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Comparative analysis of the antioxidant, antidiabetic, and anti-inflammatory potential of two bryophytes: *Apopellia endiviifolia* and *Fontinalis antipyretica*

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ABSTRACT:
Bryophytes are an ancient and ecologically important group of land plants which remain underexplored regarding their phytochemical composition and biological activities. This study aimed to determine the phenolic content and evaluate the antioxidant, antidiabetic, and anti-inflammatory potential of extracts from two bryophyte species, the liverwort *Apopellia endiviifolia* and the moss *Fontinalis antipyretica*. Spectrophotometric methods were used to determine the phenolic content, antioxidant activity (measured as the ability to donate H^+, donate electrons, and chelate metals); antidiabetic activity (the ability to inhibit α-amylase), and anti-inflammatory activity (the ability to inhibit the denaturation of bovine serum albumin, BSA). Our results indicate a significantly higher concentration of total phenolic compounds in *A. endiviifolia* (4.945 ± 0.175 mg/gDW) compared to *F. antipyretica* (2.698 ± 0.153 mg/gDW). The total antioxidant activity, assessed using the phosphomolybdenum method and expressed as equivalents of vitamin C and vitamin E, was higher for the *F. antipyretica* extract, whereas the ability to scavenge ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radicals was similar between the two samples. However, *A. endiviifolia* demonstrated superior Fe^{3+} and Cu^{2+} reduction and Fe chelation capabilities. The inhibition of the α-amylase activity was moderate for both extracts. Additionally, we report for the first time the ability of the studied bryophytes to inhibit BSA denaturation, with *F. antipyretica* showing greater anti-inflammatory activity. These findings indicate that *A. endiviifolia* and *F. antipyretica* possess considerable bioactive potential and highlight the importance of further investigations into the biological properties of bryophytes. The observed differences may reflect the functional roles of phenolic metabolites in planta, suggesting the ecological and biochemical significance of these bryophytes.

Keywords: α-amylase inhibition, Bryophyta, Marchantiophyta, phenolic compounds, protein-denaturation inhibition

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INTRODUCTION

Currently, there is a growing interest in natural substances, especially those derived from plants, due to their potential in preventing and treating various diseases (Bolat *et al.* 2024). Plant-derived secondary metabolites are particularly attractive candidates in this context, as they are generally associated with fewer adverse effects and environmental sustainability, while exhibiting a broad spectrum of biological activities, including antioxidant, antiviral, and neuroprotective effects (Gomes *et al.* 2025; Khanam *et al.* 2025; Shi *et al.* 2025). Significant attention has been focused on studying the medicinal properties of vascular plants; however, the secondary metabolites of non-vascular plants remain comparatively underexplored despite the accumulating evi-

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dence of their therapeutic relevance (Gahtori & Chaturvedi 2019; Mandić *et al.* 2021; Stojanović *et al.* 2025). Despite their small size and low biomass, they are widely used in traditional Chinese and Indian medicine (Swarnkar *et al.* 2024). According to Motti *et al.* (2023), 109 bryophyte species are utilised for ethnomedicinal purposes, underscoring their longstanding pharmacological significance.

Bryophytes synthesise various metabolites, including terpenoids (mono-, sesqui-, and diterpenoids), phenolic compounds (flavonoids), lipids, and others, all of which exhibit significant biological activities (Ćosić *et al.* 2021; Horn *et al.* 2021). Phenolic compounds represent a large and structurally diverse group of plant secondary metabolites which play crucial roles in plant defence and human health (Tatipamula & Kukavica 2021). They are produced in response to adverse environmental conditions, such as abiotic stress, pathogens, and herbivores. Exposure to biotic and abiotic stress increases reactive oxygen species (ROS), leading to oxidative stress and the disruption of redox homeostasis. The most important ROS include the superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and the highly reactive hydroxyl radical ($^{\bullet}OH$). Hydrogen peroxide, a non-radical form of ROS, has the longest half-life and can diffuse through membranes. In the Fenton reaction with redox-active metals (mainly Fe and also Cu), H_2O_2 produces an extremely reactive and dangerous hydroxyl radical. Phenolic compounds act as antioxidants by scavenging ROS through diverse mechanisms, including electron donation and the chelation of redox-active metals which promote hydroxyl radical formation. These compounds are essential for plant growth, development, and defence, and they also benefit humans by serving as antioxidants. Beyond their protective roles in plant physiology, phenolic compounds have attracted considerable attention due to their capacity to modulate oxidative stress–related pathologies in humans. Oxidative stress is implicated in the pathogenesis of diseases such as diabetes and rheumatoid arthritis, where in addition to their antioxidant properties, phenolic compounds may also exhibit antidiabetic and anti-inflammatory effects (Chang & Chuang 2010; Hussain *et al.* 2016). Although bryophytes are a valuable source of bioactive molecules, their biological properties and chemical compositions remain underexplored. They are divided into three groups: Anthocerotophyta (hornworts), Marchantiophyta (liverworts), and Bryophyta (mosses) (Hodgetts *et al.* 2020). Comparative investigations across these evolutionary lineages may therefore provide valuable insights into chemotaxonomic differentiation and lineage-specific metabolic traits.

This study focuses on two species, one liverwort, *Apopellia endiviifolia* (Dicks.) Nebel & D. Quandt (syn. *Pellia endiviifolia* (Dicks.) Dumort.), and one moss, *Fontinalis antipyretica* Hedw.

Apopellia endiviifolia (Pelliaceae) is a circumpolar, southern-temperate, thalloid liverwort (Hill & Preston 1998; Paton 1999). It grows in moist or wet, often shaded habitats, on weak to highly basic substrates (Paton 1999). According to documented ethnobotanical sources, species of the genus *Pellia* are used to treat sore throats (Motti *et al.* 2023). In addition to its traditional use, *A. endiviifolia* has also demonstrated a wide range of biological effects, including antibacterial (Dey *et al.* 2014; Ivković *et al.* 2021a), antifungal (Sharma *et al.* 2015), cytotoxic (Ivković *et al.* 2021b), genotoxic, antiproliferative, and proapoptotic effects (Ivković *et al.* 2021c).

