Exploring the Artemisia Genus: An Insight into the Phytochemical and Multi-Biological Potential of A. campestris subsp. lednicensis (Spreng.) Greuter & Raab-Straube

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Abstract: The Artemisia L. genus includes over five hundred species with great economic and medicinal properties. Our study aimed to provide a comprehensive metabolite and bioactivity profile of Artemisia campestris subsp. lednicensis (Spreng.) Greuter & Raab-Straube collected from northeastern Romania. Liquid chromatography with tandem high-resolution mass spectrometry (LC-HRMS/MS) analysis of different polarity extracts obtained from the aerial parts led to the identification of twelve flavonoids, three phenolic acids, two sesquieterpene lactones, two fatty acids, one coumarin, and one lignan. The antioxidant and enzyme inhibitory properties were shown in the DPPH (0.71–213.68 mg TE/g) and ABTS (20.57–356.35 mg TE/g) radical scavenging, CUPRAC (38.56–311.21 mg TE/g), FRAP (121.68–202.34 mg TE/g), chelating (12.88–22.25 mg EDTAE/g), phosphomolybdenum (0.92–2.11 mmol TE/g), anti-acetylcholinesterase (0.15–3.64 mg GALAE/g), anti-butyrylcholinesterase (0–3.18 mg GALAE/g), anti-amylose (0.05–0.38 mmol ACAE/g), anti-glucosidase (0.43–2.21 mmol ACAE/g), and anti-tyrosinase (18.62–48.60 mg KAE/g) assays. At 100 μg/mL, Artemisia extracts downregulated the secretion of tumor necrosis factor (TNF-α) in a lipopolysaccharide (LPS)-stimulated human neutrophil model (29.05–53.08% of LPS+ control). Finally, the Artemisia samples showed moderate to weak activity (minimum inhibitory concentration (MIC) > 625 mg/L) against the seventeen tested microbial strains (bacteria, yeasts, and dermatophytes). Overall, our study shows that A. campestris subsp. lednicensis is a promising source of bioactives with putative use as food, pharmaceutical and cosmetic ingredients.

Keywords: wormwood; LC-HRMS/MS; flavonoids; antioxidant; anti-enzymatic; pro-inflammatory cytokines

1. Introduction

The Artemisia L. genus (Asteraceae) comprises over five hundred perennial species inhabiting mainly the Northern Hemisphere, especially the arid and semi-arid temperate regions of Europe, North America, and Asia [1]. Artemisia species are small herbs or
shrubs with a specific bitter taste and pungent aroma assigned to terpenoids, mostly monoterpenes in the essential oil and sesquiterpene lactones [2]. This genus has significant economic (food, spices, beverages, and ornamental use) and medicinal properties due to its chemical diversity, which includes, aside from terpenoids, other phytochemicals such as flavonoids, phenolic acids, coumarins, sterols, and lignans [2–4]. *Artemisia* species have a long-established traditional use, being utilized to alleviate various ailments (e.g., digestive and hepatobiliary complaints, inflammatory diseases, malaria, bronchitis, helminthiasis, and cancer) [5]. The isolation of artemisinin as the active principle of *A. annua* against malaria (1971) has attracted the interest of the scientific community toward the genus and has prompted its extensive research in drug discovery and development [6]. Thus, various *in vitro*, *in vivo*, and clinical studies have revealed its versatile pharmacological profile characterized by antimalarial, anthelmintic, antitubercular, antiviral, antiemetic, hepato-protective, gastroprotective, antihyperlipidemic, antidiabetic, antihypertensive, anti-asthmatic, antidepressant, anxiolytic, anticancer, and insecticidal properties [3,7–9].

The pleiotropic pharmacology of the *Artemisia* genus resides in its vast number of species colonizing areas with different ecological conditions and types of vegetation, which translates into different morphological and biological characteristics [10–13]. Furthermore, this leads to both qualitative and quantitative variations in the phytochemical profile, consequently impacting the bioactivities of the species. Therefore, it is of great interest to further investigate the potential of *Artemisia* genus metabolites to achieve significant alleviation of various human diseases [14].

*Artemisia campestris* subsp. *lednicensis* (Spreng.) Greuter & Raab-Straube (syn. *A. lednicensis* Rochel ex Spreng.) belongs to an infraspecific taxon of *Artemisia campestris* L. [15]. This species frequently inhabits sunny meadows in steppe regions of Romania characterized by sand or loess substrate [16]. It is a perennial, more or less tomentose, and odorless plant, with ovoidal-shaped, sessile, and erect anthodia (Figure 1). *A. campestris* subsp. *lednicensis* has been used in Romanian traditional medicine as a tonic, anthelmintic, cholagogue, emmenagogue, and antiseptic agent [17]. To the best of our knowledge, no studies on the phytochemistry nor the biological attributes of *A. campestris* subsp. *lednicensis* have been undertaken.

![Image](image_url)

**Figure 1.** *Artemisia campestris* subsp. *lednicensis* (Spreng.) Greuter & Raab-Straube. (a) In its natural habitat, near Aroneanu Lake (Iasi county, Romania); (b) inflorescence; (c) herborized plant material (Photo by Adriana Trifan).

In our endeavor to promote interest in the Romanian *Artemisia* species [18], we report herein for the first time on the metabolite and biological profile of *A. campestris* subsp. *lednicensis* aerial parts. The phytochemical characterization was assessed by means of LC-
HRMS/MS. The bioactivity screening included the evaluation of (i) antioxidant effects (in vitro free radical scavenging, metal chelating and reducing power, and total antioxidant activity); (ii) enzyme inhibitory activity (in vitro anti-cholinesterase, anti-amylase, anti-glucosidase and anti-tyrosinase effects); (iii) influence upon pro-inflammatory cytokines secretion from ex vivo LPS-stimulated human neutrophils; and (iv) in vitro antimicrobial potential (against Gram-positive and Gram-negative bacteria, yeasts, and dermatophytes).

2. Results and Discussion

2.1. Total Phenolic and Flavonoid Content

In phytochemical studies, the phenolic and flavonoid content of a plant extract is usually determined to obtain the first insights into its pharmacological potential. The total content of these bioactive metabolites in the tested extracts is presented in Table 1. Apparently, the levels are dependent on the used extraction solvents. The highest concentration of the total phenolic was determined in the hydroalcoholic extract with 104.00 mg GAE/g, followed by methanol (84.42 mg GAE/g), water (71.73 mg GAE/g), dichloromethane (20.67 mg GAE/g), and hexane (17.11 mg GAE/g). Regarding the total flavonoid content, the methanol extract was the richest with a value of 23.13 mg RE/g, and the hydroalcoholic (15.08 mg RE/g) and dichloromethane (16.43 mg RE/g) extracts contained similar levels of flavonoids (p > 0.05). Interestingly, the water extract had the lowest total flavonoid content. From these results, we concluded that the methanolic and hydroalcoholic solvents could be utilized for the extraction of bioactive compounds from Artemisia campestris subsp. lednicensis. Consistent with our results, several investigators have shown the efficacy of polar solvents for preparing extracts from Artemisia plants [19,20]. In addition, the alcohol–water mixture showed synergistic effects for the extraction of phenolics and flavonoids in several studies [21,22]. In the literature, different levels of the total phenolic and flavonoid content have been reported for the species of the genus Artemisia [23–26]. However, the results of spectrophotometric assays are very controversial since not only phenolic/flavonoid compounds but also other phytochemicals (peptides, sulfides, etc.) could react with the used reagents and therefore the results obtained are not entirely accurate [27]. Therefore, to confirm the spectrophotometric results, further techniques including LC-MS, LC-HRMS/MS, or LC-NMR are needed to detect the chemical constituents of plant extracts.

