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<https://doi.org/10.5109/7395632>

出版情報 : Proceedings of International Exchange and Innovation Conference on Engineering & Sciences (IEICES). 11, pp.982-986, 2025-10-30. International Exchange and Innovation Conference on Engineering & Sciences

バージョン :

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BIOLOGICALLY ACTIVE SUBSTANCES MEDICINAL PLANTS GROWING IN KYRGYZSTAN

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Abstract: *Kyrgyzstan's rich bioecological diversity supports the growth of medicinal herbs containing flavonoids, polyphenols, and antioxidant compounds. This study investigated the biological activity and antibacterial properties of extracts from dandelion (*Taraxacum campyloides*) and plantain leaf (*Plantago major*). Ethanol extraction combined with ultrasound-assisted extraction was used to obtain bioactive compounds. Total phenolic content (TPC) in dried plant extracts was measured as follows: whole dandelion, 672.56 mg; dandelion root, 735.64 mg; dandelion leaf, 818.48 mg; and dried *Plantago major* leaf, 66.11 mg. Fresh samples showed TPC values of 136.39 mg in whole dandelion and 341.54 mg in *Plantago major* leaf. Total flavonoid content (TFC) in dried extracts ranged from 0.531 mg in *Plantago* to 1.547 mg in dandelion leaf. Antibacterial tests revealed dried extracts had the strongest activity against *Shigella*, while fresh extracts were most effective against *Escherichia coli*.*

Keywords: antibacterial activity, biological activity, medicinal plants, Dandelion, *Plantago Major*.

1. INTRODUCTION

Kyrgyzstan is known for its rich bioecological diversity. Studying herbs growing in Kyrgyzstan, based on their biochemical properties adapted to the local climate, reveals the country's internal pharmacological potential and expands possibilities for further use. Among such plants that have been used in folk medicine for centuries, dandelion (*Taraxacum campyloides* G.E. Haglund) [1] and plantain (*Plantago major*) are considered especially important. These plants are of scientific and medical interest as widespread, easily accessible, and insufficiently studied resources in Kyrgyzstan. Phytotherapeutic plants have always played an important role in folk medicine in addressing medical problems. According to the World Health Organization, about 80% of the world's population relies on herbal medicines to treat various diseases [2]. As society has evolved and technology has advanced, plants have become valuable healing resources. They contain biologically active compounds that can be used to synthesize many therapeutic substances for treating human diseases. In the field of biomedicine, phytotherapeutic drugs offer a promising alternative. These medicines are derived from plants, making them safe, sustainable, and less harmful to the environment.

They are also less likely to lead to bacterial resistance or leave behind persistent chemical residues, while effectively meeting therapeutic needs. Plants and plant-based products have shown significant promise in treating inflammatory conditions and diseases with inflammatory components. Phenolic compounds, when added to new biodegradable products, demonstrate their potential as natural preservatives, offering a viable alternative to synthetic food additives. Currently, they are used in food technology to process food products prior to packaging [3].

Taraxacum species are characterized by a rich phytochemical profile, including flavonoids, polyphenols, phytosterols, sesquiterpenoids, mineral elements, carotenoids, coumarins, polysaccharides, and inositol derivatives. Flavonoids are known for their antiviral, antioxidant, antimutagenic, anti-inflammatory, and cardioprotective properties. Polyphenols represent an important class of *Taraxacum* extracts, exhibiting antiviral effects and protecting the body from oxidative stress, diabetes, cardiovascular diseases, and cancer. Sesquiterpenoids and sterols exhibit anti-inflammatory and anticancer activities, while polysaccharides are noted for their anti-inflammatory, antioxidant, and antidiabetic effects.

