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Total phenolic content and antioxidant activity in *Frullania brasiliensis* Raddi (Marchantiophyta: Frullaniaceae) from Brazilian ironstone outcrops

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Abstract

Ironstone outcrops (Brazilian *cangas*) are high-elevation environments (> 800 m) characterized by extreme incident radiation, marked fluctuations in water availability, and shallow, oligotrophic soils developing over ferruginous bedrock. Vegetation forms mosaics ranging from exposed rock surfaces to shrub patches and small cloud-forest enclaves ("*capões*"), generating fine-scale microenvironmental heterogeneity. In these conditions, bryophytes occupy distinct microhabitats where secondary metabolites contribute to stress tolerance. We examined the leafy liverwort *Frullania brasiliensis* Raddi, a species emblematic of *cangas*, to assess its phenolic content and *in vitro* antioxidant capacity. Extracts from twenty populations were analysed for total phenolics and for radical scavenging (DPPH and superoxide anion, O_2^{-}). All samples contained phenolic compounds and exhibited antioxidant activity, with notable variation among populations. Superoxide scavenging efficiency increased with relative air humidity, suggesting environmentally modulated metabolic responses. These findings suggest phenolic metabolites may contribute to oxidative stress tolerance in *F. brasiliensis* in *canga* habitats and provide a foundation for further research on the functional ecology of bryophytes in rupestrian environments.

Keywords: rupestrian ecosystems; ferruginous rock outcrops; secondary metabolites; oxidative stress tolerance; bryophyte functional ecology.

Introduction

Ironstone outcrops (locally known as *cangas*) are high-elevation (> 800 m a.s.l.) environments characterized by shallow, nutrient-poor soils developing over ferruginous bedrock, intense solar radiation, strong winds, and pronounced fluctuations in water availability (Carmo & Jacobi, 2016; Silveira *et al.*, 2016). Vegetation forms fine-scale mosaics ranging from exposed rocky surfaces to shrub patches and small cloud-forest enclaves ("*capões*"), generating steep microenvironmental gradients (Oliveira *et al.*, 2021; 2024; Teixeira *et al.*, 2024). Such heterogeneity acts as a strong environmental filter, shaping distinct plant functional strategies associated with persistence under combined thermal, hydric, and oxidative stress (Vitt *et al.*, 2024; Peñaloza-Bojacá *et al.*, 2018; Oliveira *et al.*, 2021; 2024; Teixeira *et al.*, 2024; Oliveira *et al.*, 2025).

Bryophytes are frequent and ecologically relevant components of these environments, contributing to moisture retention, organic matter accumulation, and microhabitat formation. Their distribution across exposed and shaded sites is closely linked to species-specific mechanisms of stress tolerance (Peñaloza-Bojacá *et al.*, 2018; Oliveira *et al.*, 2021; 2024; Teixeira *et al.*, 2024; Oliveira *et al.*, 2025). Among these mechanisms, the accumulation of secondary metabolites, particularly phenolic compounds, plays a key role in modulating oxidative stress associated with desiccation–rehydration cycles and high irradiance (Martínez-Abaigar & Núñez-Olivera, 2021; Mirzoeva *et al.*, 2018; Maciel-Silva & Lima, 2020).

Although photoprotective and antioxidant metabolites are widely reported in bryophytes and contribute to their high adaptive capacity in highly stressful environments (Gahtori & Chaturvedi, 2019; Zhang *et al.*, 2023), only approximately 5% of species have been chemically characterized (Pinheiro *et al.*, 1989; Maciel-Silva & Lima, 2020), indicating that much of their secondary metabolism remains unexplored. Within liverworts, the genus *Frullania* is particularly rich in sesquiterpenoids and bibenzyl derivatives (Asakawa *et al.*, 2012; Ludwiczuk & Asakawa, 2021). Studies on *Frullania brasiliensis* Raddi from Argentina identified eremophilanolides (sesquiterpene lactones) and other terpenoids (Bardón *et al.*, 2002; Nagashima & Asakawa, 2011), and antioxidant activity has been documented in congeneric species (Simsek *et al.*, 2023). However, populations of *F. brasiliensis* from *canga* environments remain uninvestigated, despite experiencing extreme irradiance and highly dynamic hydration regimes. In these environments, this species may play an important ecological role by contributing to

moisture retention and providing habitat for microbiota and microfauna, thereby supporting biological activity and ecosystem functioning under stressful conditions. Given its frequent occurrence on exposed ferruginous rock surfaces (Peñaloza-Bojacá *et al.*, 2018; Oliveira *et al.*, 2021; 2024; Teixeira *et al.*, 2024), *Frullania brasiliensis* represents a suitable model for evaluating the potential contribution of phenolic metabolites to oxidative stress tolerance in rupestrian habitats. Therefore, this study aimed to quantify total phenolic content and assess *in vitro* antioxidant capacity using the DPPH radical scavenging assay and the superoxide anion (O_2^-) assay in *F. brasiliensis* across twenty spatially independent populations from Brazilian *cangas*.

