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PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF *BETULA VERRUCOSA* AND *BETULA PUBESCENS* BUD EXTRACTS

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Abstract: *Betula* species, particularly *Betula verrucosa* (syn. *Betula pendula*, silver birch) and *Betula pubescens* (downy birch), have been traditionally used in herbal medicine due to their diverse pharmacological properties. This study investigates and compares the flavonoid, polyphenolic, and triterpenoid content, as well as antioxidant activity, of gemmotherapy extracts from buds of both species. The extracts were prepared using a glycerol-ethanol mixture, and phytochemical content was assessed using spectral assays and thin-layer chromatography. The antioxidant potential was evaluated by DPPH radical scavenging assay and ferric reducing antioxidant power (FRAP) assay. Results revealed a significantly higher concentration of flavonoids and polyphenolic compounds in *Betula verrucosa* extract, correlating with greater antioxidant activity determined by FRAP assay, but a slightly lower antioxidant activity determined by DPPH assay in comparison to the *Betula pubescens* extract. These findings suggest that the antioxidant activity can be influenced by the synergy of polyphenolic compounds, supporting the enhanced therapeutic potential for both species and encouraging further pharmacological studies.

Keywords: *Betula verrucosa, Betula pubescens*, flavonoids, polyphenols, triterpenes, antioxidant activity, birch buds, phytochemistry, gemmotherapy.

INTRODUCTION

The genus *Betula* (birch) comprises a variety of deciduous trees and shrubs within the Betulaceae family. Encompassing over 60 species, *Betula* is widely distributed across boreal and temperate regions and plays an essential ecological and economic role. Birch species are easily recognizable by their papery bark and are valued in forestry, ornamental planting, and traditional medicine (Tutin *et al*., 2010). The studied species, *B. verrucosa* and *B. pubescens*, are the two most abundant birch species existing in the Romanian wild flora.

Betula verrucosa Ehrh. (silver birch) is a medium to large deciduous tree reaching heights of 15–25 m. It typically has an open, airy crown with slender, pendulous branches. The trunk is relatively slender and may reach up to 1 m in diameter at breast height (dbh). The bark is strikingly white to silvery, often peeling in thin, papery layers. Older trunks may develop dark, rugged fissures near the base. Young twigs are smooth and hairless, with small resinous glands that give them a rough texture to the touch. The leaves are alternate and simple, with a triangular to diamond shape. They measure about 3–7 cm long and 2–5 cm wide. The leaf base is typically wedge-shaped, and the margin is coarsely double-serrated. The upper surface is bright green and glabrous. This species is monoecious. The reproductive structures of *B.verrucosa* consist of male and female catkins. Male catkins that form in late summer and overwinter. These are long and pendulous. Female catkins appear in spring, initially upright, later becoming pendulous as they mature into cylindrical infructescence that releases numerous tiny, winged seeds. Silver birch prefers dry, light, and well-drained soils such as sandy or gravelly substrates. It is light-demanding and one of the first trees to colonize disturbed or open sites. Its canopy allows significant light penetration, supporting a rich ground flora (Ashburner, 2013).

Betula pubescens Ehrh. (downy birch) is a small to medium-sized deciduous tree, typically 10–20 m tall. The crown is more compact and upright compared to the silver birch, with branches spreading more horizontally. The bark is duller, white to greyish-white, and does not peel as readily as that of silver birch. It generally remains smooth and is less likely to develop deep fissures with age. Young twigs and buds are densely covered with fine hairs (pubescent). Resinous glands are absent, giving them a soft texture. Leaves are alternate, simple, and ovate to rhomboid in shape, about 2–6 cm long. The base is rounded or slightly tapered, and the margins are finely double-serrated. Both leaf surfaces may be slightly hairy, especially when young. Like silver birch, downy birch is monoecious, with male catkins forming in autumn and female catkins appearing in spring. The mature female catkins are cylindrical and

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pendulous, dispersing small, winged seeds. Downy birch is better adapted to wetter conditions and heavier soils than silver birch. It is commonly found in poorly drained sites such as peat bogs, riparian woodlands, and moorlands. It is more tolerant of waterlogging and acidic soil and often occurs further north or at higher elevations (Ashburner, 2013).