Fontinalis antipyretica (Fontinalaceae) is a circumpolar, boreo-temperate moss (Hill & Preston 1998). It grows submerged or emergent in neutral or alkaline water, attached to substrates (Smith 2004). Ethnobotanical investigations report that *F. antipyretica* has been traditionally used for treating fever, supporting detoxification, and as an antimicrobial remedy (Drobnik & Stebel 2021; Motti *et al.* 2023). This traditional knowledge is supported by

studies confirming its antimicrobial and antifungal activities (Veljić *et al.* 2009), allelopathic (Matić *et al.* 2024), antifeeding and mollusc-repellent effects (Matić et al. 2026), as well as its antioxidant and antidiabetic potential (Kocazorbaz *et al.* 2021). Beyond their roles in plant metabolism and cell protection, phenolic compounds are also invaluable as antioxidants in protecting the human body from various negative influences and diseases.

Both species were collected from the same habitat to minimise environmental variability and ensure a reliable comparison of their biochemical characteristics. Importantly, the selected taxa represent two distinct bryophyte lineages, Marchantiophyta and Bryophyta, which are assumed to differ in terms of morphology, evolutionary history, and secondary metabolite composition. Evaluating representatives of liverworts and mosses within the same locality and environmental context enables a more reliable differentiation between lineage-specific metabolic traits and environmentally induced responses. This comparative design therefore provides insight into whether variations in phenolic content and associated bioactivities reflect evolutionary divergence rather than habitat-related influences. Such a design strengthens the analytical framework by minimising habitat-related confounding effects and facilitates the interpretation of observed differences within an evolutionary and chemotaxonomic context. This study aimed to investigate the phenolic content and the antioxidant, antidiabetic, and anti-inflammatory activities of *Apopellia endiviifolia* and *Fontinalis antipyretica* using *in vitro* assays. By integrating comparative phytochemical assessment with functional bioactivity screening, the study seeks to provide a more comprehensive understanding of the biological potential of bryophytes and to evaluate their relevance as accessible, cost-effective, and environmentally sustainable sources of bioactive compounds.

MATERIAL AND METHODS

Plant material. *Apopellia endiviifolia* and *F. antipyretica* were collected by the authors in Eastern Herzegovina (Jazina, Trebinje, Republic of Srpska, Bosnia and Herzegovina). Both species grew in a concrete, artificial channel near the old mill by the river Sušica (330 m altitude, N 42.70378°, E 18.51181°). This canal was formerly used to bring water to the mill, which is no longer in use. *Fontinalis antipyretica* grew submerged in the water at the bottom of the widened section of the canal, while *A. endiviifolia* grew on the walls of the narrow part of the canal, just above the water level. Both species were sampled from the same site, but from their respective microhabitats. The sampling period corresponded to the active vegetative growth season under stable hydrological conditions, ensuring sufficient biomass for phytochemical analysis.

Sampling was performed manually using clean stainless-steel forceps. For each species, plant material was collected from multiple patches within the same site and pooled into three representative samples. Visible debris, sediment, and associated macroscopic organisms were carefully removed in the field. The collected material was placed in sterile polyethylene bags and transported to the laboratory in insulated containers at a low temperature (approximately 4°C).

In the laboratory, the samples were gently rinsed with distilled water to remove residual impurities, air-dried at room temperature under shaded, well-ventilated conditions until reaching a constant mass, and subsequently ground to a fine powder for extraction.

Voucher specimens collected on 14 June 2024 are deposited in the Herbarium of the Faculty of Natural Sciences and Mathematics in Banja Luka (voucher numbers AE21306, FA21307), and the nomenclature follows Hodgetts *et al.* (2020). The collection site is influenced by a modified Adriatic climate, which

is characterised by very warm summers with a small amount of precipitation, mainly occurring in autumn and winter time (Dragić *et al.* 2021).

Preparation of plant sample ethanolic extracts. The plant tissue was dried at room temperature in a shaded, well-ventilated area. After drying, the plant material (representing a pooled composite sample collected from multiple patches at the sampling site) was ground into a powder using an electric mill. The extraction ratio was 10 g of plant tissue and 200 mL of 80% ethanol for AE and 2 g and 40 mL of 80% EtOH for FA. After sonication (5 min), the homogenates were mixed on a magnetic stirrer for 30 min, and then filtered through filter paper. The obtained filtrates were labelled as F1, and the remaining precipitate was homogenised with 100 mL of 80% EtOH for AE and 20 mL for FA. The re-extraction procedure followed the same steps as the initial extraction. After the second filtration, the filtrates were labelled as F2. Filtrates F1 and F2 were then combined and concentrated using a vacuum evaporator to reduce the volume by two-thirds. The ethanol extracts were centrifuged for 10 min at 10,000 rpm and the resulting supernatants were stored at -20°C until analysis.

Determination of the total phenolic compound content (TPC). The total phenolic compound content (TPC) was determined according to Singleton & Rossi (1965), by measuring the absorbance at 724 nm. The total amount of phenolic compounds is expressed as the gallic acid equivalent (GAE) per mL of the ethanol extract of the plant.

Determination of the total flavonoid content. The total flavonoid content was determined according to Chang *et al.* (2002) by measuring the absorbance at 415 nm. The flavonoid concentration was calculated based on a quercetin standard curve and expressed in units μg quercetin mL^{-1} of the ethanol extracts.

Determination of the concentration of hydroxycinnamic acids and flavonols. The concentration of hydroxycinnamic acids and flavonols was determined in samples prepared in the same way by measuring the absorbance at 320 nm for hydroxycinnamic acids and at 360 nm for flavonols. The samples were prepared by mixing 0.250 mL of plant extract, 0.250 mL of HCl concentration of 1 g L^{-1} in 96% ethanol, and 4.55 mL of HCl concentration of 2 g L^{-1}. After mixing the samples, the absorbance was measured against a blank sample containing 0.250 mL of 80% ethanol instead of plant extracts. The concentration of hydroxycinnamic acids was determined using a caffeic acid (CA) standard curve and expressed as caffeic acid equivalents (mgCAE/gDW). The flavonol concentration was calculated based on a quercetin (Q) standard curve and expressed as quercetin equivalents (mg Q/gDW).