Materials and Methods

Study Species

We selected the liverwort *Frullania brasiliensis* Raddi (Frullaniaceae) for this study. This choice was supported by previous surveys that recorded a large occurrence of moss and liverwort species in the *cangas* (Peñaloza-Bojacá *et al.*, 2018; Oliveira *et al.*, 2021; 2024; Teixeira *et al.*, 2024), where *F. brasiliensis* is locally abundant and occurs in both shaded and sun-exposed microhabitats (Figure 1A–C). Liverworts also possess oil bodies, specialized organelles for the storage of secondary compounds, that suggest potential for the accumulation of bioactive molecules (He *et al.*, 2013). In addition, the abundance of *F. brasiliensis* ensured sufficient material for standardized collection and analyses. *Frullania brasiliensis* Raddi (Frullaniaceae) is widely distributed in Brazil (Lemos Michel, 2001; BFG, 2022) and typically occurs in sun-exposed sites, where its reddish pigmentation supports tolerance and growth under high light intensities (Gradstein *et al.*, 2001; Glime, 2013; Lima, 2025). It exhibits corticolous (living on tree trunks, Figure 1B), epiphyllous (on leaves), rupicolous (on rocks, Figure 1C), and terricolous (on soil) habits (Lemos Michel, 2001; BFG, 2022). The species is dioicous, with separate female (Figure 1D) and male (Figure 1E) individuals, and its ability to develop darker pigmentation under constant sunlight exposure (Lima, 2025) highlights its potential for secondary compound biosynthesis and justifies its selection for antioxidant investigation.

Collection and Identification

Samples of approximately 10 cm^2 were collected in March 2021 from twenty spatially independent populations, each separated by ≥ 2 m. This minimum distance is commonly adopted to ensure ecological and physiological independence among bryophyte populations in clonal species. Collections were conducted at Serra da Calçada Natural Monument, Minas Gerais, 1,000 m a.s.l., following Yano *et al.* (1984; Table 1). The samples were placed in sealed plastic bags and carefully transported to the laboratory to preserve moisture and ensure material integrity. In the laboratory, plant material was examined under a stereomicroscope and a light microscope to determine the presence of photosynthetic associates and reproductive status of the shoots (male, female, or non-sexual; Table 1).

Taxonomical identification followed Gradstein *et al.* (2001) and Gradstein & Costa (2003), and voucher specimens were deposited in the BHCB herbarium (Universidade Federal de Minas Gerais; voucher numbers 205001 –205020). The air humidity, temperature, and solar radiation in proximity to each of the twenty *F. brasiliensis* populations were registered (Table 2). A luxmeter (solar radiation in lux converted into μmol m^{-2} s^{-1}; Novotest Lx-1010b) and a thermo-hygrometer (Lnmyic HTC-2A) were used to obtain these records between 11:00 and 13:00 h on a cloudless day, in order to ensure standardized conditions (Teixeira *et al.*, 2024). These measurements were used to examine whether variation in total phenolic content and radical-scavenging activity was associated with environmental microgradients.

Sample preparation

The plants were carefully separated manually using steel tweezers to remove excess dirt and then washed in running distilled water. To eliminate smaller particles, an ultrasonic cuvette was also used. After washing, the plant material was left to air-dry for 24 h. On the following day, aiming at proper sample preservation, the material was dried in an oven at 60 °C for 48 h and subsequently stored until analysis.

Plant samples were ground using a ball mill and subjected to successive extractions with absolute ethanol for three consecutive extraction cycles. The resulting extracts were centrifuged at 9,600 × g for 5 min at 4 °C. Subsequently, samples were lyophilized (freeze-dried) and resuspended in ethanol at a concentration of 10 mg mL^{-1}. Lyophilization (rapid freezing followed by vacuum drying and removal of residual moisture; Liotop K105, Liobras, Brazil) preserves structural and biochemical integrity,

prevents thermal degradation, and enables concentration of phenolic compounds from *Frullania brasiliensis*. The resulting extracts were used for total phenolic quantification and antioxidant assays (Folin–Ciocalteu, DPPH, and superoxide anion scavenging).

