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Original Article

Antioxidative and anti-inflammatory study on the ethanolic extract of the root of *Bruckenthalia spiculifolia* (Salisb.) Reichb.

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Abstract:

Bruckenthalia spiculifolia belongs to the Ericaceae family. The aim of this study was to quantify the total content of phenols, tannins, and flavonoids spectrophotometrically and to evaluate the *in vivo* and *in vitro* antioxidant and anti-inflammatory activity of the ethanolic extract of the root of *B. spiculifolia*. The antioxidant activity with IC_{50} 2.59±0.10 µg/ml and 9.10±0.81 µg/ml in DPPH and *β*-carotene/linoleic acid assay, respectively, was measured. Significant anti-inflammatory activity was observed by inhibiting the denaturation of bovine serum albumin by 87±0.004% and reducing the inflammatory response induced by carrageenan in rats in a dose-dependent manner. In view of the demonstrated effects, the ethanolic extract from the root of *B. spiculifolia* can potentially be used as a new natural source for the pharmaceutical industry.

Key words:
BSA, carrageenan, *Erica spiculifolia*, phytochemical compounds

Apstrakt:

Antioksidativna i anti-inflamatorna studija etanolnog ekstrakta korena *Bruckenthalia spiculifolia* (Salisb.) Reichb.

Bruckenthalia spiculifolia pripada familiji Ericaceae. Cilj ovog rada je spektrofotometrijska kvantifikacija ukupnih fenola, tanina i flavonoida, kao i *in vitro* i *in vivo* procena antioksidativne i antiinflamatorne aktivnosti etanolnog ekstrakta korena *B. spiculifolia*. Etanolni ekstrakt korena je pokazao antioksidativnu aktivnost u DPPH i *β*-karoten/linoleinska kiselina testu (sa IC_{50} 2,59±0,10 µg/ml i 9,10±0,81 µg/ml). Značajna antiinflamatorna aktivnost je zabeležena uz denaturaciju goveđeg serumskog albumina od 87±0,004% i dozno zavisno smanjenje inflamatornog odgovora izazvanog karagenanom kod pacova. S obzirom na pokazanu antioksidativnu i antiinflamatornu aktivnost, etanolni ekstrakt korena *B. spiculifolia* se može potencijalno koristiti kao novi prirodni resurs u farmaceutskoj industriji.

Ključne reči:
BSA, karagenin, *Erica spiculifolia*, fitohemijska jedinjenja

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Introduction

Bruckentalia spiculifolia Salisb (syn. *Erica spiculifolia* Salisb., *Brukentalia spiculifolia* Drude) is the monospecific genus of cosmopolitan Ericaceae family (Jovanović, 1972). Today, the high mountain regions of Greece, Romania, Bulgaria, Turkey, Macedonia, and Serbia are the only places where *B. spiculifolia* can be found (Turrill, 1929; Horvat et al., 1974; Polunin & Walters, 1985). The absence of bracteoles, the partially fused sepals, and a unique type of caryology are features of this genus (Oliver 1989, 2000). The discovery of *Bruckenthalia* seeds in a deposit at Gort in County Galway, Ireland, in 1959 provided the first evidence of the plant's earlier occurrence at sites in Western Europe (Jessen et al., 1959). However, their true parentage was not discovered until Phillips & Sparks (1976) refuted their initial claim that they were descended from *Erica scoparia* var. *macrosperma*.

Bruckenthalia spiculifolia is an evergreen shrub with tiny flowers (corolla 2.5 – 3 mm long) grouped in terminal inflorescences. A low-growing, bushy shrub or subshrub has a pistillate apex that is the same diameter as the rest of the pistil, but is often mistakenly called a „stigma". This plant species

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grows on acidic soils in mountainous shrublands between 1,400 and 2,500 meters (Mitić et al., 2018). The soils of the ecosystems where this species lives are volcanic rocks with mostly acidic character (between 4 and 6) (Fagúndez & Izco, 2008). Folk uses of *Erica* species include antirheumatic, diuretic, and astringent effects, as well as treatment of urinary tract infections, wounds, snakebites, and inflammation-related diseases, including draining edema (Mitić et al., 2018).

As far as we know, the available literature data on *B. spiculifolia* are scarce, especially for the ethanolic extract. There are several studies whose aim was to investigate the primary phytochemical composition and antioxidant activity of the ethanolic extract of *B. spiculifolia*.