Determination of the total antioxidant capacity using the phosphomolybdenum method. The total antioxidant capacity was determined using the phosphomolybdenum method (PM) described by Prieto *et al.* (1999). The samples were prepared by mixing 1 mL of the working solution (0.6 M sulfuric acid, 28 mM K_3PO_4, and 4 mM ammonium molybdate) with 0.1 mL of plant extract of different concentrations. The blank sample used consisted of 1 mL of the working solution without ammonium molybdenum and 0.1 mL of the extract. The controls contained 1 mL of working solution and 0.1 mL of 80% ethanol. The samples were mixed and incubated for 2 h at 95°C. After incubation, the samples were cooled, and then the absorbance was measured at 695 nm. In addition to the extracts, vitamin E and vitamin C were also used as standards. The antioxidant capacity of the plant extracts determined by the PM method was expressed as the of vitamin E and vitamin C equivalents.

Determination of the ability to remove ABTS radicals. The ability to remove ABTS radicals was determined by the method proposed by Re *et al.* (1999). The basic ABTS solution was prepared by mixing equal volumes of 7 mM ABTS and 2.4 mM K-persulfate, then incubated for 24 hours in the dark. The working ABTS solution was obtained by diluting the basic solution with methanol and adjusting the absorbance to ~0.75 at 734 nm. The sample contained 0.5 mL of plant extracts of different concentrations and 0.5 mL of the working solution. After incubation for 7 min in the dark, the absorbance was measured at 734 nm. The control contained 0.5 mL of 80% ethanol and 0.5 mL of the working solution. The percentage of inhibition was calculated based on the equation:

$$\% \text{ inhibition} = ((\text{Acontrol-Asample})/\text{Acontrol}) \times 100$$

Determination of Cu reduction ability. The ability of the extracts to reduce Cu^{2+} was determined using the method proposed by Apak *et al.* (2004). The samples were prepared by mixing 0.3 mL of 0.01 M $CuCl_2$, 0.3 mL of 10 mM acetate buffer pH 3.6, 0.3 mL of 7.5 mM ethanolic solution of neocuprine, and 0.05 mL of ethanolic extract. The samples were mixed and incubated for 30 min at room temperature. After incubation, the absorbance was measured at 450 nm. A blank sample was prepared in the same way, where 0.05 mL of 80% ethanol was added instead of the sample. Trolox was used as the standard for concentration, and the ability of the extracts to reduce Cu^{2+} was calculated using a Trolox standard curve. The results are expressed as mg Trolox equivalents per mL^{-1} of extract.

Determination of Fe^{3+} reduction ability (FRAP). The reducing capacity of the samples was determined by the FRAP method according to Benzie & Strain (1996). The FRAP method is based on the change in absorbance at 593 nm. The working FRAP solution contained 10 volumes of 300 mmol/L acetate buffer, pH 3.6, 1 volume of 10 mmol/L 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ, 593 nm) in 40 mmol/L HCl, and 1 volume of 20 mmol/L ferric chloride. The freshly prepared FRAP reagent was heated to 37°C, and the blank value was measured at 593 nm. Samples containing 0.05 mL of the extract at different concentrations and 0.150 mL of distilled water were added to the FRAP reagent. After mixing and incubation at 37°C for 4 min, the absorbance was measured at 593 nm. The reducing capacity of the plant was calculated using a $FeSO_4$ standard curve, and the results are expressed as mmol $Fe^{2+}L^{-1}$.

Determination of Fe chelating ability. The determination of Fe chelating ability was determined by the method described by Carter (1971). The samples contained 0.938 mL ethanol, 0.0125 mL 1 mM $FeSO_4$, and 0.05 mL extracts at different concentrations. After incubation for 10 min, 0.05 mL of 2 mM ferrozine was added and incubated for an additional 10 min in the dark. The absorbance of the resulting complex was measured at 562 nm. The control contained 80% ethanol instead of the extract. The percentage of Fe chelation was calculated according to the equation:

$$\% \text{ chelating Fe} = ((A_{control}\text{-}A_{sample})/A_{control}) \times 100$$

Inhibition of α-amylase activity. The degree of α-amylase inhibition by the plant extracts was determined according to the method proposed by Zheng *et al.* (2020). The samples were prepared by mixing 0.1 mL of AE or FA extract at different concentrations (prepared by diluting the concentrated extract with 80% ethanol) and 0.2 mL of α-amylase (0.5 mg mL^{-1} dissolved in 0.1 M Na-

phosphate buffer, pH 6.8, containing 6 mM NaCl) and incubated for 10 min at 37°C. Subsequently, 0.2 mL of 0.1% starch solution was added, and the mixture was incubated for 10 min at 37°C. The reaction was stopped by the addition of 0.1 mL of 1 M HCl, and then 0.4 mL of Lugol's solution was added to colour the reaction product. The absorbance was measured at 630 nm. In addition to the samples, two blanks were also prepared. Blank 1 (B1) represents the enzyme control and contains buffer instead of the sample, while blank 2 (B2) represents the substrate control and contains buffer instead of the enzyme. Acarbose solution was used as the standard.

The percentage of α-amylase inhibition was calculated according to the following equation:

$$\% \text{ inhibition} = ((A_s - A_{B1})/A_{B2}) \times 100$$

A_s - sample absorbance; A_{B1}- blank 1 absorbance; A_{B2}- blank 2 absorbance

Ability to inhibit the denaturation of bovine serum albumin (BSA). The ability of the extracts to inhibit the denaturation of bovine serum albumin (BSA) was determined according to the method described by Kandikattu *et al.* (2013), with slight modifications. Briefly, the samples contained 0.5 mL of extract of different phenolic compound concentrations (prepared by diluting the concentrated extract with 80% ethanol) and 0.5 mL of 1% BSA. After mixing, the samples were incubated for 15 min at 37°C, followed by 15 min at 72°C. After cooling, the absorbance was measured at 660 nm. The percentage inhibition of denaturation by BSA was calculated relative to the control containing 1% BSA according to the equation:

$$\% \text{ inhibition BSA denaturation} = ((A_{control} - A_{sample})/A_{control}) \times 100$$

The extract concentrations applied in the different assays were selected based on preliminary range-finding experiments and on concentration intervals commonly used in comparable phytochemical and bioactivity studies. This ensured measurable responses within the linear range of the assays and facilitated comparison with previously published data.