Table 1. Extraction yields, total phenolic, and flavonoid content of Artemisia campestris subsp. lednicensis extracts.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Yield (%)</th>
<th>Total Phenolic Content (mg GAE/g)</th>
<th>Total Flavonoid Content (mg RE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL-HE</td>
<td>4.33</td>
<td>17.11 ± 0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.57 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>AL-DE</td>
<td>6.20</td>
<td>20.67 ± 0.52&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16.43 ± 0.37&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AL-ME</td>
<td>12.60</td>
<td>84.42 ± 0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.13 ± 0.73&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AL-MWE</td>
<td>22.59</td>
<td>104.00 ± 0.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.08 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AL-WE</td>
<td>23.55</td>
<td>71.73 ± 0.78&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.92 ± 0.31&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are given as the mean ± standard deviation of three replicates; significant differences in the investigated samples (p < 0.05) are indicated by different letters within columns. AL-HE, A. campestris subsp. lednicensis hexane extract; AL-DE, A. campestris subsp. lednicensis dichloromethane extract; AL-ME, A. campestris subsp. lednicensis methanol extract; AL-MWE, A. campestris subsp. lednicensis 50% methanol extract; AL-WE, A. campestris subsp. lednicensis water extract; GAE, gallic acid equivalents; RE, rutin equivalents.
2.2. LC-HRMS/MS Metabolite Profiling

The five *A. campestris* subsp. *lednicensis* aerial part extracts achieved with solvents of different polarities were thoroughly analyzed by LC-HRMS/MS.

The annotation of the peaks was assessed by a comparison of the spectral and chromatography data with the literature [18,28–33] and databases (KNApSaK, METLIN, NIST, etc.). Thus, 21 specialized metabolites from various phytochemical groups (phenolic acids, coumarins, flavonoids, sesquiterpenes, fatty acids, and lignans) were fully or partly ascribed (Table 2, Figure 2).

Flavonoids were the representative category of compounds, with 12 distinct congeners spotted in the analyzed extracts. Quercetin-O-deoxyhexoside-O-heoxisde (e.g., rutin) presented the pseudomolecular ion [M–H]$^-$ at m/z 609.1476 (C$_{29}$H$_{23}$O$_{10}$) and its characteristic MS/MS fragments at m/z 300.0287 [Quercetin–2H$^-$], 271.0255 [Quercetin–CH$_2$O–H$^-$], and 151.0075 (resulted after the specific Retro-Diels Alder, RDA, cleavage of quercetin). The RDA cleavage of flavonoids has been extensively detailed by Fabre et al. [34]. These fragments were in agreement with those presented by Melguizo-Melguizo et al. [31] for the structure of rutin. Galloccatechin (8) presented the [M–H]$^-$ ion at m/z 305.0671 (C$_{15}$H$_{12}$O$_{5}$) and its main MS/MS fragment ion at m/z 225.1144. Next, eriodictyol (10) was tentatively assigned based on its pseudomolecular ion at m/z 287.0567 (C$_{16}$H$_{14}$O$_{5}$) and RDA fragment ions at m/z 151.0046 and 135.0479, as proposed by Fabre et al. [34]. The presence of luteolin (12), [M–H]$^-$ at m/z 285.0567, was confirmed by standard injection and diagnostic MS/MS fragments at m/z 175.0386 [M–C$_{6}$O$_{2}$–C$_{3}$H$_{5}$O–H$^-$] and 133.0313 (RDA fragment) [34].

The remaining flavonoids were poly-hydroxylated/poly-methoxylated flavone derivatives. Due to the high isomerism, assigning the exact position of the hydroxy and methoxy group with the use of LC-HRMS/MS only is difficult. However, for simplification purposes, some possible structures have been proposed. Peak 5 with the [M–H]$^-$ ion at m/z 327.0847 (C$_{16}$H$_{14}$O$_{5}$) was suggested to have three methoxy groups, based on the RDA fragment ions at m/z 177.0415 (C$_{10}$H$_{14}$O$_{5}$) and 151.0075 (C$_{8}$H$_{10}$O$_{5}$) obtained after cleavage of the pyran ring [33]. Thus, its tentative structure was proposed as hydroxytrimethoxyflavone, possibly belonging to salvigenin. Two isobaric peaks (11 and 18) with the pseudomolecular ions [M–H]$^-$ at m/z 343.0813 (C$_{16}$H$_{13}$O$_{5}$) indicated a flavone derivative with an additional hydroxy group compared to peak 5. The MS/MS fragmentation patterns of peaks 11 and 18 showed the successive loss of three methoxy groups, as follows: 328.0382 [M–CH$_2$]–, 313.0382 [M–2×CH$_3$]–, and 298.013 [M–3×CH$_3$]–. Thus, the two dihydroxytrimethoxyflavones were putatively assigned as penteulutin (11) and eupatilin (18) [29]. In the MS/MS fragmentation of compound 13 ([M–H]$^-$ at m/z 345.0602, C$_{27}$H$_{29}$O$_{13}$), only two methoxy groups were indicated by the MS/MS fragment ions at m/z 330.0402 (M–CH$_3$)– and 315.0188 [M–2×CH$_3$]–. Thus, a number of four hydroxy groups were assumed to be attached to the flavone skeleton, suggesting a tetrahydroxydimethoxyflavone, possibly belonging to eupatolin [29]. Peak 14 with [M–H]$^-$ at m/z 315.0509 (C$_{15}$H$_{13}$O$_{5}$) indicated a flavone with one methoxy group less than eupatolin, thus a tetrahydroxymethoflavone (e.g., rhamnetin) [35]. Two isobaric peaks 15 and 16 with the pseudomolecular ions at m/z 329.0678 (C$_{17}$H$_{15}$O$_{5}$) were assigned as dihydroxydimethoxyflavones. The two methoxy groups were evidenced by the MS/MS fragment ions at m/z 314.0456 [M–CH$_3$]– and 299.0241 [M–2×CH$_3$]–. After the sequential loss of these two groups, the smaller fragments were characteristic to flavonoids structures, with the diagnostic RDA ions at m/z 151.0068 and 133.0347. In agreement with previous reports on similar compounds from the *Artemisia* genus, these two compounds were tentatively labeled as rhamnazin (15) and eupatilin (16) [29,35]. Finally, diosmetin (17), [M–H]$^-$ at m/z 299.0566, was indicated by the presence of a single methoxyl group with the characteristic MS/MS fragment ion at m/z 284.0259 [M–CH$_3$]–; all the remaining fragments confirmed the flavone structure [29]. The high abundance of poly-hydroxylated/poly-methoxylated flavone is specific to *Artemisia* species and can have chemotaxonomical importance [18]. All of these phytochemicals have been previously reported in *A. annua*, *A. austriaca*, *A. pontica*, *A. vulgaris*, or *A. absinthium* [18].
Regarding the phenolic acids from *A. lednicensis*, it is worth mentioning the presence of quinic acid derivatives such as neochlorogenic (1) and chlorogenic acid (2) as well as syringoylquinic acid (3). Confirmed by standard injection, the two caffeoylquinic isomers were also indicated by their characteristic fragmentation patterns. While neochlorogenic acid (1) presented the main MS/MS fragment ions at *m/z* 191.0484 [Quinic acid–H], 179.0252 [Caffeic acid–H] and 135.0370 [Caffeic acid–CO₂–H], chlorogenic acid (2) showed diagnostic fragments at *m/z* 191.0568 [Quinic acid–H], 173.0252 [Quinic acid–H₂O–H], and 135.0370 [Caffeic acid–CO₂–H]. These differences in the fragmentation patterns have also been reported in previous literature [36].