Total phenolic content

The quantification of total phenolics was performed to elucidate differences in antioxidant capacity among populations. The concentration of total phenols was determined using the Folin–Ciocalteu reagent (Singleton & Rossi, 1965), with slight modifications. A mixture containing 50 µL of plant extract (10 mg mL^{-1}), 50 µL of Folin–Ciocalteu solution, 100 µL of 20% sodium carbonate, and 800 µL of distilled water was incubated in the dark at room temperature for 30 min. Absorbance was then measured at 750 nm. Pyrogallol acid was used as the standard, and results were expressed as mg of pyrogallol acid per mg of lyophilized dry extract.

Assessment of antioxidant activity by scavenging of reactive species (DPPH and superoxide anion)

The antioxidant activity of the extracts was evaluated based on their ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals, a reactive nitrogen species (RNS), and superoxide anions (O_2^-), a reactive oxygen species (ROS).

The DPPH assay was performed according to Gülçin (2010), with modifications. Lyophilized dry extract (2 mg mL^{-1}) was incubated in an ethanolic medium containing 100 µM DPPH, resulting in a final extract concentration of 1 mg mL^{-1}. The mixtures were maintained under agitation in the absence of light for 30 min, and absorbance was measured at 517 nm. Resveratrol was used as a positive control, and blanks were prepared by incubating the extracts without DPPH. Each assay was carried out in three independent runs, each performed in triplicate, to minimize pipetting errors. The concentration required to scavenge 50% of the DPPH radicals (IC_{50}) was determined by testing extract concentrations ranging from 0 to 1 mg mL^{-1} (Mukhia *et al.*, 2014). The percentage inhibition was calculated as % inhibition = $[(A_0 - A_1) / A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance in the presence of the extract. Lower IC_{50} values indicate higher antioxidant activity and greater radical scavenging efficiency (Ho *et al.*, 2012).

For the superoxide anion scavenging assay, extracts (2 mg mL^{-1}; final concentration 1 mg mL^{-1}) were incubated in 50 mM phosphate buffer (pH 7.8) containing 13.3 mM L-methionine, 200 µM nitroblue tetrazolium (NBT), 100 µM EDTA, and 2 µM riboflavin

for 10 min at 25 °C under fluorescent light, a condition known to induce O_2^- formation. Control reactions were performed under the same conditions in the absence of light. Absorbance was measured at 575 nm, and scavenging activity (%) was calculated as % inhibition = 100 × [(Abs_2 sample – Abs_1 sample) / Abs control]. Each assay was carried out in three independent runs, each performed in triplicate, using a microplate reader (Thermo Scientific Multiskan FC Microplate Photometer, Finland) (Gülçin, 2010; Mukhia *et al.*, 2014).

Statistical analysis

Variation among populations in total phenolic content and radical-scavenging activity (DPPH and O_2^-) was evaluated using one-way ANOVA, followed by the Scott–Knott grouping test. When ANOVA indicated significant differences, the Scott–Knott test was applied to group treatments into homogeneous sets, represented by the letters A and B in Figures 3 and 4, according to their scavenging capacity for DPPH or O_2^- radicals. Normality and homoscedasticity were verified using the Shapiro-Wilk and Brown-Forsythe tests, respectively. Linear regression analyses were employed to determine the extract concentration required to scavenge 50% of the free radical (IC_{50}). Relationships among environmental data (i.e., solar radiation, temperature, and relative humidity), free radical scavenging activities, and total phenolic content were investigated using a correlation matrix based on the Spearman coefficient. Significant correlations ($\alpha = 5\%$) were subsequently analyzed by simple linear regression. To meet the assumptions of the regression analyses, the data were log-transformed before the test. All statistical analyses were performed using R software, version 4.0.2 (R Core Team, 2023). The *agricolae* (Mendiburu, 2021) and *plyr* (Wickham, 2011) packages were used for calculations involving DPPH, superoxide anion, and total phenolic content. The vegan (Oksanen *et al.*, 2013) and MASS (Venables & Ripley, 2002) packages were used for the analysis of environmental data.

Results

All *F. brasiliensis* populations accumulated phenolics, with an average concentration of 106.5 µg of pyrogallol mg^{-1} dry weight (Figure 2), and no significant differences were detected among populations ($P > 0.05$). Ethanolic extracts exhibited radical-scavenging activity against both DPPH (RNS) and superoxide (O_2^-; ROS), although with variable efficiency among populations (Figures 3 and 4).

For DPPH scavenging, eleven of the twenty populations (F1–F6, F8, F11, F12, F18, and F20) displayed high antioxidant capacity, with inhibition values comparable to the positive control resveratrol (≈ 97% inhibition), forming group A in the Scott–Knott test ($P < 0.001$). The remaining populations showed lower inhibition values (≈ 55%), forming group B (Figure 3).