According to Pavlović et al. (2009) significant amounts of polyphenolic compounds, including tannins and flavonoids, were found in the ethanolic extract of aboveground parts of the *B. spiculifolia*. The aforementioned authors observed the remarkable antioxidant activity of the ethanolic herbal extract as determined by three assay systems, namely total antioxidant capacity (FRAP), free radical scavenging (DPPH), and inhibition of lipid peroxidation (Pavlović et al., 2009). Qualitative and quantitative HPLC analysis of phenolic compounds, total phenylpropanoids content, and inhibition of *β*-carotene bleaching of the ethanolic leaf extract of *B. spiculifolia* was evaluated (Pavlović et al., 2013). Yet, there is no data in the literature on phytochemical compounds, and antioxidant and anti-inflammatory activity of the root extract of *B. spiculifolia.*

The aim of this study was to determine the content of the total phenolic, total tannin, and total flavonoid compounds of ethanolic extract of the root of *B. spiculifolia*. Furthermore, the antioxidant activity of the ethanolic root extract is investigated with two different complementary *in vitro* tests: DPPH radical scavenging activity and *β*-carotene/linolenic acid assay. The anti-inflammatory activity of the ethanolic root extract was determined *in vitro* by a protein denaturation assay using an aqueous solution of bovine serum albumin and *in vivo* in a carrageenan-induced rat paw oedema assay.

Materials and Methods

Plant material, extraction, and chemical analysis

The plant species was collected in Serbia (Vlasina Lake) and identified by Professor Bojan Zlatković, from the Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš. The *B. spiculifolia* (voucher No. 3364) has been deposited in the Herbarium of the Faculty of Pharmacy, University of Belgrade. The root was percolated with 70%, v/v ethanol according to Ph. Eur. 10.0 (2020) and then evaporated to dryness under vacuum at 40 °C; the extraction yield was 11.5%.

All chemicals used were obtained from Sigma Aldrich (USA), or Zorka Pharma (Šabac, Serbia). All solvents and chemicals were of analytical grade.

Determination of total phenolic contents

The total phenolic content of ethanolic extract of the root of *B. spiculifolia* was determined using the Folin-Ciocalteu method (Makkar et al., 2000; Maksimović et al., 2005). The properly diluted test samples are filled into test tubes with ethanol (70% v/v) as solvent. Then the Folin-Ciocalteu reagent, previously diluted 1:1 v/v with distilled water, and 20% Na_2CO_3 solution were added to test tubes. The tubes were then shaken vigorously for 40 min to develop the blue color and the absorbance was measured spectrophotometrically at 725 nm in comparison to a blank containing extraction solvent instead of the sample. The total phenolic content of the root extract was calculated and expressed as mg catechin equivalents (CE) per g of dry extract using a (+)-catechin calibration curve (1-5 μg/mL range). The total phenolic assay was done in triplicate.

Determination of total tannin content

The total tannin content of the ethanolic extract of the root of *B. spiculifolia* was determined using the same Folin-Ciocalteu method (Makkar et al., 2000; Maksimović et al., 2005). Polyvinylpolypyrrolidone was added to the test tubes containing the sample and then shaken vigorously. The supernatant contains all phenolic compounds except the tannins. The test was performed on the clear supernatant and the results were expressed in mg catechin equivalents (CE) per g dry extract. The experiment was done in triplicate. The content of non-tannin polyphenols is also expressed as mg catechin equivalents per g of dry extract and total tannin content is obtained from the difference.

Determination of total flavonoid content

The total flavonoid content of the ethanolic extract of the root of *B. spiculifolia* was estimated according to Lamaison & Carnat (1990). Aluminum trichloride ($AlCl_3$) in ethanol was mixed with the same volume of the extract solution. The blank sample consisted of $AlCl_3$ with ethanol without extract solution. After incubation for 1 h, the absorbance was measured spectrophotometrically at 430 nm. The total flavonoid content of the root extract was calculated and expressed as mg rutin (Ru)/g dry extract using a rutin calibration curve (0.5-5 μg/mL range). The total flavonoid assay was done in triplicate.