Statistical analysis. All the data are expressed as mean ± standard deviation (SD). All the experiments were carried out using a minimum of three to five independent replicates. Prior to statistical comparison, the data were tested for normal distribution using the Shapiro–Wilk test. The differences between the two species were analysed using a two-tailed unpaired Student's t-test. The statistical analyses were performed using Statistica 8.0 software (StatSoft Inc., USA). The differences were considered statistically significant at $P < 0.05$. The values of statistical significance were defined as follows: ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

RESULTS AND DISCUSSION

Concentration of phenolic compounds. Bryophytes are rich in secondary metabolites, with phenolic compounds playing an important role (Sabovljević *et al.* 2016; Mandić *et al.* 2021; Swarnkar *et al.* 2024). Our results showed that the TPC concentration was significantly higher in the AE sample (4.945 ± 0.175 mg/gDW) compared to FA (2.698 ± 0.153 mg/gDW) ($P < 0.0001$) (Fig. 1A). The AE sample also had a higher flavonoid content (2035 ± 122 mg/gDW) than the FA sample (655 ± 21 mg/gDW) ($P < 0.001$) (Fig. 1B). Additionally, the AE sample exhibited higher levels of hydroxycinnamic acids and flavonols ($P < 0.001$ and $P < 0.01$, respectively) (Fig. 1C & D). Specifically, the concentration of hydroxycinnamic acids in the AE sample was 0.198 ± 0.0136 mg/

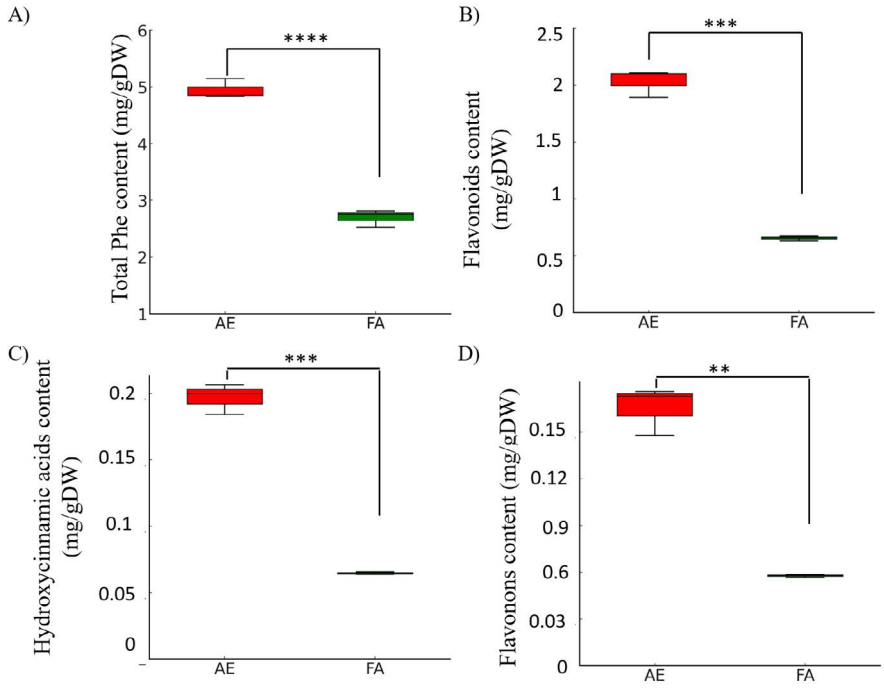

Fig. 1. A) Total phenolic compound content (Phe); B) Flavonoid content; C) Content of hydroxycinnamic acids; D) Flavonol content in ethanolic extracts of *Apopellia endiviifolia* (AE) and *Fontinalis antipyretica* (FA). Asterisks indicate statistically significant differences: ** P<0.01; *** P<0.001; **** P<0.0001

gDW, and the flavanols 0.166 ± 0.0155 mg/gDW. In contrast, the FA sample contained 0.0655 ± 0.00098 mg/gDW of hydroxycinnamic acids, and 0.0587 ± 0.000958 mg/gDW of flavonols. Flavonoids were the most abundant phenolic compounds in both samples. These findings align with previous research indicating that liverworts generally have higher flavonoid content than mosses (Wang *et al.* 2017). Bryophytes growing under lower light intensities tend to have higher flavonoid concentrations than those exposed to full sunlight. Furthermore, the total flavonoid content is highest in epiphytic bryophytes, while aquatic bryophytes exhibit the lowest levels (Wang *et al.* 2017). In this study, both AE and FA grew on the same concrete channel substrate. Our results reveal that AE, which grew above the water level, had a higher flavonoid content than FA, which was submerged. The methanol extract of *Pellia epiphylla* (L.) Corda from India had a TPC of 41.29 ± 0.18 mg GA/$g_{extract}$, with a flavonoid content of 13.56 ± 0.05 mg quercetin/$g_{extract}$ (Mukhia *et al.* 2015). The concentration of TPC (0.543 ± 0.062 mgGA/$g_{extract}$) and flavonoids (0.041 ± 0.014 mgGE/$g_{extract}$) in the methanol extract of *F. antipyretica* from Turkey (Kocazorbaz *et al.* 2021) was significantly lower than the values found in our study. In *F. antipyretica* samples from Bulgaria, TPC ranged from 0.499 mg/g in unpolluted areas to 0.273 mg/g in a polluted area (Gecheva *et al.* 2020). The authors suggested that the differences in phenolic compounds from polluted and non-polluted areas could be due to the consumption of phenolic compounds in polluted environments, emphasising the role of these compounds in bryophyte responses to environmental stress. Our findings show that the reported TPC and flavonoid contents vary widely across studies, largely due to differences in location, environmental conditions, sampling times, and extraction methods. Comparisons are further complicated by the inconsistent units used to report phenolic compounds.