Although *B.verrucosa* and *B. pubescens* can hybridize and are sometimes difficult to separate, their twig texture, bark characteristics, leaf shape, and habitat preferences are reliable distinguishing features in the field. Both species play a vital ecological role as pioneers, providing habitat and resources for a wide range of other organisms. The main differences between the two species are found in the appearance of the leaves, twigs, and bark. The twigs of *B. verrucosa* have resin glands, while the twigs of *B. pubescens* are hairy with no glands. The bark of *B. verrucosa* is brighter white and peeling, but is duller and smoother in the case of *B. pubescens.* (Atkinson,1992)

B. verrucosa and *B. pubescens* have both been studied for their bioactive secondary metabolites, especially in the bark, leaves, buds, and sap. Historically, the buds of *Betula* species have been incorporated into folk remedies for their anti-inflammatory, diuretic, and detoxifying properties. These effects are primarily attributed to their high content of polyphenolic compounds (such as flavonoids, phenolic acids, tannins) and terpenic compounds present in different structural types (pentacyclic triterpenes of lupeolic type such as betulin and its derivatives, sesquiterpenic compounds (from essential oil), sterolic compounds. Though some therapeutic claims have been validated through *in vitro* or *in vivo* studies, comparative phytochemical analyses between birch species remain limited (Rastogi *et al*., 2015).

The leaves of *B. verrucosa* contain flavonoids: notably quercetin, kaempferol derivatives, and hyperoside, phenolic acids such as chlorogenic and caffeic acids, tannins, and triterpenic saponins. Essential oils with sesquiterpenes are present in varying concentrations ranging from 0.5-1%. The flavonoid fraction is primarily responsible for the mild diuretic and anti-inflammatory properties attributed to birch leaf extracts. The outer bark is rich in triterpenoids, especially betulin (up to 30% of dry weight), betulinic acid, lupeol, and oleanolic acid. These compounds exhibit antimicrobial, antiviral, and anticancer activities *in vitro*. Betulin is also the precursor for various semi-synthetic derivatives under pharmaceutical investigation (Rastogi *et al*., 2015; Newman et.al.,2020). Birch sap, collected in early spring, contains sugars (mainly glucose and fructose), amino acids, minerals (notably potassium and calcium), and small amounts of polyphenols. It has been traditionally consumed as a refreshing tonic.

While *B. pubescens* shares a broadly similar phytochemical spectrum with *B. verrucosa*, some differences in the relative concentrations of key compounds have been noted. The leaves of *B. pubescens* contain comparable flavonoid glycosides, including hyperoside and quercitrin, as well as tannins and phenolic acids. Some studies have reported slightly higher total polyphenol contents than *B. verrucosa* in certain habitats (Raal *et al*., 2014). The bark also contains high amounts of betulin and its derivatives, but the content is often lower than in *B. verrucosa* and can vary depending on site conditions and tree age. Other identified compounds include betulinic acid, lupeol, and various lignans.

The aim of the study was to compare the total polyphenolic and flavonoid contents of the bud extracts of the most common *Betula* species from the Romanian wild flora, in order to understand the differences in bioactive compound contents between the selected species, and to offer valuable insights into their relative therapeutic efficacy based on the antioxidant activity.

MATERIALS AND METHODS

Plant material

Bud samples of *B. verrucosa* were collected from Martinesti, Cluj, Romania, and those of *B. pubescens* from Marisel, Cluj, Romania, during February 2022. The species were identified by a specialist from PlantExtrakt laboratories, and the voucher specimens were deposited in the Research and Development Department, under reference numbers 123 (*B. pubescens*) and 124 (*B. verrucosa*).

Analytical equipment, reagents

For the determination of total flavonoids, polyphenolic compounds, and antioxidant activity an UV-VIS spectrophotometer (UV-VIS Spectrophotometer, UV2500, Techcomp, Hong Kong) was used.

For TLC silica gel plates 60 F_{254}, glass support, particle size 10-12 µm from Supelco, Merck KGaA, Germany was used, and for TLC detection at 254 nm, 365 nm, and daylight, a semi-automated gel documentation system was used (Vilber Lourmat, Quantum-ST5, France).