Antioxidant activity examination
DPPH assay

The DPPH (1,1-diphenyl-2 picrylhydrazyl) free radical scavenging assay was used to measure the antioxidant activity of the ethanolic extract of the root of *B. spiculifolia* according to Pavlović et al. (2017). Increasing volumes (0.1 mL, 0.2 mL, 0.4 mL 0.6 mL, 0.8 mL) of the ethanolic extract with DPPH in 96% (v/v) ethanol were added to a 96-well microtitration plate. The plate was stored in the dark for 30 min at room temperature, then the absorbance was measured on an ELISA reader at 540 nm against 96% (v/v) ethanol as a blank. The synthetic antioxidants BHT and BHA were used as reference substances, while DPPH with 96% (v/v) ethanol was used as a control. Inhibition of DPPH free radical in percent was calculated according to:

$$\% \text{ DPPH} = (A_C - A_S) / A_C \times 100$$

where A_C is the absorbance of the control, and A_S is the absorbance of the sample. The ability to neutralize a free radical can be determined by the IC_{50} value, i.e. the concentration of the extract that removes (neutralizes) 50% of the stable DPPH• radicals from the solution (Cuendet et al., 1997).

β-carotene bleaching assay

The *β*-carotene bleaching assay was used to evaluate the ability of the ethanolic extract of the root of *B. spiculifolia* to inhibit lipid peroxidation according to Koleva et al. (2002). To perform the assay, Tween-20 and linoleic acid were combined with *β*-carotene solution in chloroform, and the mixture was evaporated under vacuum at a temperature of 40 °C. After adding distilled water, an emulsion was formed by shaking the mixture. A freshly prepared *β*-carotene-linoleic acid emulsion was added to the sample in a 96-well microtitration plate. The control contains solvent (70% v/v) instead of the extract solution. The absorbance was read on an ELISA reader at 450 nm after the plate had been shaken on a microplate shaker. After reading the initial absorbance, the plate is placed in an incubator at 55 °C for 120 min, after which the absorbance is read again. The percentage inhibition of *β*-carotene bleaching by the samples was calculated according to the formula (Barros et al., 2007):

$$\% \text{ inhibition} = 100 - (A_{120} / A_0) \times 100$$

where A_{120} is the absorbance of the sample at t=120 min and A_0 is the absorbance of the sample at t=0 min. The synthetic antioxidants BHT and BHA were used as reference substances.

Anti-inflammatory activity examination
Animals and housing

Adult male Wistar rats (200–250 g) from the animal farm of the Medical Military Academy in Belgrade, were used in this study. The experiment was conducted at the Medical Faculty of the Military Medical Academy, University of Defence in Belgrade in accordance with the internationally accepted principles for laboratory animal use and care in the European Community (EEC Directive of 1986; 86/609/EEC) adopted by Ethical Committee of Military Medical Academy, Belgrade (No. 323-07-06862/2016-05/2).

Evaluation of in vitro anti-inflammatory activity

The *in vitro* anti-inflammatory activity of the ethanolic extract of the root of *B. spiculifolia* was investigated using the protein denaturation assay according to Lavanya et al. (2010). The test solution consists of an ethanolic extract of the root and a 5% w/v aqueous solution of bovine serum albumin (BSA). A distilled water and a 5% w/v aqueous solution of bovine serum albumin served as a control. The results were compared with the standard solution, which consisted of diclofenac sodium and a 5% w/v aqueous solution of bovine serum albumin. The absorbance was measured at 340 nm using an ELISA microplate reader. The inhibition (%) of protein denaturation was calculated by applying the following equation:

Protein denaturation (%) = 100 - ((optical density of test solution - optical density of product)/optical density of test control) × 100)

The control represents 100% protein denaturation. The results were compared with diclofenac (100 µg/mL).

Evaluation of in vivo anti-inflammatory activity

The *in vivo* anti-inflammatory activity of the ethanolic extract of the root of *B. spiculifolia* was investigated in carrageenan-induced paw oedema assay according to Dobrić et al. (2017). The tested root extract was dissolved in dimethyl sulfoxide (DMSO) and administered *per os* by gastric tube at a dose of 50, 100, and 200 mg/kg body weight, 60 min before the onset of inflammation. Groups of 6 animals were formed for each dose. Oedema was induced by injection of 0.5% carrageenan solution into the right hind paw of rats. The same volume of saline solution was injected into the left hind paw of the rats. The control animals were administered a vehicle (DMSO) *per os* at 1 ml/kg. Indomethacin, which was also dissolved in DMSO and administered *per os*, served as the reference drug at a dose of 4

mg/kg. Three hours after injection of carrageenan, i.e. saline solution, and 4 h after administration of the extracts, the difference in mass between the right and left paw, indicated the size of the oedema, i.e. the degree of inflammation was measured (Dobrić et al. 2017).