Dandelion root is primarily known for its digestive and liver-supporting properties [4], while its leaves are used as diuretics and digestive stimulants. Dandelion contains terpene derivatives (taraxerol, taraxasterol, taraxacoside), sterols (β -sitosterol, stigmasterol), phenolic compounds (hydroxycinnamic acids, coumarin, lignans) [5], as well as rubber, resins, tannins, fatty acids, sugars, amino acids, carotenoids, phytosterols, and biologically active compounds such as inulin [6, 7]. Traditional Chinese medicine regards dandelion as a non-toxic herb with valuable choleretic, anti-inflammatory, diuretic, and antirheumatic properties. Studies have shown that dandelion root and leaf extracts can reduce lipid levels, mitigate oxidative stress, and protect against liver damage [8, 9]. Additionally, the Chinese medicinal compound taraxasterol has demonstrated antiarthritic effects in animal models [10, 11].

Modern therapeutic use of dandelion focuses on digestive health: it improves digestion, alleviates digestive disorders, reduces nutrient and cholesterol levels, supports liver and biliary tract function, promotes detoxification, and helps resolve bile duct problems. It also facilitates the removal of excess fluid by increasing urine production, thereby preventing kidney stones and other urinary tract issues. Some studies suggest that dandelion may help maintain healthy blood pressure.

Notably, flowers and leaves contain higher levels of polyphenols than stems and roots.

High levels of polyphenols and carotenoids have been identified in 70% ethanol extracts from dandelion roots, leaves, and the whole plant, with several new flavonoid compounds discovered in addition to previously known metabolites. These extracts, with a high ethanol concentration, revealed the presence of tartaric acid, formic acid, and sucrose for the first time. Furthermore, caffeic acid derivatives were identified as the second most abundant metabolites after flavonoids. These compounds contribute antioxidant and anti-inflammatory benefits. Research has also identified numerous other compounds, including novel ketols with C25 and C27 chains, as well as nine types of fatty acids, alcohols, aldehydes, esters, alkanes, triterpenes, sterols, and tocopherols. These bioactive substances have potential applications across various industries, including cosmetics and food [12-27].

Plantago major leaves contain flavonoids, polysaccharides, tannins, mucilage, carotenoids, citric and ascorbic acids, vitamin K, mineral salts, zinc, and oxycornic acid [6]. The seeds of *Plantago major* leaves contain mucilage, fatty acids, and the carbohydrate planteose [29]. Among the biologically active substances in its leaves are pectin, iridoids, flavonoids, and saponins. Fresh, crushed *Plantago major* leaf poultices have wound-healing properties. Saponins, pectin substances, flavonoids, and oxycornic acid contribute to lowering blood cholesterol levels and exhibit hypocholesterolemic effects [30].

The main therapeutic uses of *Plantago major* leaf derive from its expectorant, antibacterial, and hemostatic properties. Additionally, herbal preparations can improve the stomach's secretory function and provide a protective (enveloping) effect. Both preparations and fresh leaf juice exhibit bacteriostatic activity against pathogens responsible for wound infections, such as hemolytic streptococcus, *Staphylococcus*, *Pseudomonas aeruginosa*, *Proteus*, and *E. coli*.

Decoctions made from the leaves are used to treat respiratory diseases, while alcohol-based extracts reduce blood pressure. Traditional healers recommend leaf infusions for diarrhea, hay fever, bladder inflammation, hemorrhoids, and skin rashes. Oil made from dried leaf powder is effective for treating pustular skin lesions. The seeds of *Plantago major* benefit both men and women with reproductive issues. Meanwhile, root extracts are recommended for coughs related to tuberculosis, fever, pain relief, and insect or reptile bites. In folk medicine, wet compresses made from the root are used to treat cancerous tumors, and leaf infusions are applied in the treatment of stomach and lung cancers [31, 32].

Originally distributed mainly across Europe, North and Central Asia, *Plantago major* is now widespread worldwide. It has historically been used to treat a variety of diseases, including infections. Psyllium (*Plantago major*) exhibits antimicrobial, antidiabetic, antispasmodic, antiviral, anti-inflammatory, and wound-healing properties. Plant extracts are attractive alternatives to synthetic drugs because they are rich in bioactive compounds such as phenols, flavonoids, and saponins, which provide antimicrobial, anti-inflammatory, wound-healing, and antiviral effects while reducing the likelihood of disease [33-36].

The main aim of this review is to present the broad therapeutic potential of *Plantago major* extracts and explore their possible applications as medicinal herbs.

