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Chemical Features and Bioactivities of *Lactuca canadensis* L., an Unconventional Food Plant from Brazilian Cerrado

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Abstract: Throughout the world, people's diet is generally quite restricted regarding the variety of plants used in their daily regime. The Unconventional Food Plant (UFP) *Lactuca canadensis* L. is an edible species of wild lettuce sparsely described in literature and considered to be native from the eastern and central parts of North America. To valorize this species as potential alternative food, an analysis of its nutritional, chemical, and bioactive properties was performed. The results specify the occurrence of organic acids, mainly quinic acid (127.9 ± 0.6 g/kg dry weight (dw)), polyunsaturated fatty acids (65.3%), among which are linolenic acid (44.4 ± 0.4 %), and tocopherols, mostly α-tocopherol (61.2 ± 0.7 mg/kg dw). Additionally, eight phenolic compounds were also identified, among which luteolin-*O*-glucuronide was found in larger amounts in both infusion and hydroethanolic extracts (5.46 ± 0.09 and 4.6 ± 0.1 mg/g dw, respectively). Carbohydrates and proteins were the main macronutrients (603 ± 1 and 177.5 ± 0.3 g/kg dw, respectively), followed by ashes (166.5 ± 0.9), indicative of a great amount of minerals. Additionally, good antioxidant and antibacterial activities were detected in the analyzed extracts. In general, our results contribute to extend the range of different, unexploited, and nutritionally balanced plant foods, such as *Lactuca canadensis*, that can and should be included in the daily diet.

Keywords: *Lactuca canadensis* L.; unconventional food plants; wild food relatives; phytochemical characterization; bioactive properties

1. Introduction

Historically, plants have always been present in human's diet and their use has been standardized and guided by practical needs and natural predilections [1]. Despite this assumption, the world's food diet is substantially restricted to 200 plant species, of which only 9 represent 66% of all crop production by weight [2].

The growing demand for quality, quantity, and variety of foods by the consumer, which are not only nutritionally rich but also promote well-being and increase shelf life, has endorsed the investigation of other plants that were not commonly used in the human diet. In this scenario, Unconventional Food Plants (UFPs) emerged as a prospect to spread the consumption of plants in the diet, which can also be applied in the treatment of diseases, as food additives and in the promotion of health, due to the bioactive compounds present in this matrix [3].

UFPs are species characterized by having plants parts with potential inclusion in the diet but that are not commonly used as such; these can be vegetables, fruits, flowers or

herbs that grow spontaneously in nature but, because they are little known, are frequently confused with weeds or "shrubs" [4]. These plants stood out in the fight against hunger, imposing themselves as alternative foods in a balanced diet and that can replace conventional vegetables consumed widely by the general population. Additionally, they were raised as a new food option among communities where the consumption of vegetables is scarce due to the lack of resources [5].

Lettuce (*Lactuca* spp.) is one of the most popular leafy vegetable consumed as a salad in most parts of the world, contributing significantly to a good nutritional intake in the human diet. Since it is generally eaten raw, a greater amount of nutrients is retained by the organism when compared to other cooked or processed vegetables [6]. Some *Lactuca* species have been used as a dietary source and as medicinal plants since old times.

Lactuca canadensis L. is a species of lettuce recently identified [7] and considered to be a native of the eastern and central parts of North America, although its origin is not clear. It is a biennial plant that produces rosettes of leaves in the first year and tall stems in the second, reaching a maximum height of 0.5–2.0 m or more and bearing inflorescences with yellow ligules [8].

In Brazil, *L. canadensis* is popularly known as "almeirão roxo", given the presence of purple ribs in the center of the leaves, and its bitter taste, often found in the *Cichorium* genus and consumed as a salad and in soups [9].

To the best of the authors' knowledge, the present study provides a first report of about this wild lettuce, since the existing data are only of its latex composition in sesquiterpene lactones [8].

Thus, the present study intends to characterize the nutritional and chemical profile of *L. canadensis* leaves, from Góias state, Brazil, as well as the bioactive assets (antioxidant and antimicrobial activities) of its infusion and hydroethanolic extracts, thus providing a first report about the characteristics of this edible plant.

2. Materials and Methods

2.1. Samples

Ten uniform plant samples (100 g) of *L. canadensis* were collected in Orizona (Goiás, Brazil) in September 2019 from the backyards (home gardens) and lyophilized, resulting in 36.9 g, which were used for research purposes. The temperature and humidity averages were 28 °C and 20–30%, respectively. The amount of sunny in the period was 11 h and 45 min per day and the soil type is predominantly oxisol. An example specimen (71051) has been deposited at the Herbarium of the Federal University of Goiás. The fresh leaves were lyophilized (FreeZone 4.5, Labconco, MO, USA) and samples were reduced to fine particles (20 mesh).

2.2. Extract Preparations

Infusion was prepared using 1 g of freeze-dried sample and 100 mL of boiling distilled water. The mixture was leaving to rest for 5 min and then filtrated through Whatman no. 4 paper. The obtained infusion was frozen and lyophilized to get a dehydrated extract.

Hydroethanolic extract was attained extracting 1.5 g of freeze-dried sample by stirring (150 rpm) with ethanol/water (80:20, v/v, at room temperature, for 1 h) and filtered through Whatman no. 4 paper.

The residue was re-extracted and, using a rotary evaporator (Buchi R-210, Flawil, Switzerland), the combined hydroethanolic extracts were evaporated at 40 °C, and further lyophilized.