Esculetin-O-hexoside (4) was the sole coumarin putatively identified in some of the extracts of *A. lednicensis*. The pseudomolecular ion at *m/z* 339.0715 (C₁₅H₁₅O₉) generated the MS/MS fragments at *m/z* 177.0233 [Esculetin–H], 149.0157 [Esculetin–CO–H], and 133.0217 [Esculetin–CO₂–H], in agreement with Olennikov et al. [29].

In addition, one glycosylated lignan, possibly belonging to the structure of tracheloside (9), was labeled. This lignan was also previously present in *A. annua*, *A. austriaca*, and *A. vulgaris* [18]. Finally, two sesquiterpenes, artemisin hydrate (6) and cnicin (19), and two fatty acids, hydroxyoctadecatrienoic acid (20) and hydroxyoctadecadienoic acid (21), were tentatively annotated. These classes of specialized metabolites were evidenced in *A. annua*, *A. pontica*, *A. austriaca*, *A. vulgaris*, or *A. absinthium* [18].

To obtain a better view of the relative concentration of phytochemicals present in each solvent, we then performed a thorough clustered image map analysis using peak area data (Figure 3). For example, the hexane extract contained a high level of eupatilin (18). Rhamentin (14) is the main molecule of the dichloromethane extract, but important amounts of eupatilin (18), eriodictyol (10), and artemisin hydrate (6) were also determined. Both hydroalcoholic and methanol extracts contained relatively high levels of syringoylquinic acid (3).
Table 2. LC-HRMS/MS-based metabolite profiling of the *A. campestris* subsp. *lednicensis* aerial part extracts.

<table>
<thead>
<tr>
<th>No.</th>
<th>Proposed Identity</th>
<th>Class</th>
<th>TR (min)</th>
<th>[M-H]+ (m/z)</th>
<th>MF</th>
<th>MS/MS (m/z)</th>
<th>AL-HE</th>
<th>AL-DE</th>
<th>AL-ME</th>
<th>AL-MWE</th>
<th>AL-WE</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Neochlorogenic acid *</td>
<td>Phenolic acid</td>
<td>9.8</td>
<td>353.0893</td>
<td>C₁₆H₁₈O₉⁺</td>
<td>191.0484, 179.0252, 135.0370</td>
<td>–</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>2</td>
<td>Chlorogenic acid *</td>
<td>Phenolic acid</td>
<td>13.2</td>
<td>353.0893</td>
<td>C₁₆H₁₈O₉⁺</td>
<td>191.0568, 173.0429, 135.0461</td>
<td>–</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>3</td>
<td>Syringoylquinic acid</td>
<td>Phenolic acid</td>
<td>14.6</td>
<td>371.0969</td>
<td>C₁₆H₁₈O₁₀⁺</td>
<td>353.0882, 339.0740, 209.0675, 179.0358</td>
<td>–</td>
<td>–</td>
<td>×</td>
<td>×</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>Esculetin-O-hexoside</td>
<td>Coumarin</td>
<td>14.9</td>
<td>339.0715</td>
<td>C₁₆H₁₈O₉⁺</td>
<td>177.0233, 149.0157, 133.0217, 105.0327</td>
<td>–</td>
<td>–</td>
<td>×</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>5</td>
<td>Hydroxystilbene (e.g., salvigenin)</td>
<td>Flavonoid</td>
<td>16.4</td>
<td>327.0847</td>
<td>C₁₆H₁₈O₉⁺</td>
<td>241.0099, 177.0415, 151.0075</td>
<td>–</td>
<td>–</td>
<td>×</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>6</td>
<td>Artecanin hydrate</td>
<td>Sesquiterpene</td>
<td>19.6</td>
<td>295.1169</td>
<td>C₁₆H₁₈O₉⁺</td>
<td>251.1300, 207.1409, 189.1280, 151.0831</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>7</td>
<td>Quercetin-O-deoxyhexoside-O-hexoside (e.g., rutin)</td>
<td>Flavonoid</td>
<td>23.1</td>
<td>609.1476</td>
<td>C₂₇H₃₀O₁₆⁺</td>
<td>300.0287, 271.0255, 151.0035</td>
<td>–</td>
<td>–</td>
<td>×</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>8</td>
<td>Galloatechin</td>
<td>Flavonoid</td>
<td>25.0</td>
<td>305.0671</td>
<td>C₁₆H₁₈O₉⁺</td>
<td>225.1144, 177.0415, 151.0075</td>
<td>–</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>9</td>
<td>Tracheloside</td>
<td>Lignan</td>
<td>26.9</td>
<td>549.1985</td>
<td>C₁₆H₁₈O₁₀⁺</td>
<td>387.1681, 207.1037, 179.0375, 161.0251</td>
<td>–</td>
<td>–</td>
<td>×</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>10</td>
<td>Eriodictyol</td>
<td>Flavonoid</td>
<td>29.0</td>
<td>287.0567</td>
<td>C₁₆H₁₈O₇⁺</td>
<td>151.0046, 135.0479</td>
<td>–</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>11</td>
<td>Dihydroxytrimethoxyflavone I (e.g., penduletin)</td>
<td>Flavonoid</td>
<td>30.1</td>
<td>343.0813</td>
<td>C₁₆H₁₈O₇⁺</td>
<td>328.0382, 313.0382, 298.0133, 285.0421, 270.0199, 255.0318, 242.0284</td>
<td>–</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>12</td>
<td>Luteolin *</td>
<td>Flavonoid</td>
<td>31.0</td>
<td>285.0400</td>
<td>C₁₆H₁₈O₇⁺</td>
<td>175.0386, 133.0313</td>
<td>–</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>13</td>
<td>Tetrahydroxydimethoxyflavone (e.g., eupatolitin)</td>
<td>Flavonoid</td>
<td>31.5</td>
<td>345.0602</td>
<td>C₁₆H₁₈O₇⁺</td>
<td>259.0301, 259.0301, 215.0351, 175.0091, 149.0308, 121.0326</td>
<td>–</td>
<td>×</td>
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</tr>
<tr>
<td>14</td>
<td>Tetrahydroxymethoxyflavone (e.g., rhamnetin)</td>
<td>Flavonoid</td>
<td>31.7</td>
<td>315.0509</td>
<td>C₁₆H₁₈O₇⁺</td>
<td>300.0327, 271.0269, 255.0312, 243.0322, 227.0356, 215.0350, 171.0409, 147.0202</td>
<td>–</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
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<tr>
<td>15</td>
<td>Dihydroxydimethoxyflavone I (e.g., rhamnarin)</td>
<td>Flavonoid</td>
<td>33.9</td>
<td>329.0678</td>
<td>C₁₆H₁₈O₇⁺</td>
<td>314.0456, 299.0241, 271.0279, 271.0272, 243.0312, 227.0430, 215.0360, 199.0421, 185.0236, 161.0264, 151.0068, 133.0347</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>–</td>
</tr>
<tr>
<td>16</td>
<td>Dihydroxydimethoxyflavone II (e.g., eupatolitin)</td>
<td>Flavonoid</td>
<td>35.4</td>
<td>329.0678</td>
<td>C₁₆H₁₈O₇⁺</td>
<td>314.0456, 299.0241, 271.0279, 271.0272, 243.0312, 227.0430,</td>
<td>–</td>
<td>×</td>
<td>×</td>
<td>–</td>
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</tr>
<tr>
<td>No.</td>
<td>Compound</td>
<td>Class</td>
<td>Retention Time</td>
<td>Molecular Formula</td>
<td>M/z Values</td>
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<tr>
<td>17</td>
<td>Trihydroxymethoxyflavone (e.g., diosmetin)</td>
<td>Flavonoid</td>
<td>37.0</td>
<td>C_{16}H_{12}O_{6}</td>
<td>215.0360, 199.0421, 185.0236, 161.0264, 151.0068, 133.0347</td>
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<td>18</td>
<td>Dihydroxytrimethoxyflavone II (e.g., eupatilin)</td>
<td>Flavonoid</td>
<td>37.6</td>
<td>C_{16}H_{12}O_{6}</td>
<td>284.0259, 255.0179, 239.0292, 227.0330, 151.0077, 133.0252</td>
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<td></td>
<td>328.0382, 313.0382, 298.0133,</td>
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<td>285.0421, 270.0199, 255.0318,</td>
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<td>19</td>
<td>Cnicin</td>
<td>Sesquipertene</td>
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<td>C_{20}H_{26}O_{7}</td>
<td>295.1213, 251.1322, 189.1257, 151.0760</td>
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<td>20</td>
<td>Hydroxyoctadecatrienoic acid</td>
<td>Fatty acid</td>
<td>46.8</td>
<td>C_{18}H_{30}O_{3}</td>
<td>275.1973, 224.1359, 195.1381</td>
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<tr>
<td>21</td>
<td>Hydroxyoctadecadienoic acid</td>
<td>Fatty acid</td>
<td>49.3</td>
<td>C_{18}H_{32}O_{3}</td>
<td>277.2162, 195.1407, 171.1029</td>
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</tr>
</tbody>
</table>