Extracts of medicinal plants are widely used as active ingredients in herbal products and contain metabolites with diverse roles and functions. The unique and specific composition and chemical properties of each medicinal plant's extract make them nutritionally and therapeutically valuable.

Currently, more than 10,000 phenolic compounds have been isolated from plants. These phenolic metabolites are generally divided into seven groups: coumarins, flavonoids, phenolic acids, lignans, lignins, stilbenes, and tannins. Many studies report a synergistic effect among these compounds regarding their pharmacological activity. Therefore, the determination of total phenolic content (TPC) and total flavonoid content (TFC) in medicinal plant extracts remains a valuable tool for quality assessment [37-40].

Systematic research is being conducted to ensure a green way to recycle plant residues rich in flavonoids, polyphenols, saponins, alkaloids, tannins, and other antioxidant properties and biological activity and to increase their antibacterial activity. [41]

2. EXPERIMENTAL PART

2.1. Sample preparation

Medicinal plants for this study were collected in two samples: the first sample consisted of dried plants—dandelion whole plant, dandelion root, dandelion leaves, and *Plantago major* leaves—harvested from the steppe mountainous regions of the Toktogul district in the Jalal-Abad region. The second sample, collected in April, consisted of freshly harvested dandelion and *Plantago major* leaves gathered from the campus of Kyrgyz-Turkish Manas University.



Figure 1. Dried medicinal plants: (1) dandelion root, (2) dandelion leaf, (3) whole dandelion, (4) *Plantago major* leaf; fresh green crushed medicinal plants: (5) whole dandelion, (6) *Plantago major* leaf.

Dandelion (*Taraxacum campyloides* G.E. Haglund) has cylindrical stems that emerge from the center of the leaf rosette before flowering. It is a perennial plant bearing

many yellow flowers on each stem. The petals of each flower are connected by a strap-like structure. The fruit is an oblong brown achene, several millimeters long, with a textured surface and numerous cotton-like spherical seeds. The white pappus attached to each achene is dispersed by the wind, aiding reproduction. The roots are 15–50 cm long and 1.5–2.5 cm in diameter, thick and branched, with a dark gray outer surface and a shiny inner surface.

Plantago major (Latin: *Plantago major*; English: broadleaf plantain) is a perennial herbaceous plant. It has short, vertical roots with numerous fine root hairs extending from them. The stems are smooth and grooved, ranging from 15 cm to 0.5 meters in height. The leaves are broad with arching veins connected to the underground veins. The flowers are small and not showy, and the fruit is capsule-shaped. Plantain blooms from late spring to early autumn. It commonly grows in yards, abandoned weed-covered areas, along roadsides, in meadows, and near ponds. Each plant produces several thousand seeds.



(a) (b)

Figure 2. The state of medicinal herbs found in nature: (a) dandelion, (b) *Plantago major* leaf.

2.2.Extraction Procedure

To increase the contact surface and ensure complete release of biologically active substances, dried plant samples were ground prior to extraction. From each ground sample, 5 grams were extracted in 50 ml of ethanol-water mixtures at different concentrations (50%, 60%, and 70% ethanol) using an ultrasonic water bath at 40°C for 120 minutes. The extract obtained with 70% ethanol, which contained the highest amount of extractive substances and was considered the most effective, was selected for further study of biologically active substance retention. Similarly, fresh green plants harvested in spring were extracted with ethanol-water mixtures at various ratios, and the most effective extracts were chosen for further analysis.

