

PHYTOCHEMICAL CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF *ARTEMISIA ABSINTHIUM L.*

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RESEARCH ARTICLE

Abstract

Artemisia absinthium L. (Asteraceae) is a perennial aromatic herb known for its medicinal properties and rich phytochemical profile. This study aimed to evaluate the phytochemical composition and antioxidant potential of ethanolic extracts obtained from the aerial parts of *A. absinthium* collected from natural habitats in Bihor County, Romania. Extracts were prepared using Soxhlet extraction and analyzed for total phenolic content, total flavonoid content, and DPPH radical scavenging activity. The total phenolics were 96.37 ± 0.32 mg GAE/g dry extract, while the total flavonoids were 14.95 ± 0.12 mg QE/g dry extract. The extract exhibited a strong antioxidant activity, with 65.17 ± 0.73 % DPPH radical inhibition. The study highlights the potential of *Artemisia absinthium* extracts as natural antioxidants, emphasizing the contribution of phenolic and flavonoid compounds to their biological activity. The findings support the relevance of this species in the development of functional ingredients or therapeutic agents aimed at mitigating oxidative stress-related processes.

Keywords: wormwood, ethanolic extract, polyphenols, flavonoids, free radical scavenging
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INTRODUCTION

Artemisia absinthium L. (Asteraceae), commonly known as wormwood, is a perennial aromatic herb widely distributed across Europe and Asia and long recognized for its traditional medicinal applications, including digestive, antimicrobial, and anti-inflammatory uses. In recent decades, this species has attracted growing scientific interest due to its complex phytochemical composition, particularly its abundance of phenolic acids and flavonoids, compounds with well-established antioxidant potential (Pizzorno and Murray, 2021; Saunoriūtė et al., 2023).

Previous studies have reported that *Artemisia absinthium* extracts exhibit significant antioxidant and neuroprotective effects, mainly attributed to phenolic constituents such as gallic, chlorogenic, and syringic acids, as well as flavonoids including quercetin and rutin (Kosakowska et al., 2025; Liu et al., 2023). However, data concerning the variability of these compounds in plants originating from different ecological regions remain limited.

The present study aims to characterize the phytochemical composition and evaluate the antioxidant potential of *Artemisia absinthium L.* samples collected from natural habitats in Bihor County, Romania. The research

provides new insights into the chemical diversity and biological activity of this species, contributing to a broader understanding of its potential as a valuable source of natural antioxidants for pharmaceutical and nutraceutical applications.

MATERIAL AND METHOD

PLANT MATERIAL

Artemisia absinthium L. specimens were collected from natural habitats in Bihor County, Podgoria area (47.057441, 22.015630), a non-polluted site. Basal leaves and flowering tips (stem diameter ≤ 4 mm) were harvested during dry weather in July 2022 using scissors, with flowering stem tips cut to approximately 30 cm. Only healthy and clean plants were selected. A pressed specimen is preserved in the Herbarium of the Faculty of Medicine and Pharmacy, Department of Pharmaceutical Botany (code UOP 05.708).

REAGENTS AND EQUIPMENT

Analytical grade reagents were used throughout the study. Ethanol, p.a. (Chimreactiv, Romania); Folin-Ciocalteu reagent (Merck, Germany); sodium carbonate (Na_2CO_3) 20% freshly prepared (Chimreactiv, Romania); sodium nitrite (NaNO_2) 5% freshly prepared

(Chimreactiv, Romania); aluminum chloride (AlCl_3) 10% (Merck, Germany); sodium hydroxide (NaOH) 1 M (Chimreactiv, Romania); 2,2-diphenyl-1-picrylhydrazyl (DPPH) 0.1 mM (Merck, Germany); gallic acid (Silver Chemicals, Romania); quercetin (Silver Chemicals, Romania); distilled water. The study utilized a rotary evaporator Hei-VAP Advantange (Heidolph, Germany) and a UV-VIS spectrophotometer PG Instruments T70+ (UK).

METHODS

Microscopy: Plant tissues (stems and leaves) of *Artemisia absinthium L.* were examined using an optical microscope (Optika B-380 Series) with 10× and 40× objectives. Sections were stained with Genevez reagent to highlight cellular structures.