AL-HE, *A. campestris* subsp. *lednicensis* hexane extract; AL-DE, *A. campestris* subsp. *lednicensis* dichloromethane extract; AL-ME, *A. campestris* subsp. *lednicensis* methanol extract; AL-MWE, *A. campestris* subsp. *lednicensis* 50% methanol extract; AL-WE, *A. campestris* subsp. *lednicensis* water extract; *confirmed by standard. The LC-HRMS/MS analyses were performed on a Phenomenex Gemini C18 column (2 mm × 100 mm, 3 μm); mobile phase 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B); gradient 5–60% B (0–45 min), 95% B (46–55 min); flow rate 0.2 mL/min. The following MS conditions were applied: negative ionization mode; m/z range 100–1000; gas (N₂) temperature 275 °C; N₂ flow 10 L/min; nebulizer 35 psi; sheath gas temperature 325 °C; sheath gas flow rate 12 L/min; capillary voltage 4000 V; nozzle voltage 1000 V; skimmer 65 V; fragmentor 140 V; collision-induced dissociation energies 10 and 30 V.
Figure 2. Chemical structures of the compounds tentatively labeled in the *A. campestris* subsp. *lednicensis* aerial part extracts.
Figure 3. Clustered image map (red color: high content; blue color: low content) on the phytochemical dataset of the *A. campestris* subsp. *lednicensis* aerial part extracts.

2.3. Antioxidant Properties

The antioxidant activities of plants are an important marker to evaluate their pharmacological properties. This fact could provide valuable information on their defense abilities against free radical attacks. Because the term antioxidant reflects a broad spectrum of chemical effects, a singular assay does not suffice to unveil the antioxidant picture of a plant extract. Therefore, various tests with different mechanisms must be performed in phytochemical studies. With this in mind, we performed several assays to determine the antioxidant properties of *Artemisia campestris* subsp. *lednicensis* extracts and the results are shown in Table 3. These assays were classified according to the radical scavenging (ABTS and DPPH), reducing power (FRAP, CUPRAC, and PBD), and metal chelating effects. In both radical scavenging assays, the highest activity was found for the methanol extract (DPPH: 213.68 mg TE/g; ABTS: 356.35 mg TE/g), followed by the hydroalcoholic, water, dichloromethane, and hexane extracts. However, the ABTS scavenging abilities of the hydroalcoholic and water extracts did not differ statistically (*p* > 0.05). The electron-donation abilities of antioxidant compounds, namely reducing power, is one of the most relevant markers in their antioxidant mechanism. Thus, we tested the conversion of Cu$^{2+}$ to Cu$^{+}$ in the CUPRAC assay in the presence of antioxidant compounds as well as Fe$^{3+}$ to Fe$^{2+}$ in the FRAP assay. In the reducing power assays, the most active extract was methanol with values of 311.21 mg TE/g (in CUPRAC assay) and 202.34 mg TE/g (in FRAP assay). In both reducing power assays, the hexane extract displayed the weakest abilities with values of 38.56 mg TE/g in the CUPRAC and 21.68 mg TE/g in the FRAP assay, respectively. As presented in Table 3, in both radical scavenging and reducing power assays, the *Artemisia* extracts showed a similar decreasing pattern of activity. Therefore, we deduced that the same compounds might play a role in these assays.
To determine the connection, a Pearson correlation of the chemical profiles and biological abilities was undertaken. As depicted in Figure 4, it was observed that the FRAP, CUPRAC, and ABTS activities varied positively depending on the concentration of syringoylquinic acid, tracheloside, esculetin-O-hexoside, quercetin-O-deoxyhexoside-O-hexoside, and hydroxytrimethoxylavone. Syringoylquinic acid, quercetin-O-deoxyhexoside-O-hexoside, and tracheloside appeared to be involved in DPPH scavenging activity. In addition, galloallocatein played a potent role in ABTS scavenging activity. Consistent with our findings, these compounds were labeled as natural antioxidants in previous studies [35,37–40]. Additionally, a significant correlation between the total bioactive constituents and antioxidant effects, particularly free radical scavenging and reducing power, has been reported in several studies on members of the Artemisia genus [41–43].

The phosphomolybdenum (PBD) method is also considered as a reducing power assay based on the reduction of Mo(VI) to Mo(V) by antioxidants in an acidic environment. However, this test assesses the total antioxidant capacity as not only phenolic, but also non-phenolic (tocopherol, ascorbic acid) antioxidants can act as reducing agents. Contrary to radical scavenging and reducing power assays, in the PBD method, the best activity was detected for the dichloromethane extract, with 2.11 mmol TE/g. In addition, the other three extracts (hexane, methanol, and hydroalcoholic) showed similar efficacy ($p > 0.05$). In the correlation analysis, the presence of some compounds (eriodictyol, tetrahydroxydimethoxyflavone, dihydroxydimethoxyflavone I, hydroxytrimethoxyflavone II, cnicin, and both fatty acid compounds (hydroxyoctadecatrienoic acid, hydroxyoctadecadienoic acid) could be assigned to the observed PBD activity. Finally, in our study, the chelation of transition metals, which relates to the hampering of hydroxyl radical production, was measured. As seen in Table 3, the strongest metal chelating abilities were provided by the hydroalcoholic (22.25 mg EDTAE/g) and water (21.61 mg EDTAE/g) extracts. Other extracts, namely hexane, dichloromethane, and methanol had similar metal chelating effects ($p > 0.05$). In the correlation analysis, several compounds such as quercetin-O-deoxyhexoside-O-hexoside, esculetin-O-hexoside and hydroxytrimethoxylavone were related to the observed metal chelating activities. These compounds have also been described in the literature as metal chelating agents [44–48]. Taken together, *A. campestris* subsp. *lednicensis* might be considered as a source of health-promoting compounds with potential use in the development of functional ingredients.