For solid phase extraction, a Macherey-Nagel, Germany, Chromabond Spe Vacuum Manifold was used. For the drying step, a Hei-VAP Rotary Evaporator from Heidolph, Germany, and LiChrolut RP-18E (40-63µm) 500 mg 3ml standard PP-tubes was used, Supelco, Merck KGaA, Germany.

Ultrapure water was produced using the Simplicity Ultra-Pure Water Purification System, Merck Millipore, Billerica, MA, USA.

Reference substances for TLC: rutin, hyperoside, quercetin, clorogenic acid, betulinic acid, ursolic acid, betulin, lupeol, and betulin aldehyde, purchased from Phytolab GmbH & Co, KG, Germany.

Toluene, ethyl acetate, ethanol, 2-aminoethoxydiphenyl borate, polyethylene glycol 400, anisaldehyde, glacial acetic acid sulphuric acid, 2,2-diphenyl-1-picryl-hydrazyl, TPTZ (2,4,6-tripyridyl-s-triazine), hydrochloric acid, ferric chloride, aluminum chlorides, and sodium acetate were purchased from Merck KGaA, Germany. Glycerol was pharma grade from Art chemicals, and ethanol 96% from Prodvinalco, Romania.

Statistical evaluation was performed by t-test using MedCalc statistical software.

Extract Preparation

Freshly chopped buds were extracted at low temperature in a 1 to 1 glycerol-96% ethanol mixture for 20 days and filtered. The extraction ratio, 1 part plant material to 20 parts solvent mixture, was adjusted based on bud moisture content, according to the French and European Pharmacopoeias (Pharmacopée Francaise ed. 11, AN, European Pharmacopoeia ed. 11, 2025, Olah *et al*., 2022).

Determination of Total Flavonoid Content (TFC)

A spectral assay with aluminum chloride was used, according to the monographs of Romanian Pharmacopoeia 10th Edition, to quantify total flavonoids. An amount of 0,1 ml extract reacted with 3 ml of a 2.5 % solution of aluminum chloride, 5 ml solution of 10% sodium acetate, diluted to 25 ml with methanol, and the absorbance was measured at 430 nm after 30 min of repause using a UV-VIS Spectrophotometer. Blank samples were made in the same manner using 8 ml of purified water instead of the solution of aluminum chloride and sodium acetate. The calibration curve was built using standard solutions with a content of 0.6 -6.6 µg/ml rutin, R^2 =0.9989. Results were expressed as mg rutin equivalents per ml extract (mg RE/mL), with all tests performed in triplicate (Benedec *et al*., 2024)

Determination of Total Polyphenol Content (TPC)

Total polyphenol concentration was assessed using the phosphotungstic reagent method, according to Romanian Pharmacopoeia (Romanian Pharmacopoeia, ed 10). An amount of 50 µl extract was mixed with 0.5ml phosphotungstic reagent and diluted to 25 ml with 15 % sodium carbonate solution. Absorbance was recorded at 715 nm after 2 minutes, using a UV-VIS Spectrophotometer. Blank samples were made in the same manner without the phospotungstic reagent. The calibration curve was built using standard solutions with 0.3 – 5 µg/ml gallic acid and R^2 =0.9985. Results were expressed as mg gallic acid equivalents per ml extract (mg GAE/mL), and all tests were repeated in triplicate (Benedec *et al*., 2024).

Thin-Layer Chromatography (TLC)

In order to eliminate glycerol and interfering matrix components, the first step for thin-layer chromatography was a solid-phase extraction using a vacuum manifold. A portion of 3 ml extract was mixed with 27 ml of methanol and passed through a solid phase extraction column. The column was preconditioned with methanol and purified water. The sample was eluted with 3 ml acidulated methanol with 1% hydrochloric acid. The eluate was evaporated to dryness, then dissolved in 0.5 ml of methanol.

After solid-phase extraction, samples were analyzed using TLC on silica gel plates both for flavonoids and triterpenes. For the determination of flavonoids, two mobile phases were used: (1) ethyl acetate: methanol: water (77:15:8) with a development distance of 10 cm and (2) toluene: ethyl acetate (80:20) with a development distance of 17 cm, with drying at room temperature between the two steps. As reference standards, solutions were prepared with a concentration of 0,1 mg/ml of rutin, hyperoside, quercetin, and chlorogenic acid in methanol. An amount of 20 µl extract and reference solution were applied (Waksmundzka-Hajnos *et al*., 2008). Two plates were used, one of them sprayed with 1% 2-Aminoethoxydiphenyl borate (NTS) and 5% PEG 400 solution, for detection at 365 nm, and the other one with anisaldehyde–sulfuric acid reagent, heated for 10 min at 110°C for detection at daylight.