Statistical analysis

Data for both the *in vitro* and the *in vivo* assay were expressed as the mean ± SD value. A One-Way Analysis of Variance (ANOVA) followed by Tukey's post-hoc analysis (for *in vitro* tests) or Mann-Whitney U analysis (for *in vivo* tests) was used to determine whether there were statistically significant differences between treatments (GraphPad Prism version 5.03, San Diego, CA, USA).

Results and discussion

Numerous biological effects of phytochemical compounds from plant extracts have been documented in the scientific literature (Sharma et al., 2021). The most important phytochemical compounds include phenols, tannins, flavonoids, alkaloids, glycosides, steroids, and others. Most of the intended beneficial properties are due to these chemical compounds (Alzamel, 2022). Phenolic compounds have been extensively studied for their biological effects, such as antioxidant, anti-inflammatory, antimicrobial, antihypertensive, anticarcinogenic, and antimutagenic effects, which may be beneficial to human health (Shahidi & Ambigaipalan, 2015; Cosme et al., 2020). Pharmaceutical applications of tannins include astringents, diuretics, gastric and duodenal antitumor agents, antioxidants, antiseptics, anti-inflammatory agents, and hemostatics (Ribeiro et al., 2022). Various positive effects are attributed to flavonoids, including cytotoxic, anti-cancer, antibacterial, anti-inflammatory, anti-allergic, antioxidant, and vascular effects (Mutha et al., 2021).

The Folin-Ciocalteu method was used to determine the total phenolic content of the ethanolic extract of the root of *B. spiculifolia*, which was 194.41±5.74 CE/g (**Tab. 1**). The tannin compounds were also examined using the same method. We found that the extract contained 150.17±7.52 CE/g tannins (**Tab. 1**). The total flavonoid content in the ethanolic extract, expressed as mg rutin per g dry extract, was also analyzed. The results showed that the ethanolic extract of the root contains 3.51±0.06 mg Ru/g flavonoids (**Tab. 1**). As the obtained results indicate, the studied ethanolic root extract of *B. spiculifolia* contains considerable amounts of phenolic, tannic and flavonoid compounds. These phytochemical compounds may be responsible for many of the health benefits of this extract. In previous study significant amounts of phenolic compounds were found in plant materials and dry leaves extracts of *B. spiculifolia*: 3.71±0.05 and 11.79±0.25% flavonoids, 4.67±0.08 and 19.22±0.48% total polyphenols, 0.86±0.06 and 3.99±0.07% non-tannins, 3.80±0.06 and 15.23±0.07% tannins, respectively (Pavlović et al., 2009). In the study conducted by Pavlović et al. (2013) seven phenolic compounds were identified in the dry ethanolic leaf extracts. The main phenolic compounds were quercitrin (70.91 mg/g), quercetin 3-O-glucoside (32.5 mg/g), and quercetin (3.77 mg/g) (Pavlović et al., 2013).

The antioxidant activity of ethanolic extract of the root of *B. spiculifolia* was examined by two complementary tests: DPPH free radical scavenging activity and *β*-carotene bleaching assay. The investigated ethanolic extract of root showed antioxidant activity with IC_{50} 2.59±0.10 μg/mL and 9.10±0.81 μg/mL in DPPH and *β*-carotene bleaching assay, respectively (**Tab. 1**). According to Pavlović et al. (2009), the ethanolic herbal extract of *B. spiculifolia* showed anti-radical activity with IC_{50} 10.22±0.74 μg/ml. Based on the results of our study and the study conducted by Pavlović et al.

Table 1. Phenolic compounds contents and IC_{50} values in *in vitro* antioxidant assays of ethanolic extract of the root of *B. spiculifolia* (Salisb.) Reichb.