**Table 3.** The antioxidant activity of *Artemisia campestris* subsp. *lednicensis* extracts.

<table>
<thead>
<tr>
<th>Sample</th>
<th>DPPH (mg TE/g)</th>
<th>ABTS (mg TE/g)</th>
<th>CUPRAC (mg TE/g)</th>
<th>FRAP (mg TE/g)</th>
<th>Metal Chelating (mg EDTAE/g)</th>
<th>Phosphomolybdenum (mmol TE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL-HE</td>
<td>0.71 ± 0.07</td>
<td>20.57 ± 1.06</td>
<td>38.56 ± 0.62</td>
<td>21.68 ± 0.51</td>
<td>13.64 ± 1.52</td>
<td>1.46 ± 0.13</td>
</tr>
<tr>
<td>AL-DE</td>
<td>7.63 ± 0.49</td>
<td>44.96 ± 1.95</td>
<td>44.00 ± 0.94</td>
<td>25.04 ± 0.77</td>
<td>12.88 ± 0.99</td>
<td>2.11 ± 0.11</td>
</tr>
<tr>
<td>AL-ME</td>
<td>213.68 ± 7.30</td>
<td>356.35 ± 9.46</td>
<td>311.21 ± 3.66</td>
<td>202.34 ± 3.26</td>
<td>13.35 ± 0.33</td>
<td>1.42 ± 0.02</td>
</tr>
<tr>
<td>AL-MWE</td>
<td>61.74 ± 0.12</td>
<td>152.40 ± 0.21</td>
<td>275.79 ± 0.47</td>
<td>166.59 ± 3.85</td>
<td>22.25 ± 0.24</td>
<td>1.41 ± 0.06</td>
</tr>
<tr>
<td>AL-WE</td>
<td>58.78 ± 0.09</td>
<td>151.76 ± 0.15</td>
<td>143.32 ± 1.08</td>
<td>91.93 ± 0.30</td>
<td>21.61 ± 0.34</td>
<td>0.92 ± 0.01</td>
</tr>
</tbody>
</table>

Results are given as the mean ± standard deviation of three replicates; significant differences in the investigated samples ($p < 0.05$) are indicated by different letters within columns. AL-HE, *A. campestris* subsp. *lednicensis* hexane extract; AL-DE, *A. campestris* subsp. *lednicensis* dichloromethane extract; AL-ME, *A. campestris* subsp. *lednicensis* methanol extract; AL-MWE, *A. campestris* subsp. *lednicensis* 50% methanol extract; AL-WE, *A. campestris* subsp. *lednicensis* water extract; EDTAE, EDTA equivalents; TE, trolox equivalents.
2.4. Enzyme Inhibitory Properties

In the last decade, the term enzyme inhibition has gained scientific interest. With the increase in the human population, people need effective therapeutic strategies against serious human ailments called “global health diseases”. For example, the prevalence of Alzheimer’s disease increased from 20.2 million in 1990 to 43.8 million in 2006 [49]; a similar trend was also observed in the case of diabetes mellitus [50]. Considering this information, enzymes are believed to be the cornerstone in alleviating such diseases. Several key enzymes that are frequently screened in the enzyme inhibition assays include cholinesterase for Alzheimer’s, amylase for diabetes, and lipase for obesity. In this respect, several inhibitors have been chemically designed, but many of them exhibit various side effects [51,52]. Thus, safe and effective inhibitors derived from natural sources represent a promising research direction. In the current work, the enzyme inhibitory abilities of *A. campestris* subsp. *lednicensis* extracts against cholinesterase, tyrosinase, amylase, and glucosidase were assessed. The obtained results are presented in Table 4. The strongest AChE inhibitory ability was observed for dichloromethane extract (3.64 mg GALAE/g), followed by hexane (3.26 mg GALAE/g), methanol (2.66 mg GALAE/g), hydroalcoholic (1.21 mg GALAE/g), and water extracts (0.15 mg GALAE/g). Regarding the BChE inhibitory effect, two extracts (hexane and dichloromethane) were active against the enzyme, while the polar extracts showed no inhibitory activity. In the correlation analysis, eriodictyol and hydroxytrimethoxyflavone II strongly correlated with the observed anti-cholinesterase inhibitory activity (Figure 5). Our results also agree with the data reported by Uddin et al. [53] and Uriarte-Pueyo and Calvo [54]. Tyrosinase plays a key role in the synthesis of melanin and is therefore a checkpoint in the treatment of hyperpigmentation [55]. The highest tyrosinase inhibitory activity was displayed by the methanol extract (48.60 mg KAE/g), while the weakest was shown for the water extract (18.62 mg KAE/g) (Table 4). As depicted in Figure 4, flavone derivatives were strongly correlated with the demonstrated tyrosinase inhibitory effects. These data are also supported by findings from several studies that reported significant tyrosinase inhibitory abilities of flavonoids and their derivatives [56,57]. In both the amylase and glucosidase inhibition assays, the dichloromethane extract was the most active, followed by the hexane, methanol, hydroalcoholic,
and water extracts (Table 4). The observed anti-amylase and anti-glucosidase abilities could be attributed to the presence of several compounds, among which special attention must be paid to eriodictyol. In fact, literature data support the potential of eriodictyol in the treatment of diabetes due to its insulin secretagogue properties [38].

**Table 4.** Enzyme inhibitory activity of *Artenisia campestris* subsp. *lednicensis* extracts.

<table>
<thead>
<tr>
<th>Sample</th>
<th>AChE (mg GALAE/g)</th>
<th>BCHE (mg GALAE/g)</th>
<th>Tyrosinase (mg KAE/g)</th>
<th>Amylase (mmol ACAE/g)</th>
<th>Glucosidase (mmol ACAE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL-HE</td>
<td>3.26 ± 0.06</td>
<td>3.18 ± 0.06</td>
<td>35.89 ± 2.11</td>
<td>0.33 ± 0.01</td>
<td>2.16 ± 0.03</td>
</tr>
<tr>
<td>AL-DE</td>
<td>3.64 ± 0.09</td>
<td>2.82 ± 0.15</td>
<td>41.53 ± 0.34</td>
<td>0.38 ± 0.02</td>
<td>2.21 ± 0.02</td>
</tr>
<tr>
<td>AL-ME</td>
<td>2.66 ± 0.08</td>
<td>n.a.</td>
<td>48.60 ± 0.67</td>
<td>0.29 ± 0.02</td>
<td>2.06 ± 0.13</td>
</tr>
<tr>
<td>AL-MWE</td>
<td>1.21 ± 0.02</td>
<td>n.a.</td>
<td>40.38 ± 0.48</td>
<td>0.20 ± 0.01</td>
<td>0.88 ± 0.02</td>
</tr>
<tr>
<td>AL-WE</td>
<td>0.15 ± 0.05</td>
<td>n.a.</td>
<td>18.62 ± 1.28</td>
<td>0.05 ± 0.00</td>
<td>0.43 ± 0.03</td>
</tr>
</tbody>
</table>

Results are given as the mean ± standard deviation of three replicates; significant differences in the investigated samples (p < 0.05) are indicated by different letters within columns. ACAE, acarbose equivalents; AL-HE, *A. campestris* subsp. *lednicensis* hexane extract; AL-DE, *A. campestris* subsp. *lednicensis* dichloromethane extract; AL-ME, *A. campestris* subsp. *lednicensis* methanol extract; AL-MWE, *A. campestris* subsp. *lednicensis* 50% methanol extract; AL-WE, *A. campestris* subsp. *lednicensis* water extract; GALAE, galanthamine equivalents; KAE, kojic acid equivalents; n.a., not active.