Extraction: Dried aerial parts of *Artemisia absinthium L.* were finely ground. Approximately 18 g of plant material underwent Soxhlet extraction with ethanol for 4 h (10 cycles). Extracts were concentrated using a rotary evaporator at 40°C, 80 rpm, and 200 atm until a thin residue remained, then reconstituted in 10 mL ethanol for further analyses.

Total Phenolic Content: The phenolic content of extracts was determined using the Folin-Ciocalteu method. Samples were mixed with Folin-Ciocalteu reagent and sodium

carbonate solution, incubated in the dark, and absorbance measured at 765 nm. A calibration curve was prepared using gallic acid solutions in the range of 10–50 mg/mL (Hbika et al., 2022).

Total Flavonoid Content: Flavonoids were quantified using a colorimetric assay based on the formation of a complex with aluminum chloride. Absorbance was read at 510 nm, and quercetin solutions (2–10 mg/mL) were used to construct a standard calibration curve (Msaada et al., 2015).

DPPH Radical Scavenging Assay: The antioxidant activity of extracts was evaluated using the DPPH method. Extracts were mixed with a 0.1 mM DPPH solution and incubated in the dark. Absorbance was recorded at 517 nm after 30 minutes, and the percentage of radical scavenging was calculated relative to a blank sample.

RESULTS AND DISCUSSIONS

Transverse sections of the stem revealed the defensive tissues, collateral vascular bundles, and mechanical tissues. The stem is circular with 8–10 ridges, showing collenchyma and numerous collateral xylem-phloem bundles, with a pluristratified fibrous pericycle forming caps above the phloem, as shown in Figure 1. The epidermis is densely covered with tector hairs.

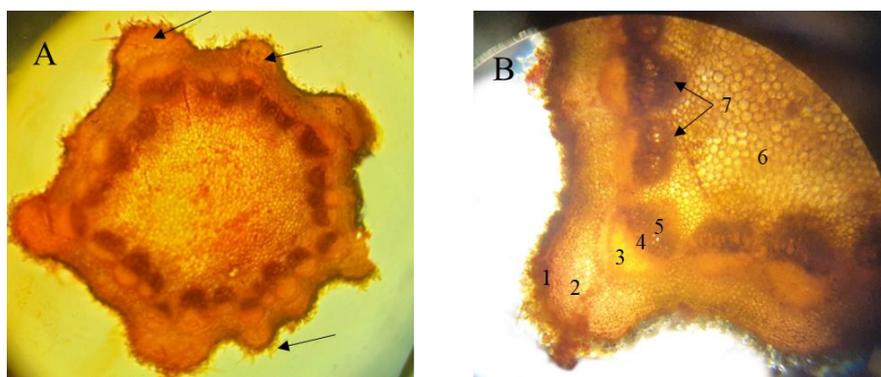


Figure 1. Transverse section of the stem of *Artemisia absinthium L.*
A – circular outline with 8–10 ridges and tector hairs on the epidermis (10X),
B – 1-epidermis, 2-collenchyma, 3-sclerenchyma, 4-secondary phloem, 5-secondary xylem, 6-parenchymatous pith, 7-collateral vascular bundles (40X)

Leaf sections highlighted key diagnostic features, including hairs, glands, secretory cavities, and epidermal structure. The lamina exhibits a heterogeneous, asymmetric structure with unequal collateral vascular bundles. Both epidermises have T-shaped tector hairs with

short multicellular stalks and elongated terminal cells with thin walls, as illustrated in Figure 2. Glandular hairs are biseriate with a short unicellular stalk and a multicellular gland, more abundant on the abaxial epidermis. No fibers were observed.

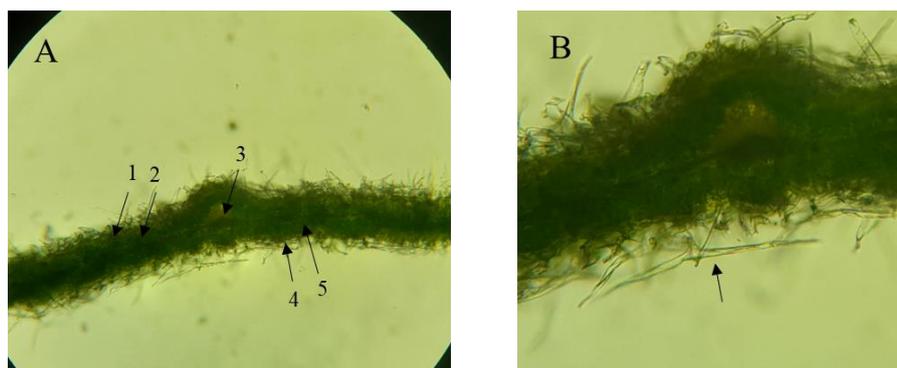


Figure 2. Transverse section of the leaf of *Artemisia absinthium L.*

A – 1-upper epidermis, 2-palisade tissue, 3-collateral vascular bundle, 4-spongy tissue, 5-lower epidermis (10X), B – T-shaped tector hairs (40X)