	Phenolic content (mg CE/g)			Total flavonoid content (mg Ru/g)	Radical scavenging assay IC_{50} (μg/mL)	β-carotene linoleic assay IC_{50} (μg/mL)
	Total phenolic content (mg CE/g)	Total nontannin content (mg CE/g)	Total tannin content (mg CE/g)			
	194.41±5.74	44.23±12.29	150.17±7.52	3.5±0.06	2.59±0.10	9.10±0.81
BHT					22.82±2.07	0.03±0.00
BHA					2.44±0.09	0.04±0.01

Data are given as mean ± SD (n=3)

(2009) the ethanolic extract of the root has a higher DPPH radical scavenging activity. The plant species were collected from different locations, which could be one reason for the different results. Further, the different antioxidant activity could be due to the fact that different plant parts were used to prepare the ethanolic extract and the phytoconstituents are different (Pavlović et al., 2009). The dry ethanolic herbal extract in concentration of 62.5 µg/ml reduced the bleaching of *β*-carotene by more than 95% (Pavlović et al., 2013). These results are similar to those found for the same extracts tested to inhibit lipid peroxidation in liposomes (Pavlović et al., 2009). In a recent study by Pavlović et al. (2024), the ethanolic extract of the leaves, flowers, and aboveground parts as well as different fractions of the extract, except for the petroleum ether fraction, showed excellent antioxidant activity in the two complementary assay methods: DPPH radical scavenging activity and inhibition of lipid peroxidation in the *β*-carotene-linolenic acid assay. For comparison, the abilities of the commercially available synthetic antioxidants BHT and BHA were investigated. Under the same conditions under which the extract was tested, the IC_{50} values for BHT (22.82±2.07 µg/mL; 0.03±0.00 µg/mL) and BHA (2.44±0.09 µg/mL; 0.04±0.01 µg/mL;) were determined in DPPH and *β*-carotene bleaching assay, respectively (**Tab. 1**). By comparing the concentrations obtained, it can be concluded that the ethanolic root extract showed a better ability of free radicals removal from the commercial antioxidant BHT. The reaction mechanism between antioxidants and $DPPH^{\bullet}$ radicals depends on the structural conformations of antioxidants, a large number of compounds react slower, and more complex mechanisms are involved (Prior et al., 2005). If the radical formed by the antioxidant is not stable enough, many other reactions are also possible. Due to the complex reactions involving three different reaction pathways between BHT and $DPPH^{\bullet}$ radicals, the obtained results can be explained (Bondet et al., 1997). On the other hand, the synthetic antioxidants showed significantly greater activity in stopping the chain reaction of lipid peroxidation. A significant correlation between antioxidant capacity and total phenolic content suggests that phenolic substances are the most responsible for the antioxidant capacity. The antioxidant activity of phenols comes from their high reactivity as electron or hydrogen donors, from properties of radicals formed from phenols to stabilize and delocalize the unpaired electron and to chelate transition metal ions (Gebicki & Nauser, 2021; Andrés et al., 2023).

According to Cui et al. (2013) and Dharmadeva et al. (2018), one of the causes of inflammation is the production of autoantigens through protein denaturation. Changes in hydrophobic, disulfide, hydrogen, and electrostatic bonds may be part of the mechanism of protein denaturation (Cui et al., 2013; Dharmadeva et al., 2018). Consequently, the inhibition of protein denaturation can reduce inflammatory activity. The standard drug in this study was diclofenac, a non-steroidal anti-inflammatory drug (NSAID). NSAIDs have an anti-inflammatory effect by preventing the function of the enzyme cyclooxygenase. However, these drugs can also cause constipation, bleeding, ulcers, and perforations (Sohail et al., 2023). In the current study, ethanolic extract of the root of *B. spiculifolia* showed a significant anti-inflammatory effect with a percentage inhibition of BSA denaturation of 87±0.004%, which is comparable although lower than the standard value for diclofenac (95.6±0.001%). The secondary metabolites like phenolic and tannin compounds which were found in preliminary phytochemical screening might be responsible for this activity (Fürst & Zündorf, 2014; Ghosh, 2015). Therefore, it is necessary to explore plant-based anti-inflammatory drugs that are natural, safe, and have few side effects. Pavlović et al. (2024) discovered that the ethanolic extract of leaves, flowers, and aboveground part of *B. spiculifolia* and the various fractions, with the exception of petroleum ether, exhibited significant and similar inhibition of BSA denaturation as diclofenac. In addition, the anti-inflammatory effect of the ethanolic extract of the root of *B. spiculifolia* was investigated in an *in vivo* assay of carrageenan-induced paw oedema. Based on the difference between rats treated with the active substance and rats treated with the vehicle (control), the anti-inflammatory effect of the tested extract and reference drug was found in a carrageenan-induced paw oedema assay. Oral administration of the ethanolic extract of the root of *B. spiculifolia* statistically significantly inhibited the carrageenan-induced inflammatory response in rats in a dose-dependent manner, but differences in the effect between individual doses only in some cases reach the level of statistical significance (far right column of **Tab. 2**). The strongest inhibition 52.37±7.03% was observed at a dose of 200 mg/kg ethanolic extract of root, which was higher than that of the reference drug at a dose of 4 mg/kg, while the vehicle (DMSO) at a dose of 1 ml/kg showed no effect. The mechanism of action of carrageenan-induced inflammation is biphasic, with the first phase characterized by the release of histamine, serotonin, and bradykinin, and to a lesser extent prostaglandins produced by cyclooxygenase enzymes (COX) in the first hour, while the second phase is attributed to neutrophil infiltration, and the continuing of