![Figure 5. Pearson correlation between enzyme inhibitory activity and individual phytochemicals.](image)

**2.5. Multivariate Analysis**

PCA is a common statistical tool to analyze compositional data in biopharmaceutical studies [59]. In this study, PCA was used to test the similarity of extraction solvents in terms of their antioxidant and enzyme inhibition activity. First, the number of significant dimensions was obtained based on the Kaiser criterion [60]. Accordingly, PCA showed that about 92% of the data variation could be explained by a total of two dimensions with proportions of 57.9% (Dim1) and 34.3% (Dim2) (Figure 6A). The remaining dimensions made very small contributions and were not retained for the following analysis. From the data in Figure 6B, it can be shown that dimension 1 (Dim1) predominantly discriminated the samples for both neurodegenerative and diabetes-related enzymes. In contrast, dimension 2 (Dim2) differentiated the samples according to their potential to inhibit the tyrosinase enzyme and scavenge DPPH and ABTS radicals. After describing the dimensions, a scatterplot of Dim1 vs. Dim2 was examined (Figure 6C). The extracts obtained
with non-polar solvents (dichloromethane and hexane) were separated from the polar solvents (methanol, hydroalcoholic, water) along the first dimension. The dichloromethane and hexane extracts were very close and showed remarkable enzyme inhibitory activity. In contrast, the three polar solvent extracts were clearly separated from each other. Methanol extracts had excellent antioxidant activity, followed by the hydroalcoholic and water extracts.

![Figure 6](image)

**Figure 6.** Principal component analysis on the biological activities of the *A. campestris* subsp. *lednicensis* aerial part extracts. (A) Eigenvalue and percentages of the explained variances of each dimension. (B) Contribution of variables on the dimensions of PCA. (C) Scatter plot showing the distribution of the samples in the two retained dimensions.

2.6. Inhibitory Activity on the Cytokine Secretion

Neutrophils play a significant role in the initiation, progression, and resolution of the inflammatory response in the human body. Biofunctional ingredients endowed with both antioxidant and anti-inflammatory properties were prompted to reduce the risks of modern lifestyle diseases [61,62]. Considering that the *A. campestris* subsp. *lednicensis* extracts acted as significant antioxidant and anti-enzymatic effectors, we aimed to assess their influence on the inflammatory response. Thus, the neutrophils derived from healthy volunteers were stimulated with LPS, followed by the measurement of key pro-inflammatory cytokine (IL-1β, TNF-α and IL-8) levels in the presence of *Artemisia* extracts. Preliminary studies showed that over the tested concentration range (5–100 μg/mL), most samples exhibited no cytotoxicity toward human neutrophils (cell viabilities >96.38%), except for the hydroalcoholic and water extracts, which slightly altered their viability (88.74% and 91.87%, respectively, at 100 μg/mL) but still not significantly when compared to the non-stimulated control (Figure 7).
Neutrophils showed good viability and LPS stimulation promoted the secretion of functionally pro-inflammatory cytokines (e.g., TNF-α, IL-1β, and IL-8) (Figure 6). Interleukin-1β is the main pyrogenic molecule produced by leucocytes in response to noxious stimuli and promotes the secretion of adhesion molecules and thrombogenic mediators as well as the production of pro-inflammatory and tissue-remodeling enzymes [63]. After its release by neutrophils and macrophages, IL-8 acts as a chemotactic agent for basophils and lymphocytes and promotes their degranulation, followed by endothelial adhesion [61]. Synthetized in neutrophils, macrophages, natural killers, and mast cells, TNF-α induces apoptosis and modulates the expression of stress-activated protein kinases and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [64]. Thus, the suppression of IL-1β, IL-8, and TNF-α could be targeted in both acute and chronic inflammatory processes.

Our study showed that A. campestris subsp. lednicensis extracts inhibited, with different degrees of potency, the release of cytokines in LPS-stimulated neutrophils. Except for the dichloromethane extract, all samples significantly inhibited the secretion of TNF-α (Figure 8). At 100 μg/mL, the following decreasing order of inhibition was observed: methanol extract (29.05% of LPS+ control) > hydroalcoholic extract (35.86% of LPS+ control) > hexane extract (45.98% of LPS+ control) > water extract (53.08% of LPS+ control). One must note that the effects displayed by the methanol extract were comparable to those of the positive control dexamethasone at 1 μM (25.30% of LPS + control). The investigated samples did not interfere with the production of IL-1β and IL-8 (Figures 8 and 9). Our results are in agreement with previous in vitro and in vivo studies that reported on the ability of Artemisia species to impair the release of pro-inflammatory cytokines (e.g., TNF-α, IL-1, IL-6, IL-8, IL-11) [14,65]. Moreover, sesquiterpene lactones are acknowledged to act as anti-inflammatory and immunomodulatory effectors and their underlying mechanism relates to the inhibition of the NF-κB pathway [66].

As depicted in Figures 9 and 10, the investigated Artemisia extracts tended to induce the overproduction of IL-1β and IL-8 in the LPS-stimulated neutrophils. Therefore, A. campestris subsp. lednicensis extracts might not protect neutrophils from the deleterious effects
of these mediators, which are involved in the initiation and progression of the inflammation process. Moreover, the overproduction of such pro-inflammatory cytokines or the “inflammatory cascades” is the first response of host defense in the case of invading pathogens [67]. Thereby, phytoconstituents from the investigated Artemisia extracts could act as anti-infectious agents and consequently, an assessment of their antimicrobial was further undertaken.

**Figure 8.** The effects of Artemisia campestris subsp. lednicensis extracts (5–100 μg/mL) and dexamethasone (Dex) (0.01–1 μM) on TNF-α release in LPS-stimulated (100 ng/mL) neutrophils. Results are presented as the mean ± SEM of three separate experiments performed with cells isolated from six independent donors; # p < 0.001 vs. non-stimulated control (LPS-); * p < 0.05; ** p < 0.001 vs. stimulated control (LPS+). Abbreviations: AL-HE, A. campestris subsp. lednicensis hexane extract; AL-DE, A. campestris subsp. lednicensis dichloromethane extract; AL-ME, A. campestris subsp. lednicensis methanol extract; AL-MWE, A. campestris subsp. lednicensis 50% methanol extract; AL-WE, A. campestris subsp. lednicensis water extract; LPS, lipopolysaccharide.

**Figure 9.** The effects of Artemisia campestris subsp. lednicensis extracts (5–100 μg/mL) and dexamethasone (Dex) (0.01–1 μM) on IL-1β release in LPS-stimulated (100 ng/mL) neutrophils. Results are presented as the mean ± SEM of three separate experiments performed with cells isolated from six independent donors; # p < 0.001 vs. non-stimulated control (LPS-); * p < 0.05; ** p < 0.001 vs. stimulated control (LPS+). Abbreviations: AL-HE, A. campestris subsp. lednicensis hexane extract; AL-DE,
A. campestris subsp. lednicensis dichloromethane extract; AL-ME, A. campestris subsp. lednicensis methanol extract; AL-MWE, A. campestris subsp. lednicensis 50% methanol extract; AL-WE, A. campestris subsp. lednicensis water extracts; LPS, lipopolysaccharide.