The total phenolic content of the ethanolic extract of *Artemisia absinthium L.* was calculated using the calibration curve of gallic acid ($Y = 0.007X + 0.0671$, $R^2 = 0.9918$). The determined values are presented in Table 1. According to data reported in the literature, the

total phenolic content of *Artemisia absinthium L.* extracts generally ranges between 69.01 and 180.33 mg GAE/g dry extract, depending on extraction conditions, solvent polarity, and geographical origin of the plant material (Bora and Sharma, 2011; Boudjelal et al., 2020).

Table 1

Total phenolics in ethanolic extract of *Absinthii herba*

| Sample | Sample absorbance measured at 765 nm | Sample concentration (mg GAE/1 g dry extract) | Mean concentration (mg GAE/g dry extract) \pm SD |
|---|--------------------------------------|---|--|
| Ethanolic extract of <i>Absinthii herba</i> | 0,823 | 96,41 | 96,37 \pm 0,32 |
| | 0,820 | 96,03 | |
| | 0,825 | 96,67 | |

The ethanolic extract of *Artemisia absinthium L.* was analyzed for its flavonoid content, with concentrations calculated based on the quercetin calibration curve ($Y = 0.033X + 0.1418$, $R^2 = 0.9873$). The results of the measurements are summarized in Table 2. Comparisons with literature data indicate that

flavonoid levels in *Artemisia absinthium L.* extracts can vary widely, generally ranging from 2.61 to 35.81 mg QE/g dry extract, depending on factors such as solvent type, extraction conditions, and geographical origin of the plant material (Hbika et al., 2022; He et al., 2023).

Table 2

Total flavonoid content of the ethanolic extract of *Absinthii herba*

| Sample | Sample absorbance measured at 510 nm | Sample concentration (mg QE/1 g dry extract) | Mean concentration (mg QE/g dry extract) \pm SD |
|---|--------------------------------------|--|---|
| Ethanolic extract of <i>Absinthii herba</i> | 0,736 | 15,00 | 14,95 \pm 0,12 |
| | 0,729 | 14,82 | |
| | 0,738 | 15,05 | |

The DPPH radical scavenging activity of the ethanolic extract of *Artemisia absinthium L.* was evaluated by measuring the absorbance of the samples and the blank at 517 nm. The percentage of inhibition was calculated for each sample, and the mean values with standard

deviation are presented in Table 3. The ethanolic extract exhibited a high DPPH inhibition (65.17 % \pm 0.73), indicating a strong free radical scavenging capacity, consistent with values reported in the literature, ranging between 60.34 % and 79.30 % (Amin et al, 2025; Moacă et al, 2019).

Table 3

DPPH radical scavenging activity of the ethanolic extract of *Absinthii herba*

| Sample | Blank absorbance | Sample absorbance | Inhibition % | Mean inhibition % \pm SD |
|---|------------------|-------------------|--------------|----------------------------|
| Ethanolic extract of <i>Absinthii herba</i> | 0,895 | 0,312 | 65,13 | 65,17 \pm 0,73 |
| | | 0,316 | 64,46 | |
| | | 0,305 | 65,92 | |

CONCLUSIONS

The phytochemical analysis of *Artemisia absinthium* L. ethanolic extract revealed a significant content of phenolic and flavonoid compounds, with total phenolics of 96.37 ± 0.32 mg GAE/g dry extract and total flavonoids of 14.95 ± 0.12 mg QE/g dry extract. The extract also exhibited a marked antioxidant capacity, as shown by the 65.17 ± 0.73 % DPPH radical inhibition, demonstrating its efficiency in scavenging free radicals.

Overall, these results underscore the biological relevance of *Artemisia absinthium* L. as a rich source of bioactive constituents, whose synergistic interactions contribute to its pronounced antioxidant potential and support its role as a functional agent in counteracting oxidative processes.

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