Evaluation of Antioxidant Potential and Total Phenolic Content of *Leucodon sciuroides* (Hedw.) Schwager.

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ABSTRACT

Bryophytes have been attracting increasing attention as potential sources of natural bioactive compounds due to their diverse ecological adaptations and secondary metabolite profiles. In this study, the antioxidant potential and total phenolic content of the moss species *Leucodon sciuroides* (Hedw.) Schwägr. were evaluated. Antioxidant activity was assessed using the 2,2-diphenyl-1 picrylhydrazyl (DPPH) radical scavenging assay and the cupric ion reducing antioxidant capacity (CUPRAC) method, while phenolic content was determined by total phenolic content analysis

The DPPH assay was applied at eight different concentrations ranging from 0.0078125 to 1 mg/mL. Scavenging activity increased in a concentration-dependent manner, reaching a maximum inhibition of 40.48% at 1 mg/mL. The EC_{50} value, representing the concentration required to scavenge 50% of DPPH radicals, was calculated as 5.00 mg/mL, indicating a moderate level of antioxidant capacity.

In the CUPRAC method, absorbance values expressed in terms of ascorbic acid equivalents (AAE, mM) increased with rising concentrations. At concentrations of 1.0, 2.5, 5.0, 7.5, and 10.0 mg/mL, the AAE values were determined as 0.258±0.01, 0.540±0.02, 0.722±0.02, 0.889±0.03, and 0.940±0.07, respectively. These results demonstrate the extract's ability to reduce Cu (II) to Cu (I), thus confirming its antioxidant power.

The total phenolic content was determined in terms of gallic acid equivalents. As a result of the analysis, the total phenolic content of *L. sciuroides* was found to be 51.18±4.28 mg GAE/g extract. This indicates that phenolic compounds contribute significantly to the antioxidant activity observed.

In conclusion, *L. sciuroides* exhibits measurable antioxidant activity in terms of both radical scavenging and reducing power and contains a notable amount of phenolic compounds. These findings provide a scientific basis for the evaluation of mosses as natural antioxidant sources for potential use in pharmaceutical or nutraceutical applications.

Keywords: Antioxidant Activity, CUPRAC, DPPH, *Leucodon sciuroides (Hedw.) Schwager.*

INTRODUCTION

Antioxidants are defined as compounds that prevent the formation of free radicals and reactive oxygen species or inhibit the biological damage caused by them [1]. Although oxygen is essential for life, its utilization during energy production leads to the formation of reactive oxygen and nitrogen species, which can cause structural damage to nucleic acids, proteins, and lipids [2]. This condition results in oxidative stress, defined as a shift in the balance between oxidants and antioxidants in favor of oxidants, leading to oxidative damage [3]. With the understanding of the relationship between oxidative stress and various diseases, interest in natural compounds that provide protection against free radicals has increased [4].

The investigation of natural antioxidant sources is therefore of great importance. Bryophytes (mosses), due to their phenolic compounds and diverse secondary metabolites, are considered potential natural antioxidant sources. Previous studies have reported that mosses exhibit effective antioxidant activity through radical scavenging and metal ion reducing capacities [5].

In this study, the antioxidant activity and total phenolic content of the species *L. sciuroides* were determined in order to evaluate the potential of mosses as natural antioxidant sources.