**Figure 10.** The effects of Artemisia campestris subsp. lednicensis extracts (5–100 μg/mL) and dexamethasone (Dex) (0.01–1 μM) on IL-8 production in LPS-stimulated (100 ng/mL) neutrophils. Results are presented as the mean ± SEM of three separate experiments performed with cells isolated from six independent donors; # p < 0.001 vs. non-stimulated control (LPS-); * p < 0.05; ** p < 0.001 vs. stimulated control (LPS+). Abbreviations: AL-HE, A. campestris subsp. lednicensis hexane extract; AL-DE, A. campestris subsp. lednicensis dichloromethane extract; AL-ME, A. campestris subsp. lednicensis methanol extract; AL-MWE, A. campestris subsp. lednicensis 50% methanol extract; AL-WE, A. campestris subsp. lednicensis water extract; LPS, lipopolysaccharide.

2.7. Antimicrobial Properties

The results of the anti-inflammatory screening prompted us to explore the putative antimicrobial properties of A. campestris subsp. lednicensis extracts. The criteria proposed by de Oliveira Lima et al. [68] were used to rank the antimicrobial screening results: strong activity (MIC 50–500 mg/L), moderate activity (500 mg/L > MIC < 1500 mg/L), and weak activity (MIC >1500 mg/L). Overall, the investigated Artemisia extracts showed weak activity toward the tested strains (Table 5). H. pylori was the most sensitive, with the hexane, hydroalcoholic and water extracts displaying a moderate activity toward this Gram-negative bacterium (MIC = 625 mg/L). The anti-H. pylori activity of Artemisia species was previously reported. The water extract of A. douglasiana and its main constituent dehydroelecodeine, a guaianolide-type sesquiterpene lactone, displayed significant inhibitory effects against the reference and clinical isolates of H. pylori, with MIC values of 1–8 mg/L and 60–120 mg/L, respectively [69]. In addition, an aqueous extract of A. ludoviciana subsp. mexicana was shown to exert anti-H. pylori properties (MIC = 250 mg/L). Further fractionation of the extract afforded the isolation of the sesquiterpene lactone estafiatin and polymethoxylated flavone eupatilin, which were the main bioactive constituents responsible for the inhibition of H. pylori (MIC values of 15.6 and 31.2 mg/L, respectively) [70]. In the case of A. campestris subsp. lednicensis, the LC-MS/MS analysis annotated the presence of both sesquiterpenes and polyhydroxy- and poly-methoxylated flavone derivatives in the extracts displaying anti-H. pylori activity. Therefore, we can hypothesize that these constituents might contribute to the observed effects, but the synergistic effects among various classes of compounds identified in the studied extracts (e.g., phenolic acids, flavonoids, sesquiterpenes, coumarins, lignans and fatty acids) cannot be excluded.
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Table 5. Antimicrobial properties of the Artemisia campestris subsp. ledricensis extracts.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>MIC (mg/L)</th>
<th>Positive Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AL-HE</td>
<td>AL-DE</td>
</tr>
<tr>
<td>Staphylococcus aureus ATCC 25923</td>
<td>&gt;5000</td>
<td>5000</td>
</tr>
<tr>
<td>Staphylococcus epidermidis ATCC 12228</td>
<td>&gt;5000</td>
<td>5000</td>
</tr>
<tr>
<td>Micrococcus luteus ATCC 10240</td>
<td>&gt;5000</td>
<td>5000</td>
</tr>
<tr>
<td>Enterococcus faecalis ATCC 29212</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
</tr>
<tr>
<td>Bacillus subtilis ATCC 6633</td>
<td>&gt;5000</td>
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<tr>
<td>Bacillus cereus ATCC 10876</td>
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<tr>
<td><strong>Gram-negative bacteria</strong></td>
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<tr>
<td>Salmonella Typhimurium ATCC 14028</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922</td>
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<td>Proteus mirabilis ATCC 12453</td>
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<tr>
<td>Klebsiella pneumoniae ATCC 13883</td>
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</tr>
<tr>
<td>Pseudomonas aeruginosa ATCC 90271</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
</tr>
<tr>
<td>Helicobacter pylori ATCC 43504</td>
<td>625</td>
<td>2500</td>
</tr>
<tr>
<td><strong>Yeasts</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida albicans ATCC 2091</td>
<td>5000</td>
<td>5000</td>
</tr>
<tr>
<td>Candida parapsilosis ATCC 22019</td>
<td>5000</td>
<td>5000</td>
</tr>
<tr>
<td>Candida glabrata ATCC 90030</td>
<td>10,000</td>
<td>10,000</td>
</tr>
<tr>
<td><strong>Dermatophytes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trichophyton rubrum ATCC 28188</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes ATCC 9533</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

AL-HE, A. campestris subsp. ledricensis hexane extract; AL-DE, A. campestris subsp. ledricensis dichloromethane extract; AL-ME, A. campestris subsp. ledricensis methanol extract; AL-MWE, A. campestris subsp. ledricensis 50% methanol extract; AL-WE, A. campestris subsp. ledricensis water extract; MIC, minimum inhibitory concentration.

3. Materials and Methods
3.1. Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 5,5-dithio-bis(2-nitrobenzoic acid (DTNB), α-glucosidase (EC. 3.2.1.20, from Saccharomyces cerevisiae), α-amylose (EC. 3.2.1.1, from porcine pancreas), acarbose, acetylthiocholine iodide (ATCh), ammonium acetate, ammonium molybdate, butyrylthiocholine chloride (BTCh), cupric chloride, dichloromethane, electric eel acetylcholinesterase (AChE) (type-VI-S, EC 3.1.1.7), ethylene-diaminetetraacetate (EDTA), ferric chloride, ferrozine, ferrous sulfate hexahydrate, Folin-Ciocalteu reagent, galantamine, gallic acid, hexane, horse serum butyrylcholinesterase (BChE) (EC 3.1.1.8), hydrochloric acid, kojic acid, lipopolysaccharide (LPS, from Escherichia coli 0111:B4), methanol, neocuproine, rutin, sodium hydroxide, sodium molybdate, sodium nitrate, sodium carbonate, trolox, and tyrosinase (EC1.14.18.1, mushroom) were obtained from Merck KGaA (Darmstadt, Germany). Citric acid, glucose for citrate dextrose solution (ACD), and sodium citrate tribasic dihydrate were purchased from Chem-pur (Piekary Ślaskie, Poland). Chloramphenicol, cycloheximide, glucose, RPMI 1640 medium, and terbinafine hydrochloride were acquired from Sigma-Aldrich (Steinheim, Germany). Dextran from Leuconostoc mesenteroides, propidium iodide (PI), dexamethasone (Dex), Triton X-100, and amphotericin B were bought from Sigma-Aldrich (Saint Louis, MO, USA). Fetal bovine serum (FBS), RPMI 1640 enriched by 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineneethanesulfonic acid), and 2 mM L-glutamine as well as penicillin-streptomycin were obtained from Biowest (Nauillé, France). Pancoll Human (P04-601000) was purchased from PAN-Biotech (Aidenbach, Germany). Liquid chromatography (LC) grade acetonitrile, formic acid, and water were obtained from Merck KGaA (Darmstadt,
Germany). Phosphate buffered saline (PBS) was purchased from Gibco (Gibco, HK, China), whereas the calcium-free PBS was from Biomed (Lublin, Poland). Buffy coats, used for neutrophil isolation, were provided by the Warsaw Blood Donation Centre (Warsaw, Poland). Human ELISA sets (TNF-α, IL-1β, IL-8) were bought from BD Biosciences (Erembodegem, Belgium).