For the determination of triterpenes, the developing system consists of methanol, diethyl ether, and toluene (5:10:85), and the developing distance was 10 cm. Standards included betulinic acid, oleanolic acid, and ursolic acid, each with a concentration of 0.1 mg/ml in methanol, were applied. For detection, the plate was sprayed with anisaldehyde–sulfuric acid reagent, visualized at 365 nm, then heated for 10 minutes at 110°C, and visualized at daylight.

Antioxidant Activity *In vitro* Assessment

Antioxidant capacity was evaluated via the DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical scavenging method and FRAP (Ferric Reducing Antioxidant Power) method.

In the first case, changes in absorbance at 517 nm were used to calculate IC_{50} values (the concentration required to inhibit 50% of DPPH radicals), indicating the antioxidant strength of each extract. Different volumes of extract were diluted to 10 ml of methanol in order to obtain a concentration of 30, 60, 90 µl/ml, and 5 ml of these solutions were mixed with a 0.25 mM solution of DPPH. Absorbance was measured after 30 minutes of incubation at 40°C. For the reference solution, 5 ml of 0.25 mM DPPH solution was mixed with 5 ml of methanol and incubated for 30 minutes at 40°C (Gulcin I, 2023). Antioxidant activity (AA%) was calculated using the formula: AA% = [(A_control − A_sample)/A_control] × 100, where *A_control* represents the absorbance of the DPPH solution without extract, and *A_sample* the absorbance with extract. Results were expressed using the half-maximal inhibitory concentration (IC_{50}) (µg/mL), and all determinations were performed in triplicate.

For the determination of antioxidant effect by the Ferric Reducing Antioxidant Power (FRAP) method, 24 µl of extract was diluted to 2 ml purified water and mixed with 6 ml FRAP reagent. Absorbance was measured at 593 nm. Blank solution was prepared by mixing 2 ml purified water and 6 ml FRAP reagent. A calibration curve was made using Trolox solutions containing 10, 20, 30, and 40 µg Trolox/ml. Results were expressed as µmol equivalents of Trolox/ ml extract (µmol ET/ ml); all determinations were performed in triplicate.

RESULTS

B. verrucosa bud extract revealed higher levels of both flavonoids and total polyphenols than *B. pubescens*. In the case of *B. verrucosa* extract, the total flavonoid concentration was 1.78 mg RE/ml extract, while for *B. pubescens* extract concentration was 1.50

mg RE/ml (Table 1). The total polyphenolic compounds followed the pattern in the case of *B. verrucosa* extract, the determined concentration being 2.37 mg GAE/ml, while in the case of *B. pubescens* extract, it was 1.99 mg GAE/ml (Table 1). The antioxidant potential determined with the DPPH method of the two gemmotherapy extracts was appropriate, while the evaluation with the FRAP method showed a better capability of reducing ferric ion for *B. verrucosa* extract (Table 1).

The TLC of the extract revealed the presence of chlorogenic acid, hyperoside (Fig. 1), betulinic and ursolic acids (Fig. 2).

Table 1.

Results of quantitative analysis of TPC, TFC, and assessment of *in vitro* antioxidant activity

	TFC, mg RE/mL	TPC, mg GAE/mL	DPPH, IC_{50}, µg/mL	FRAP, µmol ET/ ml
B. verrucosa bud extract	1.78 ± 0.30	2.37 ±0.05	51.82*±0.56	29.2*±0.12
B. pubescens bud extract	1.50 ±0.10	1.99±0.13	43.32*±0.32	14.4* ±0.11

Notes: TPC: total polyphenols content; TFC: total flavonoids content; GAE: gallic acid equivalents; RE: rutin equivalents. Each value is the mean of three replicates ± SD, *p<0.0001