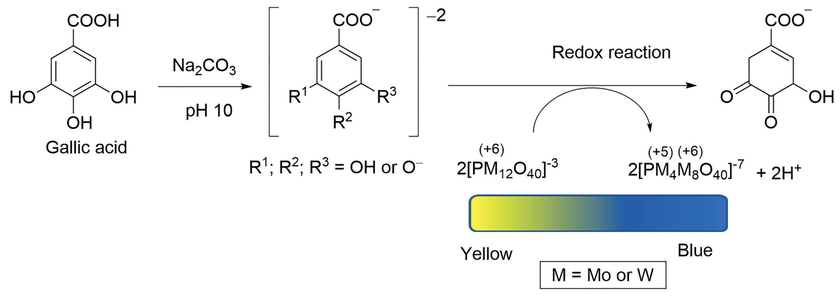
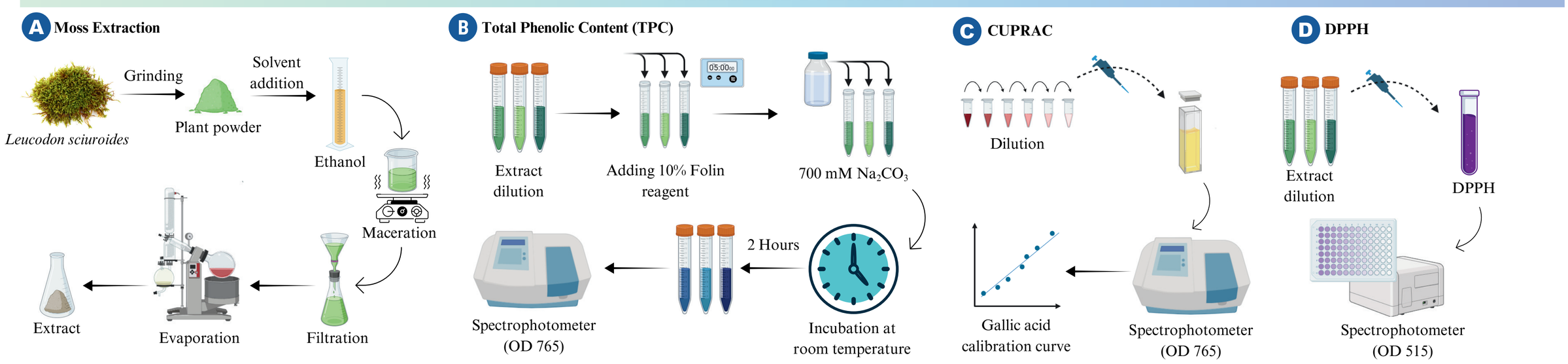


Figure 1. The redox reaction and color change in the Folin–Ciocalteu assay [6].

MATERIALS AND METHODS



After drying and grinding *L. sciuroides*, 40 g of the sample was extracted with 300 mL of methanol by shaking at 160 rpm for 72 hours. The filtrate was then concentrated using a rotary evaporator at 35 °C to remove the solvent [6]. To dissolve the dried residue, 35 mL of 99.9% pure ethanol was added, and the moss extracts were prepared.

In the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, 0.0039 g of DPPH was weighed and dissolved in 50 mL of ethanol within a 250 mL glass Erlenmeyer flask to prepare the radical solution [7]. The microplate loading process consisted of sequential steps: ethanol addition, extract loading and dilution, followed by the addition of DPPH solution. As a positive control, ascorbic acid (vitamin C), a compound with known antioxidant activity, was prepared at a concentration of 1 mg/mL in ethanol and used in the assay. All tests were conducted in triplicate, and absorbance readings were taken at 515 nm using a microplate reader [8].

The CUPRAC (Cupric Ion Reducing Antioxidant Capacity) assay was adapted from the method described by Apak et al. [9]. Moss extracts were prepared at concentrations of 10 mg/mL, 7.5 mg/mL, 5 mg/mL, 2.5 mg/mL, and 1 mg/mL. The Cu(II) chloride solution (10^{-2} M) was prepared by dissolving 0.4262 g of $CuCl_2$ in 250 mL of distilled water. Neocuproine (Nc) solution (7.5×10^{-3} M) was prepared by dissolving 0.039 g of Nc in 25 mL of 96% ethanol.

The ammonium acetate buffer (pH 7.0) was prepared by dissolving 19.27 g of ammonium acetate in 250 mL of distilled water. As a positive control, ascorbic acid solutions were prepared at concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 mM. All polyphenolic compounds and vitamin solutions were freshly prepared in 96% ethanol at a concentration of 1 mM (1.0×10^{-3} M) immediately before measurement. In each test tube, 1 mL of Cu(II) solution, 1 mL of Nc solution, and 1 mL of ammonium acetate buffer were added, followed by the addition of the sample and distilled water to bring the final volume to 4.1 mL. After incubation for 1 hour, the absorbance was recorded at 450 nm. A standard calibration curve was constructed using gallic acid.