Antioxidant activity. The antioxidant activities of phenolic compounds depend not only on their structure, but also on their qualitative and quantitative content (Gecheva *et al.* 2020). Our results showed that the total antioxidant activity, measured by the PM method, increases with phenolic compound

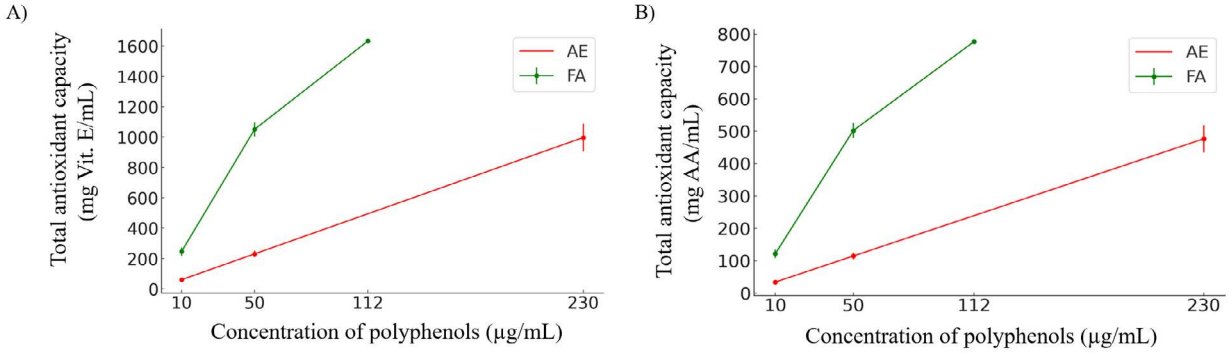

Fig. 2. Total antioxidant capacity measured by the phosphomolybdenum method in extracts of *Apopellia endiviifolia* (AE) and *Fontinalis antipyretica* (FA); A) expressed as equivalents of vitamin E and B) equivalents of vitamin C.

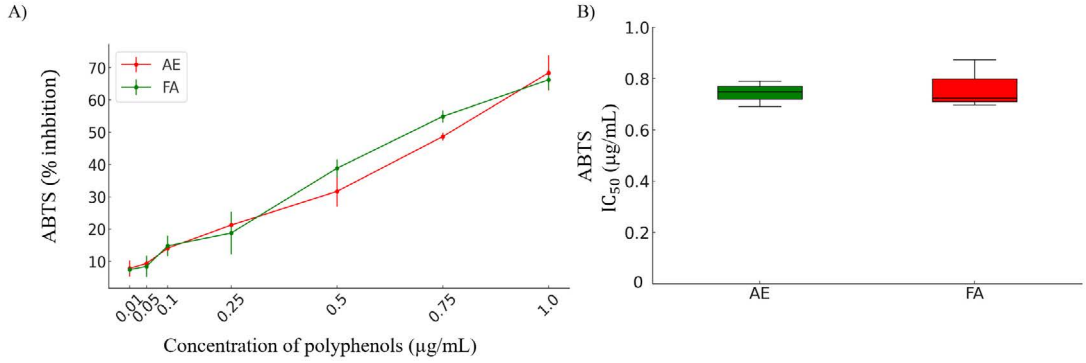

Fig. 3. Antioxidant activity of extracts *Apopellia endiviifolia* (AE) and *Fontinalis antipyretica* (FA) measured by the ability to scavenge ABTS radicals. A) Changes in the percentage of ABTS radical inhibition with the concentration of phenolic compounds; B) IC_{50} values of the extracts.

concentration (Fig. 2). In addition, for both samples, the total antioxidant activity was expressed as equivalents of vitamin E. In the concentrated extracts (AE 0.230 mg mL^{-1} and FA 0.112 mg mL^{-1}), the total antioxidant activity expressed as vitamin E equivalents reached 1632 ± 1.5 mg mL^{-1} for FA and 997 ± 90 mg mL^{-1} for AE. When expressed as vitamin C equivalents, the activity was 776 ± 2 mg mL^{-1} for FA and 477 ± 0 mg mL^{-1} for AE (Fig. 2). Overall, FA exhibited higher total antioxidant capacity in both the vitamin E (Fig. 2A) and vitamin C equivalents (Fig. 2B), suggesting that not only the overall phenolic content, but also the specific qualitative composition of phenolic constituents contribute to antioxidant effectiveness.

The ability to scavenge ABTS radicals was comparable between the extracts, with IC_{50} values of 0.743 ± 0.049 µg mL^{-1} for AE and 0.765 ± 0.0953 µg mL^{-1} for FA, despite the markedly higher TPC concentration in AE (Fig. 3A & B). This pattern suggests the potential role of specific phenolic compounds, rather than total phenolic levels alone, in ABTS radical neutralisation. The methanol extract of *Pellia epiphylla* from India had an IC_{50} below 0.5 µg mL^{-1} (Mukhia *et al.* 2015), which is slightly lower than the IC_{50} results we obtained for the AE ethanol extract. The ability of phenolic compounds in the AE and FA extracts to reduce ABTS radicals by donating a hydrogen atom indicates their ability to remove ROS and demonstrates significant antioxidant activity. Cu and Fe are