Table 2. *In vitro* and *in vivo* anti-inflamatory activity of ethanolic extract of the root of *B. spiculifolia* (Salisb.) Reichb.

	The inhibition of protein denaturation (%)	Percent of edema inhibition (%)			Statistical comparison between the doses
		50	100	200	
Extract (mg/kg; p.o.)	87±0.004	38.50±9.95 [c]	40.77±8.67 [c]	52.37±7.03 [c]	50:100 [ns]; 100:200 [a]; 50:200 [a]
Diclofenac	95.6±0.001				
DMSO (1 ml/kg; p.o.)			0,00±10.23		p*
Indometacin (4 mg/kg; p.o.)			50.28±6.4		

Data are given as mean ± SD (n=6); Comparison was done using One Way ANOVA followed by Tuckey's post hoc test.
[a] p<0.05; [b] p<0.01; [c] p<0.001 *vs.* DMSO treated rats,
*p significance of the difference between individual doses within the same group,
ns – no statistically significant difference between the doses

the prostaglandin generation (Brooks & Day, 1991; Gilligan et al., 1994). The delayed phase of carrageenan-induced acute inflammation involves the release of pro-inflammatory cytokines such as tumor necrosis factor (TNF-*α*) and interleukin-1 *β* (IL-1 *β*) as well as neutrophil-derived free radicals and nitric oxide (NO) (Halici et al., 2007). The results of the current study show that the extract reduces the acute inflammation caused by carrageenan. This effect can be explained by the inhibition of the release or synthesis of cyclooxygenase and/or lipoxygenase products since the production of arachidonic acid metabolites is an important factor involved in both phases of carrageenan-induced inflammation (Guay et al., 2004). In addition, the anti-inflammatory effect of the ethanolic extract of the root of *B. spiculifolia* could be related to the inhibition of neutrophil infiltration and the elimination of free radicals, which are characteristics of the second phase of inflammation (Halici et al., 2007). A statistically significant anti-inflammatory effect, ranging from 17.8% to 63%, was obtained from ethanol extracts of the leaves, flowers, and aboveground parts as well as from petroleum ether, ethyl acetate, and water fractionations of the ethanol extract of *B. spiculifolia* (Pavlović et al., 2024). In the *in vivo* study, it was found that the studied gel containing 2% dry leaf extract of *B. spiculifolia* significantly reduced the degree of skin irritation and reversed the pH of the skin disturbed by the pre-irritation with sodium lauryl sulfate (Pavlović et al., 2013). Further, ethanolic extracts of leaves, flowers, and herbs of *B. spiculifolia* significantly inhibited NO production by macrophages stimulated with lipopolysaccharide (Pavlović et al., 2022).

Our study of the ethanolic extract of the root of *B. spiculifolia* has shown that the investigated extract contains significant amounts of phenolic, tannin, and flavonoid compounds, as well as considerable *in vitro* antioxidant and *in vitro*/*in vivo* anti-inflammatory activity, providing a basis for future investigations. Identification of the molecules responsible for the pharmacological activity requires phytochemical studies to separate the compounds. Additional spectral evaluation of the isolated chemicals will also yield a potential drug for later use.

Conclusion

The studied ethanolic root extract of *B. spiculifolia* contains considerable amounts of phenolic, tannic, and flavonoid compounds. These phytochemical compounds may be responsible for many of the health benefits of this extract. The studied ethanolic extract of the root showed significant antioxidant and anti-inflammatory activity in both *in vitro* and *in vivo* studies. However, further studies are required to isolate the active constituents responsible for the observed effect and find potential applications of *B. spiculifolia* root in light of these and future findings.

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