For O_2^- scavenging, extracts from populations F3, F4, F8, and F12–F19 presented inhibition levels similar to resveratrol (≈ 56%), forming group A, while the remaining populations averaged ≈ 31% inhibition and formed group B (Figure 4). The mean concentrations required to achieve 50% radical scavenging (IC_{50}) were 750 ± 31.5 μg mL^{-1} for DPPH and 955 ± 61.4 μg mL^{-1} for O_2^-, with no significant differences among populations.

A significant positive correlation was detected between relative air humidity and O_2^- scavenging ($R^2 = 0.25$, $P = 0.01$), indicating that antioxidant activity increases under higher moisture availability (Figure 5). No significant associations were found between solar radiation or temperature and radical-scavenging activity. Antioxidant responses showed no clear differentiation across sex expression and were not consistently associated with the presence of photosynthetic associates; a slight tendency for higher superoxide anion scavenging activity was observed in populations expressing both sexes (Supplementary Table S1, S2, Figure S1).

Discussion

The consistent total phenolic content across the twenty *Frullania brasiliensis* populations suggests a stable antioxidant potential in this species and reinforces the role of secondary metabolites in coping with oxidative stress under the environmental conditions typical of *cangas*. However, although total phenolic content was quantified, the antioxidant activity observed cannot be attributed exclusively to phenolic compounds and may also involve other ethanol-extractable metabolites, as DPPH and superoxide scavenging varied among populations despite similar phenolic levels. Phenolics are well known for their ability to neutralize free radicals by donating hydrogen atoms or electrons and stabilizing unpaired electrons (Antolovich *et al.*, 2002; Sánchez-Moreno, 2002; Villaño *et al.*, 2005). In addition, the dark reddish pigmentation commonly observed in *F. brasiliensis* has been associated with the accumulation of phenolic pigments in bryophytes adapted to high-irradiance environments (Newsham,

2003; von Konrat & Braggins, 2003; Asakawa *et al.*, 2012; Glime, 2013; Fernandes *et al.*, 2015; 2018; Mejía-Giraldo *et al.*, 2015; 2022; Maciel-Silva & Lima, 2020), although the specific compounds were not characterized in our study.

Our results show extracts of *F. brasiliensis* exhibited significant scavenging activity for both DPPH and superoxide radicals, with IC_{50} values around 0.75 mg mL^{-1} and 0.95 mg mL^{-1}, respectively. When compared with vascular plants commonly recognized for medicinal or antioxidant uses, the activity of *F. brasiliensis* is of similar magnitude to that reported for *Anacardium occidentale* L., *Senna occidentalis* (L.) Link*, and Newbouldia laevis* (P.Beauv.) Seem. ex Bureau*.* (Tsado *et al.*, 2016 a;b; Niwoye, 2019). When compared to other liverworts, *F. brasiliensis* showed higher antioxidant performance than *Marchantia paleacea* Bertol., *Marchantia linearis* Lehm. & Lindenb., *and Conocephalum conicum* (L.) Dumort. (Mukhia *et al.*, 2014), reinforcing that liverwort lineages differ markedly in their secondary metabolite profiles (Mirzoeva *et al.*, 2018; Martínez-Abaigar & Núñez-Olivera, 2021).

Chemical studies in *Frullania* indicate a diversity of secondary metabolites, including phenolic derivatives such as bibenzyls, as well as terpenoids and frullanolides (Bardón *et al.*, 2002; Nagashima & Asakawa, 2011; Ludwiczuk & Asakawa, 2021). These compounds have been linked to defense against herbivory and microorganisms and have also been reported to contribute to photoprotection and antioxidant responses, illustrating potential exaptive functions, where molecules originally selected for one function are later co-opted for another (Agati *et al.*, 2020). This multifunctionality aligns with the selective pressures imposed by *cangas*, where *F. brasiliensis* frequently experiences extreme irradiance, rapid desiccation–rehydration cycles, and wind exposure (Carmo & Jacobi, 2016; Silveira *et al.*, 2016; Peñaloza-Bojacá *et al.*, 2018).