2.3.Total phenol content (TPC)

The total phenolic content was determined using a modified version of the method described by Conde-Hernandez (2017). Briefly, 0.4 ml of the extract was mixed with 2 ml of distilled water and 0.8 ml of Folin–Ciocalteu reagent. The mixture was incubated at room temperature (25 °C) for 5 minutes, then 1 ml of 8% sodium carbonate (Na_2CO_3) solution was added. After incubating at room temperature for 2 hours, the sample was ready for analysis using a UV spectrophotometer at 765 nm. Gallic acid (GA) was used as a reference standard, and results are expressed as mg of gallic acid

equivalent (GAE) per gram of sample. The standard calibration curve equation is:

$$y = 0.0011 x - 0.0079.$$

2.4.Total flavonoid content (TFC)

Total flavonoid content was determined using a modified method based on Feng et al. Briefly, 0.5 ml of the alcohol extract was mixed with 4 ml of distilled water and 0.15 ml of 5% sodium nitrite (NaNO_2) solution. After 5 minutes, 0.3 ml of 10% aluminum nitrate ($\text{Al}(\text{NO}_3)_3$) solution was added. Six minutes later, 1 ml of 1 M sodium hydroxide (NaOH) solution was added. The solution was then incubated at room temperature (25 °C) for 15 minutes. Flavonoid content was measured using a spectrophotometer at 510 nm. Rutin was used as the reference standard, and results are expressed as mg of rutin equivalent (RE) per gram of sample. The standard calibration curve equation is:

$$y = 0.0945 x + 0.0276.$$

2.5. Determination of antibacterial properties

The antibacterial activity of the nanoparticles in the samples was evaluated using the standard Kirby-Bauer method against *Escherichia coli*, *Staphylococcus aureus*, and *Shigella* bacteria. Sterile metal rods were used to evenly spread bacterial suspensions on Mueller-Hinton agar plates. Then, 1 ml of each sample was applied to the respective Petri dishes. After incubation at 37 °C for 24–48 hours, bacterial sensitivity was assessed by measuring the diameter of the zones of inhibition (lysis zones).

3. RESULTS OBTAINED AND THEIR DISCUSSION

The extraction of medicinal herbs, collected and dried from the steppe-mountainous areas of the Toktogul district in the Jalal-Abad region, as well as freshly harvested plants from the Kyrgyz-Turkish University campus in April, was performed using a 70% ethanol-water mixture in an ultrasonic water bath for 120 minutes. The total phenolic content (TPC) and total flavonoid content (TFC) of the extracts were then determined using a SPECORD 50 spectrophotometer. According to the described methodology, TPC was measured at 765 nm and TFC at 510 nm. The resulting values, calculated based on the standard calibration equations, are presented in the following tables.

Table 1. Quantitative manicure of TPC and TFC dried plant extracts.

№	Plant name	TPC, mg	TFC, mg
1	<i>Plantago major</i> leaf	66.11	0.531
2	Dandelion leaf	818.48	1.547
3	Dandelion root	735.64	0.159
4	Dandelion all (whole)	672.56	0.721

Table 2. Quantitative manicure of TPC and TFC of freshly green plant extracts.

№	Plant name	TPC, mg	TFC, mg
1	Plantago major leaf	341.54	0.087
2	Dandelion (whole) all	136.39	0.061

As a result, we found that dried plants, particularly dandelion, contain more biologically active substances than fresh green ones, especially in the dandelion leaves. While the flavonoid content is higher in dried Plantago major leaves, the phenolic content is greater in fresh green leaves. This suggests that the antibacterial and anti-inflammatory properties of Plantago major are most effective immediately after harvesting. For this reason, fresh Plantago major juice or poultice is commonly applied directly to wounds or cuts

4. CONCLUSION

This scientific work focused on medicinal plants. We analyzed the presence of biologically active substances in dried and fresh green dandelion and Plantago major leaves and studied their antibacterial properties.

A 70% ethanol-water mixture proved to be the most effective solvent for extracting bioactive substances from medicinal plants, using an ultrasonic water bath (s30h Elmasonic) at 40°C for 120 minutes. Total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent, measured on a spectrophotometer at 720–750 nm, and calculated using the gallic acid calibration curve. Total flavonoid content (TFC) was measured spectrophotometrically at 460–495 nm and calculated based on the rutin calibration curve.

Antibacterial activity tests of the extracts against Staphylococcus aureus, Escherichia coli, and Shigella bacteria showed the highest inhibition zones for dried plants, particularly Plantago major leaves and then dandelion leaves, against Shigella. Fresh green plants exhibited the strongest antibacterial activity against Escherichia coli, followed by Shigella and Staphylococcus.

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