The TPC (Total Phenolic Content) was determined according to the Folin–Ciocalteu method for phenolic reactions, adapted from Singleton et al. [10] and Ainsworth & Gillespie [11]. The moss sample was prepared at a concentration of 7.5 mg/mL. The Folin–Ciocalteu reagent (FCR) was diluted to 10% with distilled water. Gallic acid standards were prepared in distilled water at concentrations of 0, 25, 50, 75, 100, 125, 150, 175, 200, and 250 mg/L. Finally, sodium carbonate was prepared in distilled water at a concentration of 700 mM. From each sample, standard, or negative control, 200 µL was transferred into a clean cuvette, and the absorbance was measured at 765 nm. The standard calibration curve was generated from the absorbance values of gallic acid standards at 765 nm.

RESULTS

B TPC (Total Phenolic Content)

The total phenolic content of the *L. sciuroides* extract was determined to be 51.18 ± 4.28 mg GAE/L.

C CUPRAC

Concentration (mg/mL)	*L. sciuroides* (AAE mM)
10.0	0.940±0.07
7.5	0.889±0.03
5.0	0.722±0.02
2.5	0.540±0.02
1.0	0.258±0.01

Table 1. *L. sciuroides* CUPRAC absorbance table

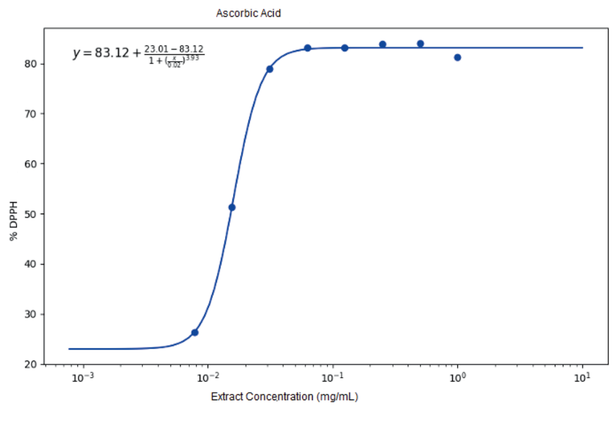
Concentration (mM)	Ascorbic Acid
1.0	3,733±0,18
0.9	3,642±0,08
0.8	3,402±0,11
0.7	2,796±0,14
0.6	2,446±0,04
0.5	1,855±0,20
0.4	1,679±0,03
0.3	1,191±0,12
0.2	0,999±0,09
0.1	0,541±0,05
0.0	0,152±0,01

Table 2. Ascorbic acid CUPRAC absorbance table

D DPPH (2,2-diphenyl-1-picrylhydrazyl)

Concentration (mg/mL)	*L. sciuroides* %
1	40,478
0,5	32,442
0,25	30,457
0,125	23,990
0,0625	19,819
0,03125	18,659
0,015625	17,902
0,0078125	17,041

Table 3. *L. sciuroides* DPPH scavenging table



Graphic 1. Ascorbic acid DPPH scavenging table

DISCUSSION

The results of this study indicate that *L. sciuroides* possesses significant antioxidant activity based on both radical scavenging and reducing power assays. The DPPH assay demonstrated a concentration-dependent increase in radical scavenging activity, suggesting that the extract is capable of effectively neutralizing free radicals at higher concentrations. This activity is closely linked to the presence of proton-donating phenolic compounds.

The total phenolic content of the extract was determined to be 51.18 ± 4.28 mg GAE/L, which confirms that phenolic secondary metabolites contribute substantially to the antioxidant potential of *L. sciuroides*. Phenolic compounds are known to act as natural antioxidants by transferring electrons or hydrogen atoms to reactive species, thereby preventing oxidative damage.

The CUPRAC assay further supported these findings. The extract exhibited increasing cupric ion reducing capacity with rising concentration (0.258±0.01 to 0.940±0.07 mM AAE across tested concentrations), indicating strong electron-donating ability and reductive potential. This suggests that *L. sciuroides* is not only capable of scavenging free radicals but also plays an active role in reducing oxidized intermediates, contributing to overall redox balance.

Taken together, the results confirm that *L. sciuroides* is a promising natural source of antioxidant compounds. The consistency between DPPH radical scavenging, CUPRAC reducing capacity, and total phenolic content highlights the biochemical richness of this bryophyte. Further studies focusing on isolation and structural characterization of the active constituents, as well as biological activity-guided fractionation, would help clarify the specific compounds responsible for the observed antioxidant effects. Additionally, these findings suggest potential applications of *L. sciuroides* extracts in pharmaceutical, food, and cosmetic formulations aimed at preventing oxidative damage.

REFERENCES