The positive association observed between relative air humidity and superoxide scavenging efficiency suggests antioxidant responses in *F. brasiliensis* may be modulated by hydration dynamics. This is consistent with the well-established desiccation tolerance of bryophytes, which involves rapid metabolic down- and up-regulation during drying and rehydration (Proctor & Tuba, 2002; Alpert & Oliver, 2002; Wood, 2007; Koster *et al.*, 2010; Glime, 2015; Oliveira *et al.*, 2021). Under low humidity, oxidative stress is expected to increase during rehydration, and the observed antioxidant responses may involve the action of multiple metabolites, including phenolic compounds. Conversely, no consistent relationships were detected with the other

environmental variables measured, nor with sex expression or the presence of photosynthetic associates. Although a slight tendency for higher superoxide anion scavenging activity was detected in populations expressing both sexes, this pattern was weak and should be interpreted cautiously given the limited sampling across categories. From an ecological perspective, our findings highlight how secondary metabolites contribute to stress tolerance in bryophyte species occupying exposed microhabitats within *canga* mosaics. Because bryophytes facilitate moisture retention, surface stability, and organic matter accumulation, the persistence of species such as *F. brasiliensis* has implications for early successional processes and microhabitat formation in rupestrian ecosystems (Oliveira & Maciel-Silva, 2022; Teixeira *et al.*, 2024; Oliveira *et al.*, 2025).

Finally, the biochemical consistency observed across populations, combined with variation in antioxidant responses, suggests *F. brasiliensis* maintains a stable baseline of secondary metabolites while harbouring underlying chemical diversity that may not be fully captured by total phenolic content alone. This pattern highlights the importance of integrating functional assays with detailed chemical characterization to better understand the ecological roles of metabolites in bryophytes. Future studies combining metabolomic approaches and experimental assays will be essential to disentangle the contributions of different compound classes to oxidative stress responses in *canga* environments.

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Author's Contributions

Pablo Oliveira Santos, Adaíses S. Maciel da Silva, and Mateus Fernandes Oliveira conducted the fieldwork and collected the ecological data. Pablo Oliveira Santos and Thamara Ferreira Silva Ávila performed the biochemical analyses in the laboratory. Plant identification was carried out by Pablo Oliveira Santos and Mateus Fernandes

Oliveira. Pablo Oliveira Santos wrote the first draft of the manuscript. Revisions of the manuscript were performed by Adaíses S. Maciel da Silva and Mateus Fernandes Oliveira. Luzia Valentina Modolo critically revised the manuscript and edited the final version. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there are no conflicting interests.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

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Supplementary Material

The following online material is available for this article:

Supplementary Figure S1. Envfit analysis showing the significant influence of solar radiation on the ordination of *Frullania brasiliensis* communities.

Supplementary Table S1. Effect of environmental variables and antioxidant activity of *Frullania brasiliensis* on the species composition of *F. brasiliensis* communities and their associated organisms. Asterisks indicate significant results in the Envfit test (*, $P < 0.05$).

Supplementary Table S2. Mean antioxidant responses (DPPH scavenging activity, superoxide anion scavenging - SAS, and total phenolic content) across sex expression in *Frullania brasiliensis* populations. Values represent simple arithmetic means and are presented to illustrate general trends.