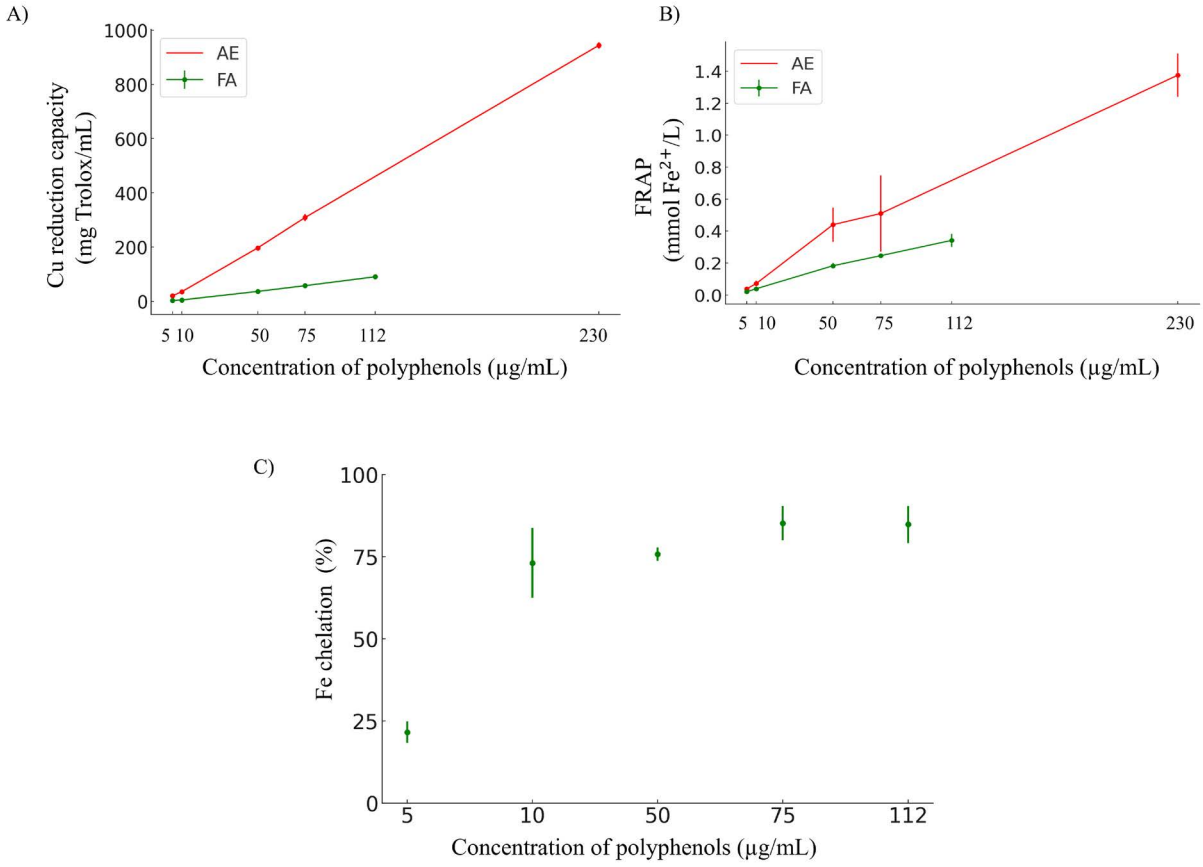

Fig. 4. Antioxidant capacity of ethanol extracts of *Apopellia endiviifolia* (AE) and *Fontinalis antipyretica* (FA): A) ability to reduce Cu^{2+}; B) ability to reduce Fe^{3+}; C) ability to chelate Fe.

redox-active metals which can participate in the Fenton reaction, leading to the increased production of harmful hydroxyl radicals. The reducing capacity of the plant extracts is likely attributable to phenolic compounds, which act as electron donors capable of converting oxidized Cu^{2+} and Fe^{3+} to Cu^{+} and Fe^{2+}, respectively. In the concentrated samples, Cu^{2+}-reducing activity reached 943.591 ± 7.0 mg Trolox mL^{-1} in AE and 90.561 ± 2 mg Trolox mL^{-1} in FA. This represents almost a tenfold higher reduction potential in AE compared with FA (Fig. 4A), consistent with the observation that AE contains approximately twice as much TPC (Fig. 1). The ability of FA extracts from various Bulgarian localities to reduce Cu^{2+} has also been reported (Gecheva *et al.* 2020). Free Fe^{3+} ions contribute to pro-oxidant activity through redox cycling, making their reduction and control important. The ability to reduce Fe^{3+} increases almost linearly with increasing concentrations of TPC in the sample for both the AE and FA extracts (Fig. 4B). At lower concentrations (5–75 mg mL^{-1}), the ability to reduce Fe^{3+} was higher in the AE sample, a trend also observed for the concentrated samples. For the concentrated samples, the ability to reduce Fe^{3+} for the AE sample reached 943.6 ± 11.6 mmolFe^{2+} L^{-1}, and for FA 90.56 ± 2.04 mmolFe^{2+} L^{-1}. In the study carried out by Mukhia *et al.* (2015), the ability of *Pellia epiphylla* extract to reduce Fe^{3+} was shown to be in the range of 400-800 mgAAE/g of extract at a phenolic compound concentration range of 1–3 mg mL^{-1}.

Most phenolic compounds, such as catechol (a benzene ring with two -OH groups) and gallol (a benzene ring with three -OH groups) derivatives, can chelate Fe and prevent its participation in the Fenton reaction (Perron & Brumaghim 2009). The Fe^{2+} chelation capacity of the AE sample could only be assessed at a concentration of 10 mg mL^{-1} (59 ± 8%), as higher concentrations inhibited chelation activity. In contrast, the Fe^{2+} chelating ability of FA increased

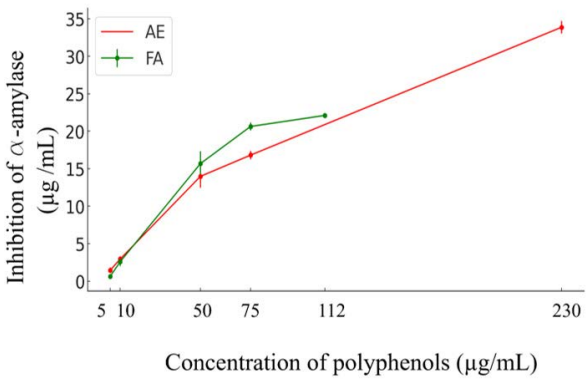

Fig. 5. Antidiabetic ability of extracts of *Apopellia endiviifolia* (AE) and *Fontinalis antipyretica* (FA) expressed as the ability to inhibit α-amylase.