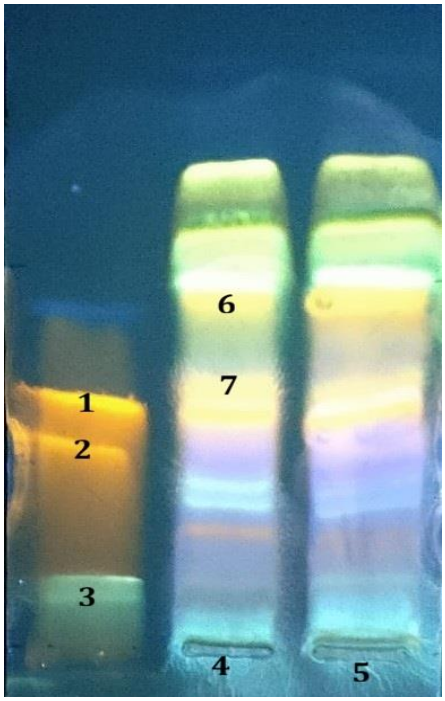

Fig. 1. TLC for polyphenols and flavonoids (1 - hyperoside, 2 - rutin, 3 - chlorogenic acid, 6 - quercetin, 7 – isoquercitrin).

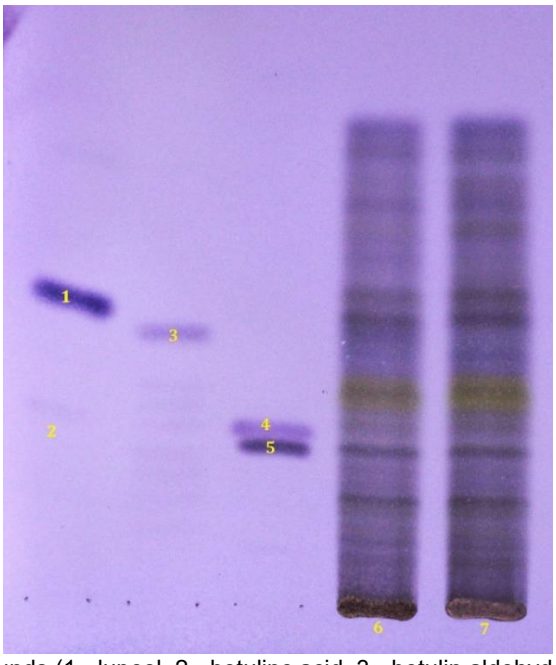

Fig. 2. TLC for triterpenoid compounds (1 - lupeol, 2 - betulinc acid, 3 - betulin aldehyde, 4 - betulin, 5 - ursolic acid).

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DISCUSSIONS

Gemmotherapy uses extracts prepared from young plant organs such as buds, young shoots (micro/macroblasts), or rootlets that present meristematic tissues. These tissues contain a unique biochemical profile, rich in growth factors, phytohormones, and precursors of secondary metabolites, which are not present in mature plant organs. Extracts from *Betula* species are widely used due to their detoxifying, anti-inflammatory, and renal-supportive properties. The phytochemical analysis of bud extracts from *B. verrucosa* and *B. pubescens* demonstrated a higher concentration of flavonoids and total polyphenolic compounds in *B. verrucosa*. These findings are consistent with previous reports suggesting interspecies variability in secondary metabolite production within the *Betula* genus (Raal *et al*., 2013).

The results obtained by the TLC method revealed the presence of flavonoids, polyphenols, and triterpenes like hyperoside, chlorogenic acid, betulinic acid, and ursolic acid. Besides hyperoside, quercetin, and isoquercetin, as well as flavonol aglycons, were also identified in the two extracts. Based on the TLC, chlorogenic acid is more present in *B. pubescens* extract (Fig. 1, (5)) than in the *B. verrucosa* extract (Fig.1, (4)). Also, in the *B. pubescens* extract, the presence of rutin can be noticed, but in a low concentration. All these compounds are therapeutically effective, especially for their anti-inflammatory and antioxidant effect. Taking into consideration the TLC of triterpenoidic compounds, there is no significant difference between the intensity of bands of the two extracts (Fig.2., (6- *B. verrucosa*) (7 – *B. pubescens*)).