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Table and Figure Captions

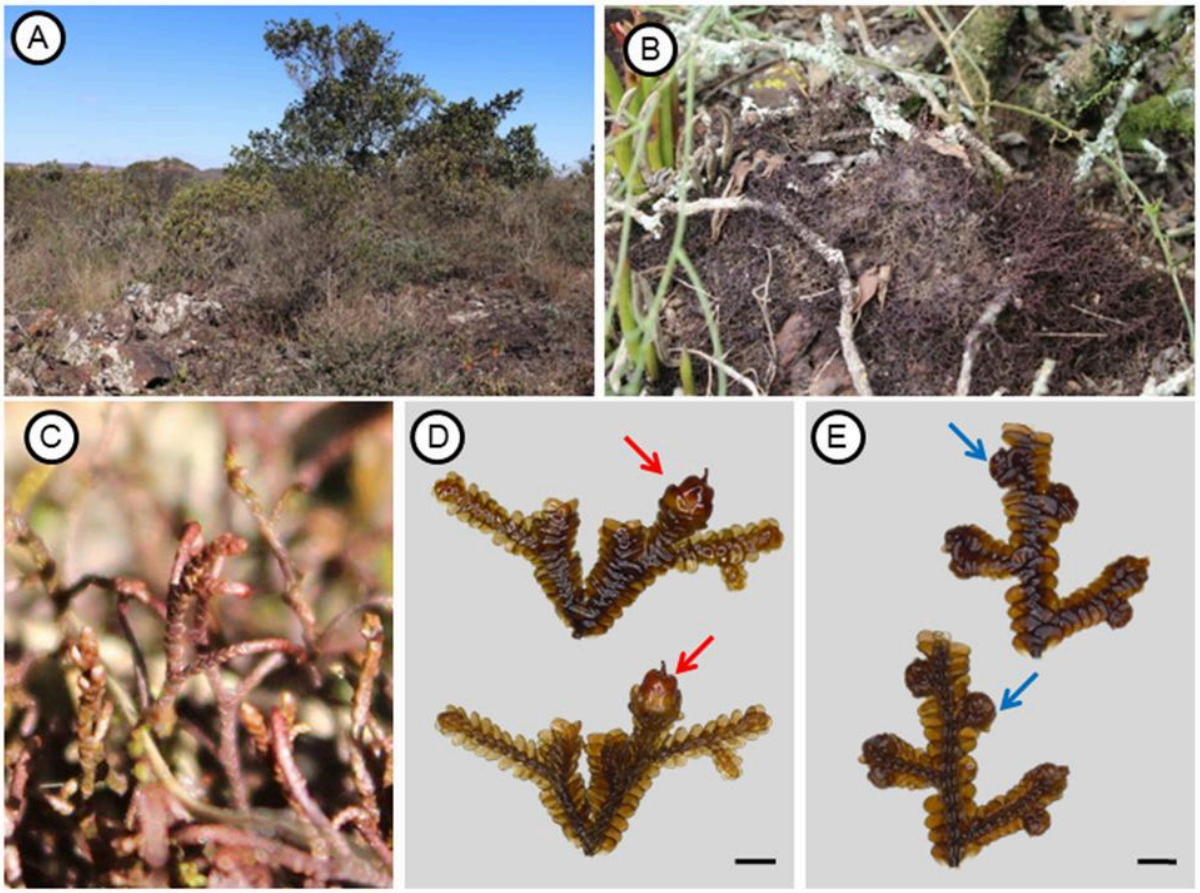

Figure 1. Ironstone outcrop ("*canga*") and morphological features of *Frullania brasiliensis*. (A) General view of a *canga* outcrop. (B) Habitat where *F. brasiliensis* occurs. (C) Population of *F. brasiliensis* under natural light conditions. (D) Female gametophyte in dorsal and ventral views; red arrow indicates the perianth (female reproductive branch). (E) Male gametophyte in dorsal and ventral views; blue arrow indicates the androecia (male reproductive branches). Scale bars = 1 mm.

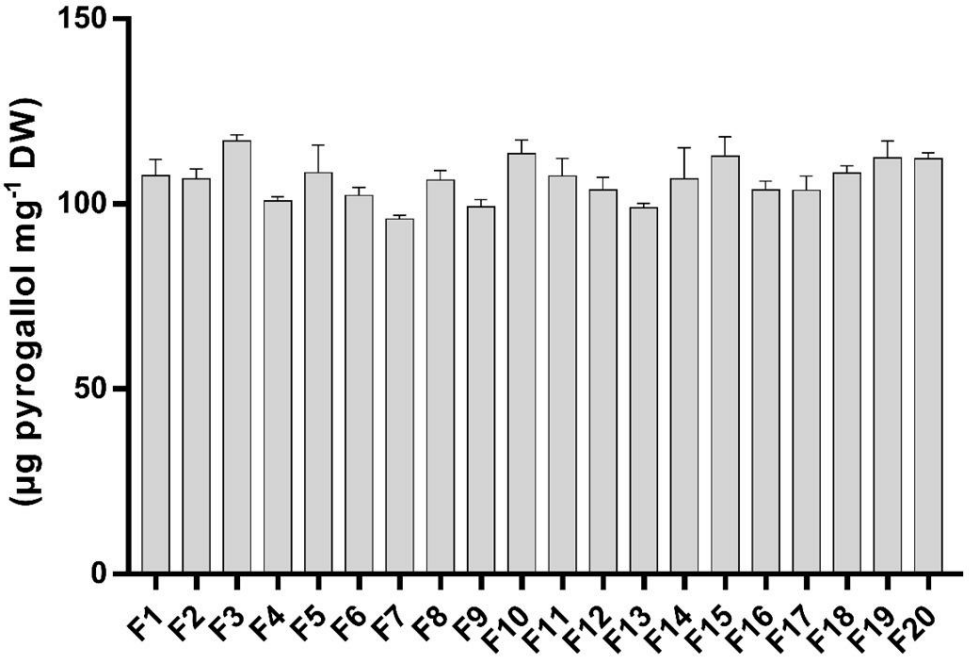

Figure 2. Total phenolic content in *Frullania brasiliensis* populations (F1–F20) collected from a Brazilian *canga*. Values represent mean ± standard deviation. No significant differences were observed among populations ($P < 0.05$). Pyrogallol was used as the standard.