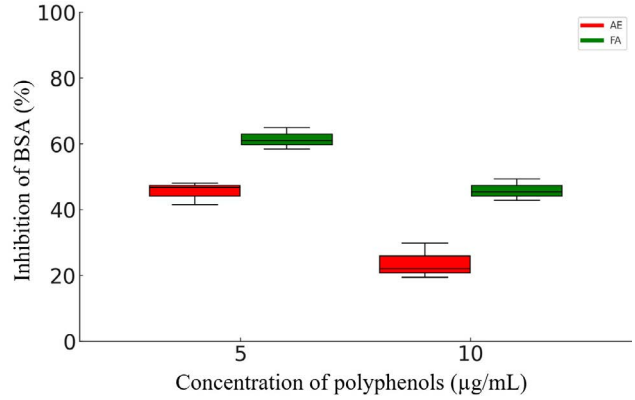

Fig. 6. Anti-inflammatory activity of ethanolic extracts of *Apopellia endiviifolia* (AE) and *Fontinalis antipyretica* (FA) expressed as the ability to inhibit the denaturation of bovine serum albumin.

with TPC content (Fig. 4C). At concentrations between 10 and 112 mg mL^{-1}, the percentage of Fe^{2+} chelation was in the range of 75–85%, indicating that the relationship between TPC concentration and Fe^{2+} chelating capacity is not strictly linear (Fig. 4C). Regardless of the differences in the concentration dependence of Fe chelating ability, both extracts demonstrate the ability to chelate Fe, which can prevent the formation of hydroxyl radicals and the oxidation of important biomolecules. The methanolic *Pellia epiphylla* extract from India was shown to have a stronger Fe chelation ability with an IC_{50} of 1 mg mL^{-1} (Mukhia *et al.* 2015), in comparison to our results for AE, which may be a consequence of the use of different solvents. According to data from the literature, extracts of liverworts (*Marchantia polymorpha* L. and *Plagiochasma appendiculatum* Lehm. & Lindenb.) and mosses (*Ceratodon purpureus* (Hedw.) Brid. and *Dryptodon pulvinatus* (Hedw.) Brid.) exhibited significant antioxidant activity (Rana *et al.* 2018; Wolski *et al.* 2021; Pandey & Rana 2022). Our results corroborate these findings, indicating that both the AE and FA extracts may serve as effective antioxidant agents.

Inhibition of α-amylase. Diabetes mellitus is a metabolic disorder characterised by alterations in carbohydrate, lipid, and protein metabolism. Type 2 diabetes (T2D) begins with the resistance of peripheral tissues (fat tissue, liver, skeletal muscles) to insulin, and progresses to the failure of pancreatic β-cells. Due to peripheral insulin resistance, plasma glucose concentration increases, leading to hyperglycaemia (Chang & Chuang 2010). Diabetes is associated with oxidative stress, which can arise from enzymatic, non-enzymatic, and mitochondrial sources. Non-enzymatic sources of ROS include the pro-oxidative properties of glucose; therefore, hyperglycaemia leads to oxidative stress. The autoxidation of glucose produces $^{\bullet}OH$ radicals, while the metabolism of glucose through sorbitol leads to the increased production of $O_2^{\bullet-}$. Controlling postprandial glucose levels after ingestion is an important therapeutic strategy for treating T2D. Therefore, the inhibition of enzymes which catalyse the hydrolysis of complex carbohydrates and increase the concentration of glucose, such as α-amylase, is an effective approach for preventing and treating T2D (Hieu *et al.* 2020). Synthetic digestive enzyme inhibitors are expensive and associated with adverse effects, making it imperative to identify natural compounds as potential enzyme inhibitors. The changes in the percentage of α-amylase inhibition with increasing concentrations of TPC in the AE and FA extracts are shown in Fig. 5. The maximum inhibition of α-amylase activity for both extracts was achieved for

the concentrated samples, reaching 34 ± 1% for the AE extract and 22 ± 0.3% for the FA sample. Although the concentrated AE extract contained twice the concentration of phenolic compounds compared to the FA extract, the percentage of α-amylase inhibition was only 10% higher, indicating that the FA extract has a greater capacity to inhibit α-amylase. Based on the results obtained, we calculated the possible IC_{50} values for α-amylase inhibition for the AE (335.5 mg mL^{-1}) and FA (208.63 mg mL^{-1}) extracts. These results show that the FA extract exhibits a stronger α-amylase inhibitory capacity. Although the IC_{50} values obtained for both extracts are higher than that of the standard acarbose (127 μg mL^{-1}), they still fall within the micromolar range, demonstrating notable inhibitory potential. Previous research reported that *Pellia epiphylla* extract inhibits α-amylase with an IC_{50} of 1.58 mg mL^{-1} (Mukhia *et al.* 2015). However, no information was found in the available literature on the α-amylase inhibitory activity of *F. antipyretica*. Our *in vitro* findings indicate that both the AE and FA extracts exhibit α-amylase inhibitory activity, suggesting their potential as antidiabetic agents. However, this *in vitro* assay does not capture the complexity of glucose regulation *in vivo*, as it does not account for intestinal absorption, bioavailability, metabolic transformation, hormonal regulation, or interactions with other digestive enzymes. Therefore, although the observed inhibition suggests potential antidiabetic activity, extrapolation to physiological or clinical effects should be approached with caution and requires further *in vivo* validation in appropriate *in vivo* models.

Inhibition of denaturation of bovine serum albumin. Inflammation is the body's immune response to injury and foreign invaders (infections, harmful chemicals, and drugs) (Rana *et al.* 2018; Esho *et al.* 2021). The role of inflammation is to contain and limit the injury site, destroy microorganisms, inactivate toxins, and heal and recover tissue (Rana *et al.* 2018). However, inflammation can also be potentially harmful, causing life-threatening hypersensitivity reactions and progressive tissue damage. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to mitigate inflammation by inhibiting the production of prostaglandins and preventing protein denaturation. Despite their capacity to relieve pain, these NSAIDs are also associated with some serious side effects, especially in the elderly (Rana *et al.* 2018). Therefore, there is a growing interest in identifying plant metabolites with anti-inflammatory properties to minimise adverse effects. The physiology of inflammation involves protein denaturation because most biological proteins lose their biological function when denatured. One common method for evaluating the anti-inflammatory properties of plant extracts is to test their ability to inhibit the denaturation of BSA. Both the AE and FA extracts exhibit the ability to inhibit BSA at phenolic compound concentrations of 5 and 10 mg mL^{-1}, with the FA extract demonstrating significantly higher inhibition ($P < 0.001$) (Fig. 6). At an extract concentration of 5 mg mL^{-1}, the percentage of BSA denaturation inhibition was 45 ± 3% for the AE extract and 61 ± 3% for FA. However, an increase in concentration (10 mg mL^{-1}) resulted in a decrease in the percentage of BSA denaturation inhibition for both extracts: AE extract 24 ± 5% and FA extract 46 ± 3%, indicating the existence of components in the extracts which promote BSA denaturation. The results indicate that the AE extract significantly inhibits BSA denaturation, similar to other liverwort extracts. For instance, the extract of the thalloid liverwort *Marchantia polymorpha* showed inhibition of egg albumin denaturation within a concentration range of 50–1000 mg mL^{-1} with a percentage range of 18–75% (Rana *et al.* 2018). In addition, at a concentration of 1000 μg mL^{-1}, *M. polymorpha* extract showed high inhibition capacity (75.49%), while diclofenac sodium, the standard, showed 85.1% inhibition of protein denaturation (Rana *et al.* 2018). Furthermore, the methanolic extract of liverwort *Plagiochasma appendiculatum* at a concentration of 800 μg mL^{-1} showed the highest ability to

