Diglycerol, various kinds of acids etc. (Jamilah et al. 2012; Gao et al. 2022).

According to studies conducted to determine the chemical composition of other varieties of the genus *Plantago*, comparing the results of these studies, we can conclude that the main phytochemical components of plants of the genus *Plantago* do not differ much in composition from each other, the difference is the amount of one or another component depending on the species and place of growth (Jurisic et al. 2004; Piyaviriyakul et al. 2017; Fayera et al. 2018; Hasan et al. 2018).

Phytoconstituents of *Plantago major*: minerals and amino acids

Several *Plantago* species including *Plantago major* contain significant amounts of indispensable amino acids and minerals, enhancing their potential as food or food additives (Guil-Guerrero 2001; Haddadian et al. 2014).

In this regard, minerals and amino acids were determined in the comestible organs of *P. major*, namely the leaves, and the results are reported in Tables 2, 3, respectively. *Plantago major* has high levels of Na (44.7 mg/g dw), is a good source of Ca (12.5 mg/g), K (10.36 mg/g dw) and Mg (6.34 mg/g dw). Additionally, it was found that the presence of some essential chemical elements like Fe (0.29 mg/g), Mn (0.03 mg/g) and Zn (0.07 mg/g). Furthermore, potentially harmful minerals such as Cu, Cr, Ni, Cd, and Pb were not found in this investigation.

According to the amino acid profile of *Plantago major* leaves (Table 3) arginine was the major essential amino acid (8.29 mg/g dw) followed by lysine (6.27 mg/g dw), threonine (4.10 mg/g dw), phenylalanine (3.77 mg/g dw) and isoleucine (3.34 mg/g dw) while histidine (1.13 mg/g dw), methionine (1.08 mg/g dw) were the minor amino acids. Arginine is a conditionally essential amino acid, meaning it is required in higher amounts during growth than it can be generated, and a dietary shortfall might cause metabolic, neurological, reproductive, immunity and cardiovascular pathologies (Kirk and Barbul 1990; WU 2009; Kelly and Pearce 2020; Rashid et al. 2020). *Plantago* leaves, like lettuce or asparagus, are a rich source of arginine for young/developing mammals, according to our findings (USDA 2015). Although non-essential amino acids are generated by the body, some are conditionally required in newborns (e.g. glutamine, taurine), during stress (e.g. glutamine), or for carnivores and some fish (taurine) (WU 2009). *Plantago major* leaves can be used as a supplemental supply of these amino acids (alanine 3.53 mg/g, aspartic acid 0.38 mg/g, cysteine 0.83 mg/g, glutamic acid 0.23 mg/g, glutamine 0.30 mg/g, glycine 4.48 mg/g, ornithine 0.15 mg/g, proline 2.48 mg/g, serine 4.37 mg/g and taurine 0.13 mg/g dw) in certain situations.

Table 2. Mineral content (mg/g dw) of *Plantago major* leaves.

Mineral	Chemical symbol	Dry weight content
Sodium	Na	44.7 ± 2.13
Calcium	Ca	13.5 ± 0.42
Potassium	K	10.36 ± 0.07
Magnesium	Mg	6.34 ± 0.28
Iron	Fe	0.29 ± 0.02
Manganese	Mn	0.03 ± 0.00
Zinc	Zn	0.07 ± 0.00

Note: Data represent the mean ± sd (n = 3).

Conclusions

Plantago major has been used widely since ancient times, to manage a wide range of diseases including constipation, coughs and wounds. These include ailments involving the skin, respiratory organs, stomach-related organs, generation, circulation, cancer prevention, pain relief, and various other disease prevention. Previously, *P. major* extracts have been reported to have a variety of biological properties, including wound healing, anti-inflammatory, analgesic, antioxidant, mild antibacterial, immunomodulating, hepatoprotecrive, cardioprotective and antiulcerogenic activity. A few of these impacts may explain the utilization of this plant in traditional medicine.

Our results indicate that the edible *Plantago major* leaves contain a high phytochemicals with various biological activities and current study proved the presence of 31 bioactive compounds. In addition, the leaves are a good source of minerals and amino acids. The phytochemical diversity associated with a wide range of biological

Table 3. Amino acid profile (total amino acids, mg/g leaves dw) of *Plantago major* leaves.

Group	Amino acids	Total amino acids	Group	Amino acids	Total amino acids
Indispensable amino acids (essential)	Isoleucine	3.34 ± 0.11	Dispensable amino acids (nonessential)	Alanine	3.53 ± 0.02
	Histidine	1.13 ± 0.04		Aspartic acid	0.38 ± 0.01
	Leucine	3.24 ± 0.05		Cysteine	0.83 ± 0.01
	Lysine	6.27 ± 0.03		Glutamic acid	0.23 ± 0.01
	Methionine	1.08 ± 0.08		Glutamine	0.30 ± 0.02
	Phenylalanine	3.77 ± 0.07		Glycine	4.48 ± 0.06
	Threonine	4.10 ± 0.07		Ornithine	0.15 ± 0.09
	Tryptophan	0.61 ± 0.01		Proline	2.48 ± 0.05
	Valine	2.88 ± 0.09		Serine	4.37 ± 0.06
	Arginine*	8.29 ± 0.08		Taurine	0.13 ± 0.10
	Total indispensable amino acids	**34.71 ± 0.31**		**Total dispensable amino acids**	**16.88 ± 0.17**

Note: Data represent the mean ± sd (n = 2). *Conditionally essential: required in greater amounts than can be produced in growth development.

activities can explain the various traditional uses, and these findings highlight *Plantago major*'s potential as a source of bioactive substanses, particularly useful for the prevention of oxidative stress-related diseases, leading to the development of new drugs and biological active supplements. Carveol was firstly identified in *Plantago major* leaves, and its presence may explain antioxidant, anti-hyperlipidemic, antidiabetic, anti-inflammatory and hepatoprotective properties of *Plantago major*.

This paper presents a study of phytochemical compounds of the *Plantago major* plant. The study was carried out to determine the chemical composition of this type of plant growing on the territory of Kazakhstan, which has not been studied until now. The plant was collected in the mountains of Almaty, Kazakhstan, in the spring, at the time of glow, then divided into 3 main parts (root, flowers and leaves) and dried. Determination of the chemical composition was carried out by the method of gas chromatography. As a result, 30 chemical compounds were isolated which are shown in Table 1 and their chemical structures in Fig. 1. The predominant components, in percentage terms, contained in the methanol extract are lupeol 21.46%, benzimidazo[2,1-a]isoquinoline 17.41%, β- amyrin 9.88% and lup-20(29)-en-3-ol, acetate, (3β)-acid 9.46%, β-sitosterol 6.23% and α-amyrin 4.91%. carveol was found for the first time in the leaves of *Plantago major* in an amount of 1.01% which makes this study valuable. Carveol has been identified in other *Plantago* species, but in *Plantago major* composition was found for the first time. The presence of this kind of component will expand the use of *Plantago major* in pharmaceutical practice. Our results show that leaves of *Plantago major* contains a large number of phytochemicals with various biological activities, and the current study has proven the presence of 31 biologically active compounds. In addition, the leaves are a good source of minerals and amino acids. The phytochemical diversity explains the various traditional uses of this plant for medicinal purposes, and these results show the potential of *Plantago major* as a source of bioactive substances, especially useful for the prevention of diseases associated with oxidative stress. Also it leads to the development of new drugs and dietary supplements. Carveol was first discovered in the leaves of *Plantago major*, and its presence may show the antioxidant, antihyperlipidemic, antidiabetic, anti-inflammatory, and hepatoprotective properties of.

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