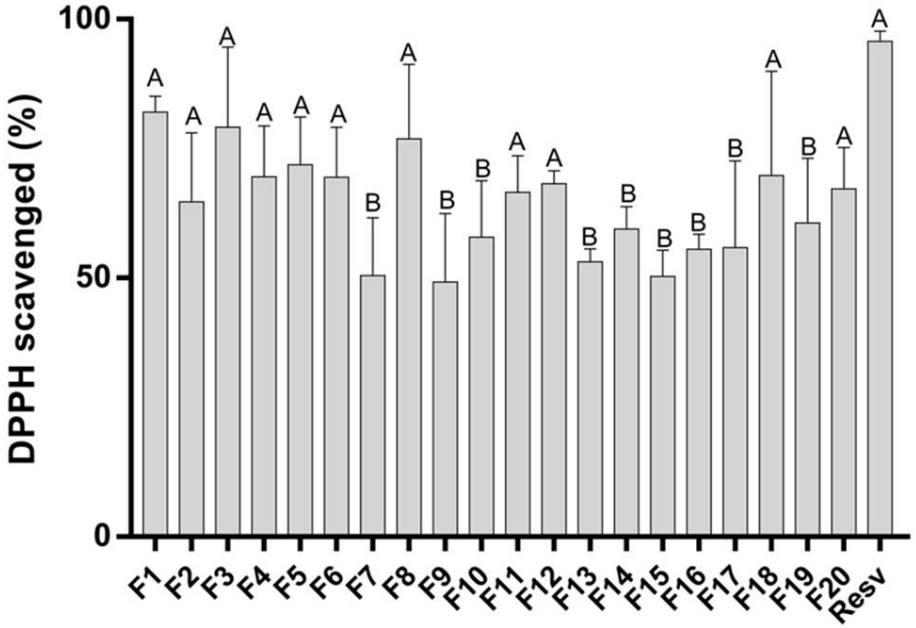

Figure 3. DPPH radical scavenging activity of ethanolic extracts (1 mg mL^{-1}) from *Frullania brasiliensis* populations (F1–F20). Values represent mean ± standard deviation. Different letters indicate significant differences among populations

(Scott–Knott test, P < 0.05). Populations labeled with "A" showed scavenging activity comparable to resveratrol (97% radical scavenging), whereas those labeled with "B" showed lower activity (mean = 55%). Resveratrol (1 mg mL^{-1}) was used as a reference compound.

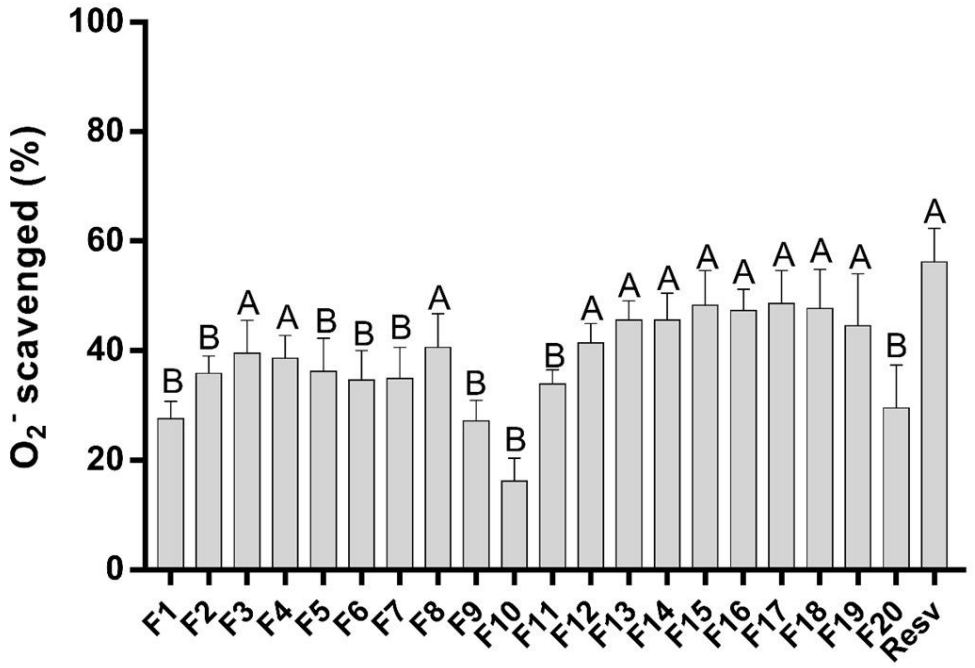

Figure 4. Superoxide anion scavenging activity of ethanolic extracts (1 mg mL^{-1}) from *Frullania brasiliensis* populations (F1–F20). Values represent mean ± standard deviation. Distinct letters indicate statistically significant differences among samples (Scott-Knott test; P < 0.05). Populations labelled with "A" showed scavenging activity similar to resveratrol (55.7% radical scavenging), whereas populations labelled with "B" showed lower activity (mean = 31%). Resveratrol (1 mg mL^{-1}) was used as a reference phenolic compound.