3.2. Plant Material and Extraction

The aerial parts of Artemisia campestris subsp. lednicensis were collected during September 2020 from Aroneanu Lake area, Iasi county, Romania (GPS coordinates 47.203117, 27.593671). The plant material was authenticated by Dr. Constantin Mardari and Dr. Adrian Oprea, Botanic Garden “Anastasie Fatu”, Iasi, Romania. A voucher specimen (ACL/2021) was deposited in the Department of Pharmacognosy, “Grigore T. Popa” University of Medicine and Pharmacy Iasi (Romania). The aerial parts were dried, ground, and then 5 g were separately extracted with solvents of different polarities (hexane, dichloromethane, methanol, 50% methanol, and water) by ultrasonication (three cycles of 30 min. each, at room temperature). The extracts were evaporated to dryness under vacuum (the obtained yields are shown in Table 1) and kept at -20 °C until subsequent experiments.

3.3. Phytochemical Analysis

The total phenolic and flavonoid content were evaluated using the Folin–Ciocalteu and AlCl₃ tests, respectively [71]. Results were expressed as gallic acid equivalents (mg GAE/g dry extract) and rutin equivalents (mg RE/g dry extract) for these assays. The LC-HRMS/MS analysis was performed following the methodology extensively described in Trifan et al. [18].

3.4. Antioxidant Assays

Antioxidant assays were performed using previously described methods [72,73]. The antioxidant potential was reported as follows: mg Trolox equivalents (TE)/g extract in the DPPH and ABTS radicals scavenging, CUPRAC, FRAP, and MCA tests; mmol TE/g extract in the PBD assay.

3.5. Enzyme Inhibitory Assays

The enzyme inhibition experiments were performed following the previously described methodologies [72,73]. Amylase and glucosidase inhibition were expressed as mmol acarbose equivalents (ACAE)/g extract, while AChE and BChE inhibition was expressed as mg galanthamine equivalents (GALAE)/g extract. Tyrosinase inhibition was expressed as mg kojic acid equivalents (KAE)/g extract.

3.6. Cytokine Secretion in Human Neutrophils

The influence of the tested samples on cytokine secretion was assessed in human neutrophils using a methodology previously described [61]. Briefly, neutrophils were isolated by dextran sedimentation and centrifugation in a Panoll gradient, providing the neutrophil preparation (purity >97%). The A. campestris subsp. lednicensis extracts (5, 20, and 100 μg/mL) were added 30 min before stimulation with LPS (100 ng/mL). Neutrophils (2 × 10⁶/mL) were co-incubated with extracts for 18 h in RPMI 1640 medium supplemented with 10% FBS and 1% penicillin-streptomycin. Then, the neutrophils were centrifuged and washed twice with calcium-free PBS, re-suspended in PI solution (0.5 μg/mL), and followed by incubation (15 min in the dark, at room temperature). Within one hour, the neutrophils were analyzed by flow cytometry (BD FACSVerse, BD Biosciences, San Jose, CA, USA). The viability of neutrophils was calculated as % = 100% – %PI+ cells; Triton X (0.1%) was the positive control. The release of cytokines (IL-1β, IL-8, and TNF-α) was evaluated by ELISA using a Synergy 4 microplate reader (BioTek, Winooski, VT, USA), following
the manufacturer’s indications. The secretion of cytokines was expressed as a percentage of the cytokines released into the supernatant compared to the LPS+ stimulated cells; dexamethasone (0.01, 0.1, and 1 μM) was used as the positive control.

3.7. Antimicrobial Assays

The antimicrobial assays were performed by the microdilution method according to the European Committee on Antimicrobial Susceptibility Testing [74, 75]. Mueller-Hinton broth and MH broth with 7% lysed horse blood were used for the growth of non-fastidious bacteria and H. pylori, respectively, whereas MH broth with 2% glucose was used for the growth of yeasts. RPMI 1640 medium 2% glucose buffered with 0.165 M MOPS and supplemented with cycloheximide 300 mg/L and chloramphenicol 50 mg/L was utilized for the growth of dermatophytes [74, 75]. Minimum inhibitory concentrations (MIC) of the samples were determined in Gram-positive bacteria (Staphylococcus aureus ATCC 25923, S. epidermidis ATCC 12228, Micrococcus luteus ATCC 10240, Enterococcus faecalis ATCC 29212, Bacillus subtilis ATCC 6633, and B. cereus ATCC 10876); Gram-negative bacteria (Salmonella Typhimurium ATCC 14028, Escherichia coli ATCC 25922, Proteus mirabilis ATCC 12453, Klebsiella pneumoniae ATCC 13883, Pseudomonas aeruginosa ATCC 90271, and Helicobacter pylori ATCC 43504); yeasts (Candida albicans ATCC 2091, C. glabrata ATCC 90030, and C. parapsilosis ATCC 22019) and dermatophytes (Trichophyton rubrum ATCC 28188 and T. mentagrophytes ATCC 9533). All experiments were performed in triplicate.

3.8. Statistical Analysis

Antioxidant and enzyme inhibitory experiments were performed in triplicate, with the data presented as the mean ± standard deviation. The phytochemical dataset was logarithm transformed, scaled, and submitted to clustered image maps (CIMs). For CIMs, “Ward’s rule” and “Euclidean distance” were used. Then, the antioxidant and anti-enzymatic activities dataset was also scaled, centered, and submitted to the principal component analysis (PCA). Then, the relationship between phytochemical and antioxidant/anti-enzymatic activities was evaluated by calculating the Pearson correlation coefficient. A Pearson’s coefficient greater than 0.7 was considered significant. CIMs, PCA, and correlation analysis were conducted under R v 4.1.2 software. The evaluation of neutrophil viability and cytokine release was determined in three independent experiments performed with cells isolated from six separate donors. Data were expressed as the mean ± standard error of the mean. Statistical analysis was evaluated by one-way ANOVA, with Tukey’s and Dunnett’s multiple comparison tests. If necessary, non-parametric methods such as the Mann–Whitney test were employed; p < 0.05 was considered significant. Statistica 13 software (TIBCO Software Inc., Palo Alto, CA, USA) was employed.

4. Conclusions

We report herein for the first time a comprehensive metabolite and biological profiling of Artemisia campestris subsp. lednicensis aerial part extracts. By using a LC-HRMS/MS-based platform, 21 specialized metabolites belonging mostly to polyphenolic compounds were annotated such as twelve flavonoids (mostly polyhydroxy-/poly-methoxylated flavones), three phenolic acids (quinic acid derivatives), two sesquiterpene lactones, two fatty acids, one coumarin, and one lignan. PCA revealed that polar extracts (in particular the methanol extract) were more active than the non-polar extracts in the antioxidant assays (radical scavenging, metal reducing, chelating, and phosphomolybdenum). In contrast, the non-polar extracts exhibited higher anti-enzymatic activity compared to the polar extracts against key enzymes targeted in the management of chronic diseases such as Alzheimer’s (acetylcholinesterase, butyrylcholinesterase), type 2 diabetes mellitus (amylase, glucosidase), and skin disorders (tyrosinase). Furthermore, the Artemisia extracts
were shown to downregulate the release of TNF-α in the LPS-stimulated human neutrophil model. Finally, moderate anti-\(H.\) pylori effects were displayed by the investigated samples.

Our study reveals \(A.\) campestris subsp. \textit{lednicensis} as a valuable source of compounds endowed with important biological activities and brings additional data that support the pleiotropic pharmacology of the \textit{Artemisia} genus. In conclusion, the obtained results represent a starting point in the further development of \(A.\) campestris subsp. \textit{lednicensis} extracts as nutraceutical, cosmeceutical, and pharmaceutical ingredients addressing modern life-related diseases.


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**Data Availability Statement:** Not applicable.

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**Conflicts of Interest:** The authors declare no conflict of interest.

**References**