Chlorogenic acid is a well-documented bioactive compound with significant therapeutic potential. Studies have shown a strong antioxidant capacity, regulatory action on metabolic pathways, and its ability to modulate inflammatory and cellular signaling cascades (Huang *et al*., 2023).

Hyperoside is another frequently studied flavonol glycoside found in various medicinal plants with a wide spectrum of biological activities, including antioxidant, anti-inflammatory, cytoprotective, organ-protective, anticancer, neuroprotective, and vascular protective effects. Studies have shown that its actions are mediated through enhancement of endogenous defense, inhibition of oxidative stress and inflammation, modulation of key signaling pathways, and regulation of cell survival (Park *et al*., 2016).

Another well-studied and widely distributed in many medicinal plants flavonol aglycone is quercetin. It has been studied for its pharmacological activity, such as antioxidant, anti-inflammatory, cytoprotective, antimicrobial, metabolic, cardiovascular, and anticancer effects, due to its capacity to interact with cellular redox systems, regulate transcription factors, and modulate key signaling cascades involved in inflammation, metabolism, and cell survival (Frenț *et al*., 2024).

Rutin is a naturally occurring flavonol glycoside, present in many medicinal plants, fruits, and vegetables. It has drawn substantial scientific interest because of a broad spectrum of biological activities, including antioxidant, anti-inflammatory, cytoprotective, and metabolic effects (Rahmani *et al*., 2022). Studies show that rutin enhances the cellular antioxidant defense by upregulating antioxidant enzymes, like superoxide dismutase, catalase, or glutathione-related enzymes, and increasing reduced glutathione levels. Concurrently, it reduces markers of oxidative damage such as malondialdehyde. Besides antioxidant effect, rutin has been shown to suppress the expression and release of pro-inflammatory mediators such as Tumor necrosis factor alpha (TNF-α), Interleukin-6 (IL-6), as well as enzymes such as Cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), which are critical in inflammatory responses (Muvhulawa *et al*., 2022).

Betulinic acid is a naturally occurring pentacyclic triterpenoidic compound predominantly isolated from the bark of *Betula* species and various medicinal plants. Over the past decades, it has attracted substantial research interest due to its broad pharmacological profile, particularly its anticancer, antiviral, anti-inflammatory, and metabolic effects. Its unique structure, derived from the lupane-type triterpene framework, contributes to its membrane-modifying properties and interaction with numerous molecular targets (Lou *et al*., 2021; Zheng *et al*., 2025).

Another, a naturally occurring triterpenoid is ursolic acid, which demonstrates significant anti-inflammatory and antioxidant effects in preclinical models. Evidence from a systematic review and meta-analysis shows reduced pro-inflammatory cytokines, enhanced antioxidant enzyme activity, and decreased oxidative damage, likely through regulation of inflammatory and redox signaling pathways (Zhao *et al*.,2023).

The results of antioxidant potential are in concordance with the presence of these bioactive compounds. The DPPH assay is one of the most commonly applied methods for determining the free radical scavenging activity of vegetal extracts, purified compounds, and food matrices. Despite the higher concentration of polyphenolic compounds in *B. verrucosa* buds extract, *B. pubescens* buds extract showed a slightly stronger antioxidant potential ($p<0.0001$) determined with DPPH assay. Studies showed that extracts obtained from leaves of *B. verrucosa* can present a strong antioxidant activity by the DPPH method at a moderate concentration (~98% scavenging at 80 µg/mL). Other studies reported moderate to strong antioxidant activity measured by DPPH assay on bark extracts of different species of *Betula*. The bark extract of *Betula alba* var. *pendula* displayed moderate DPPH scavenging capacity ($IC_{50} \approx$ 73.6 µg/mL), likely related to its rich polyphenolic composition, while the methanolic extract of *Betula platyphylla* var. *japonica* showed substantially higher antioxidant potency, achieving an IC_{50} of approximately 2.4 µg/mL (Penkov *et al*., 2018; Ghica *et al*., 2023; Ju *et al*., 2004). However, these results cannot be compared with the results obtained from our determinations, due to the difference in the extraction method, species, solvent, and plant part.

FRAP assay is a widely utilized analytical technique for quantifying the total antioxidant capacity of biological samples, plant extracts, and food products.