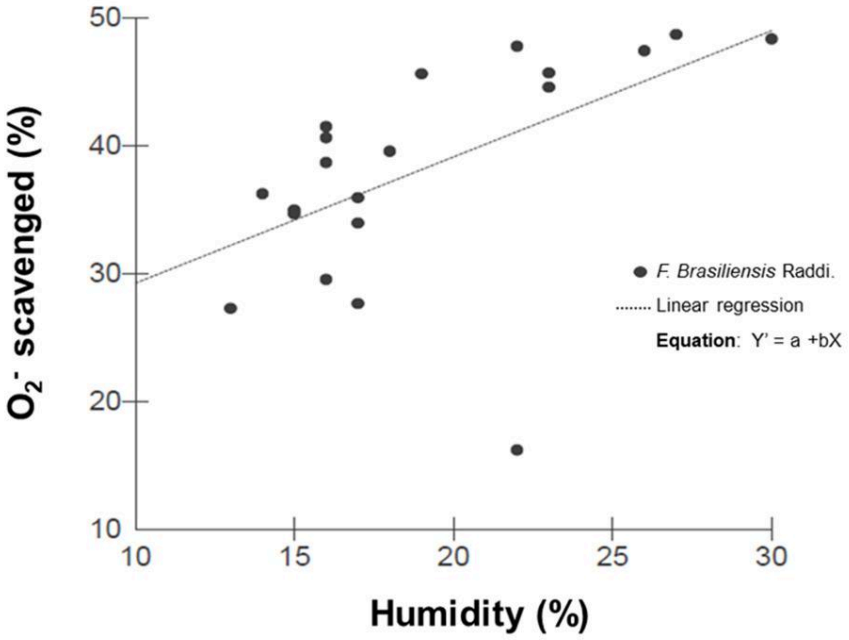

Figure 5. Relationship between superoxide anion scavenging activity (%) and relative humidity (%) in *Frullania brasiliensis* populations from Brazilian *cangas*. The dotted line represents the linear regression (R^2 = 0.297, r = 0.545, P = 0.0125).

Table 1. Sex expression and geographic location of the liverwort *Frullania brasiliensis* Raddi populations collected in Brazilian ironstone outcrops (cangas).

Populations	Sex	Latitude	Longitude
F1	Male	20°05'43.6"S	43°58'58.7"W
F2	Male and female	20°05'46.3"S	43°58'58.9"W
F3	Non sex-expressing	20°05'44.8"S	43°58'58.7"W
F4	Female	20°05'46.2"S	43°58'59.2"W
F5	Male	20°05'46.2"S	43°58'59.5"W
F6	Male	20°05'45.8"S	43°58'59.7"W
F7	Non sex-expressing	20°05'45.5"S	43°59'00.4"W
F8	Male	20°05'45.5"S	43°59'00.4"W
F9	Non sex-expressing	20°05'45.5"S	43°59'00.4"W
F10	Non sex-expressing	20°05'45.3"S	43°59'00.9"W
F11	Non sex-expressing	20°05'46.3"S	43°58'59.6"W
F12	Non sex-expressing	20°05'43.5"S	43°58'58.3"W
F13	Male	20°05'53.5"S	43°59'01.9"W

F14	Male	20°05'53.9"S	43°59'01.6"W
F15	Male and female	20°06'43.7"S	43°58'54.5"W
F16	Male and female	20°06'43.7"S	43°58'54.5"W
F17	Female	20°06'43.7"S	43°58'54.5"W
F18	Male	20°06'48.3"S	43°58'54.4"W
F19	Male	20°06'48.6"S	43°58'56.3"W
F20	Male and female	20°06'46.7"S	43°58'56.5"W

Table 2. Characterization on temperature, relative air humidity, and light intensity of *F. brasiliensis* Raddi.

Populations	Temperature (°C)	Humidity (%)	PPFD ($\mu mol/m^{-2}.s$)
F1	32.7	17	130
F2	28.6	17	917
F3	32.4	18	424
F4	32.6	16	120
F5	36.7	14	524
F6	35.5	15	261
F7	45.9	15	920
F8	36.8	16	440
F9	45.7	13	700
F10	30.5	22	40
F11	27.4	17	30
F12	66.3	16	734
F13	24.1	23	34
F14	26.5	19	31
F15	27.4	30	151
F16	31.9	26	975
F17	30.3	27	526
F18	43.9	22	741
F19	27.8	23	32
F20	35.5	16	425

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