inhibit BSA denaturation (74.854 ± 1.547%), while at the same concentration, the standard drug indomethacin showed 79.238 ± 1.523% inhibition (Pandey & Rana 2022). The FA extract exhibited a higher percentage of BSA denaturation inhibition than AE, suggesting potentially superior anti-inflammatory activity. According to the available literature data, this is the first report of a moss exhibiting anti-inflammatory activity by inhibiting BSA denaturation. Although the *in vitro* assay used to determine the inhibition of BSA denaturation suggests the possible anti-inflammatory potential of the extracts, it does not fully replicate the complexity of inflammatory processes *in vivo*, including cell signalling, metabolism, and immune system interactions. These results should therefore be interpreted as preliminary indicators of potential activity which should be verified *in vivo*. Previous studies have demonstrated the anti-inflammatory ability of bryophyte extracts through the stabilisation of erythrocyte cells and the inhibition of LPS-induced NO production (Oyedapo *et al.* 2015; Marques *et al.* 2022).

Although the *in vitro* assays provide valuable preliminary insights into the antioxidant, α-amylase-inhibitory, and anti-inflammatory potential of the investigated extracts, certain limitations should be acknowledged. *In vitro* models do not fully replicate the complexity of physiological systems, including factors such as bioavailability, metabolic transformation, tissue distribution, and systemic interactions. Enzyme inhibition and protein denaturation assays represent simplified biochemical systems and may not directly translate into *in vivo* therapeutic efficacy. Hence, the observed activities should be interpreted as indicative of biological potential rather than definitive evidence of pharmacological effectiveness. Further studies involving detailed phytochemical characterisation, cellular assays, and *in vivo* models are necessary to confirm and better understand the biological relevance of these findings.

Obtained results indicate that the bryophytes *Apopellia endiviifolia* and *Fontinalis antipyretica*, as collected from the investigated habitat, contain notable concentrations of phenolic compounds, predominantly flavonoids, associated with pronounced antioxidant activity, along with moderate antidiabetic and anti-inflammatory potential, under the experimental conditions applied in this study. Since oxidative stress is a key factor in the development of various diseases, including type 2 diabetes and inflammatory disorders, identifying natural plant sources with combined antioxidant, antidiabetic, and anti-inflammatory activities remains of scientific interest. Within the scope of this preliminary screening study, the investigated bryophyte species may represent promising sources of biologically active compounds and provide a foundation for further research aimed at exploring their phytochemical diversity and potential medicinal applications, including detailed chemical characterisation and biological validation.

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COMPREHENSIVE PLANT BIOLOGY

REZIME

Komparativna analiza antioksidativnog, antidijabetičkog i antiinflamatornog potencijala dve briofite: *Apopellia endiviifolia* i *Fontinalis antipyretica*

Siniša Škondrić, Biljana Kukavica i Nataša Lukić

Briofite predstavljaju drevnu i ekološki značajnu grupu kopnenih biljaka koja je i dalje nedovoljno istražena u pogledu svog fitohemijskog sastava i bioloških aktivnosti. Cilj ovog istraživanja bio je da se utvrdi sadržaj fenolnih jedinjenja i proceni antioksidativni, antidijabetički i antiinflamatorni potencijal ekstrakata dve vrste briofita, jetrenjače *Apopellia endiviifolia* i mahovine *Fontinalis antipyretica*. Za određivanje sadržaja fenolnih jedinjenja korišćene su spektrofotometrijske metode, kao i za procenu antioksidativne aktivnosti (sposobnost doniranja H^+ jona, doniranja elektrona i heliranja metala), antidijabetičke aktivnosti (sposobnost inhibicije α-amilaze) i antiinflamatorne aktivnosti (sposobnost inhibicije denaturacije goveđeg serumskog albumina, BSA). Rezultati su pokazali značajno višu koncentraciju ukupnih fenolnih jedinjenja kod vrste *A. endiviifolia* (4,945±0,175 mg/g SM) u poređenju sa vrstom *F. antipyretica* (2,698±0,153 mg/g SM). Ukupna antioksidativna aktivnost, određena fosfomolibdenskom metodom i izražena kao ekvivalenti vitamina C i vitamina E, bila je viša kod ekstrakta vrste *F. antipyretica*, dok je sposobnost neutralizacije ABTS (2,2′-azino-bis(3-etilbenzotiazolin-6-sulfonska kiselina)) radikala bila slična kod obe vrste. Međutim, vrsta *A. endiviifolia* je pokazala izraženiju sposobnost redukcije Fe^{3+} i Cu^{2+} jona, kao i heliranja gvožđa. Inhibicija aktivnosti α-amilaze bila je umerena kod ekstrakta obe vrste. Pored toga, po prvi put je utvrđena sposobnost ekstrakata ispitivanih briofita da inhibiraju denaturaciju BSA, pri čemu je vrsta *F. antipyretica* pokazala veći antiinflamatorni potencijal. Dobijeni rezultati ukazuju na značajan bioaktivni potencijal vrsta *A. endiviifolia* i *F. antipyretica* i naglašavaju potrebu za daljim istraživanjima bioloških svojstava briofita. Uočene razlike mogu odražavati funkcionalne uloge fenolnih metabolita u biljnom organizmu, što ukazuje na njihov ekološki i biohemijski značaj.

Ključne reči: inhibicija α-amilaze, Bryophyta, Marchantiophyta, fenolna jedinjenja, inhibicija denaturacije proteina