The method is based on the principle that antioxidants act as reducing agents, capable of transferring electrons to oxidized molecules. In the FRAP system, this electron-donating ability is evaluated by monitoring the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) under acidic conditions. Tests conducted by researchers from Spain on ethanol extracts of *B. verrucosa* leaves (50% w/w) showed values of 1.52 mmol TE/g dry raw material, and those from Poland on ethanol extracts of leaves (70% w/w) obtained values of 640 ± 10 mg TE/g dry raw material. The values obtained for *B. pubescens* leaf extracts from plant material harvested from Poland showed a value of 40 µmol TE/l. extract (Kucharski *et al*., 2025, Grabek-Lejko *et al*., 2017, Azman *et al*., 2017). Comparing the results obtained by them with our samples becomes difficult due to the non-uniformity of the way of expressing the results, the extraction methods, or the different nature of the plant material.

Flavonoids are vital for their free-radical scavenging and anti-inflammatory properties (Panche *et al*., 2016). Their abundance in *B. verrucosa* buds may reflect a more active biosynthetic pathway or a greater physiological need for defense compounds during early tissue development. Given that buds represent a vulnerable stage in plant development, the higher flavonoid content likely plays a role in protecting against environmental stressors such as UV radiation, pathogens, and oxidative damage (Treutter, 2006).

Total polyphenol content, another indicator of antioxidant potential, was also notably higher in *B. verrucosa*. Polyphenols, including phenolic acids and tannins, contribute to the plant's ability to neutralize free radicals and modulate inflammatory pathways (Scalbert *et al*., 2005). The greater presence of these compounds enhances the therapeutic prospects of *B. verrucosa* buds as gemmotherapeutic products.

However, the higher content of flavonoids and polyphenols does not explain the antioxidant potential on its own. Although some studies show that flavonoids are superior in direct antioxidant activity, while triterpenoids (including betulinic acid, betulin, and ursolic acid) are stronger modulators of mitochondrial apoptosis and membrane integrity, with quercetin excelling in signaling modulation, and betulinic acid excelling in mitochondria-targeted cytotoxicity (Szlasa *et al*., 2023). Other studies identified potential synergy between quercetin/rutin and betulinic acid, improving anti-inflammatory outcomes (Gangwar *et al*., 2021). These can partially explain the different behavior in the antioxidant mechanism of the two extracts. The synergy of these bioactive compounds remains to be studied in more detail.

CONCLUSIONS

Results obtained in this study show that *B. verrucosa* and *B. pubescens* bud extracts have high polyphenol and flavonoid contents, contributing to the antioxidant activity of neutralizing the DPPH radical and reducing the ferric ion to the ferrous ion by the FRAP method. Following the phytochemical analysis, a higher concentration of flavonoids and total polyphenolic compounds was observed in the *B. verrucosa* buds extract.

B. pubescens bud extract presented a higher antioxidant potential determined by the DPPH radical neutralization method, and *B. verrucosa* bud extract showed a stronger activity in reducing the ferric ion. These different results could be related to the preponderance of certain polyphenolic components with different antioxidant activity that act *in vitro* through different antioxidant mechanisms. Additional analysis by more advanced methods (e.g., LC/MS) would be necessary to be able to qualitatively and quantitatively characterize the individual polyphenolic profile and explain the difference in antioxidant response.

These findings suggest that the antioxidant activity is mediated by a synergy of compounds and also reinforce the traditional medicinal use of birch buds, while also highlighting the potential of *B. verrucosa* and *B. pubescens* in developing gemmotherapeutic products. Future research should focus on the identification of individual bioactive constituents, evaluating their pharmacokinetics, and conducting clinical trials to substantiate therapeutic claims.

AUTHORS CONTRIBUTIONS

Conceptualization, T.H.B., V.T., N.K.O. and D.H.; methodology, T.H.B., V.B.B., C.E. R.B., V.T., N.K.O., I.I. and D.H.; data collection, T.H.B., V.B.B., C.E., R.B. and I.I..; data validation, V.T. N.K.O and D.H.; data processing, T.H.B., V.B.B., C.E. R.B., V.T., N.K.O., I.I. and D.H.; writing—original draft preparation, T.H.B., N.K.O. and I.I.; writing—review and editing, T.H.B., B.V.B, V.T., N.K.O. and D.H.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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