2.3. Chemical Composition

2.3.1. Nutritional Value

Ash, protein, fat, carbohydrates, and energy were established in the lyophilized plant material according to the AOAC procedures [10]. In brief, the crude protein content ($N \times 5.14$) was assessed by macro-Kjeldahl method, the crude fat content was determined

by Soxhlet extraction with petroleum ether, and the ash content was determined by incineration at 550 ± 10 °C. Total carbohydrates content was calculated by difference: Total carbohydrates = 100 − (g fat + g ash + g proteins).

The results were articulated as g kg^{-1} of dried weight (dw) and the energetic value was estimated giving to the Atwater system through the formula: Energy (kcal kg^{-1} dw) = 4 × (g proteins + g carbohydrates) + 9 × (g fat).

2.3.2. Free Sugars

Soluble free sugars were determined according to a formerly described procedure [11] on the lyophilized plant material. The investigation was achieved using a high-performance liquid chromatography (HPLC) system coupled with a refraction index detector and compounds identified and quantified by contrast with genuine standards and by the internal standard (IS) method using melezitose (Sigma Chemical Co., St. Louis, MO, USA), respectively. Results were treated in a Clarity Software and expressed in g kg^{-1} of dw.

2.3.3. Organic Acids

Organic acids were determined by ultra-fast liquid chromatography (UFLC) coupled to a photodiode array detector (PDA), operating in the conditions previously described [12]. The compounds were identified and quantified by comparing the area of the sample' peaks recorded at 215 nm with calibration curves obtained from commercial standards (Sigma-Aldrich, St. Louis, MO, USA). The results were recorded and processed using LabSolutions Multi LC-PDA software and were expressed in g kg^{-1} of dw.

2.3.4. Fatty Acids

Fatty acid methyl esters (FAME) were verified by gas-liquid chromatography (GC) with flame ionization detection (FID), using a YOUNG IN Crhomass 6500 GC System [10]. Fatty acids identification and quantification were done by relating the comparative retention times of FAME peaks from samples with standards (standard mixture 47885-U; Sigma Chemical Co., St. Louis, MO, USA), the results were logged and processed using the Software Clarity DataApex 4.0 Software and stated in relative percentage of each fatty acid.

2.3.5. Tocopherols

From the lyophilized plant material, tocopherols were determined following a procedure before described by Spréa et al. [11] using a HPLC system coupled to a fluorescence detector and using the IS (tocol, Matreya, Pleasant Gap, PA, USA) method for quantification. The results were logged and processed using Clarity 2.4 software and the results were expressed as mg kg^{-1} of dw.

2.4. HPLC-DAD-ESI/MS^n Analysis of Phenolic Compounds

Phenolic compounds were determined in the infusion preparations and hydroethanolic extracts, which were re-dissolved in 2 mL of water and ethanol/water (80:20, v/v), respectively, to a final concentration of 10 mg mL^{-1}. The resulting extracts were then analysed for their phenolic composition by HPLC coupled with to a diode-array detector and mass spectrometry with electrospray ionization (HPLC-DAD-ESI-MS/MS) working with the settings defined and optimized by Bessada et al. [13].

Phenolic compounds identification was performed by comparison the retention time, UV–Vis, and mass spectra with existing standard compounds (Extrasynthese, Genay, France) and with available data described in the literature. For quantitative analysis, a 7-level calibration curve for each available standard phenolic compound was created based on the UV signal. When the commercial standard was not available, the quantification was executed through the calibration curve of the most similar available standard compound. The results were expressed in mg g^{-1} of extract.

2.5. Bioactive Properties

2.5.1. Antioxidant Activity Evaluation

For the thiobarbituric acid reactive substances (TBARS) assays, the infusion preparations and hydroethanolic extracts were dissolved in water and ethanol/water (80/20, *v*/*v*), respectively, and subjected to dilutions from 5 mg/mL to 0.15625 mg mL^{-1}. Porcine (*Sus scrofa*) brain homogenates were used to obtain lipid peroxidation inhibition and were evaluated by the decline in TBARS formation. The color intensity of the malondialdehyde–thiobarbituric acid (MDA–TBA) complex was measured by its absorbance at 532 nm [14]. The results were expressed in EC_{50} values (mg mL^{-1}, sample concentration providing 50% of antioxidant activity).

The antihaemolytic potential of the lyophilized infusion and hydroethanolic extracts redissolved in PBS (0.005–4 mg mL^{-1}) was assessed through the oxidative haemolysis inhibition assay (OxHLIA), as mentioned in detailed Lockowandt et al. [15]. The results were expressed as IC_{50} values (μg mL^{-1}) for Δt of 60 and 120 min (extract concentration required to keep 50% of the erythrocyte population intact in the time mentioned). Trolox (3.91–125 μg mL^{-1}; Sigma Chemical Co., St. Louis, MO, USA) was used as positive control in both assays.

2.5.2. Antibacterial Activity

The antibacterial activity of the infusion preparations and hydroethanolic extracts was determined following the microdilution method described by Pires et al. [16]. The evaluated microorganisms are Gram-positive (*Enterococcus faecalis*, *Listeria monocytogenes*, and methicillin-resistant *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Morganela morganii*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*). The minimum inhibitory concentration (MIC) was determined based on the reduction of *p*-iodonitrotetrazolium chloride (0.2 mg mL^{-1}; Panreac AppliChem, Barcelona, Spain).

The minimum bactericidal inhibitory concentration (MBC) was evaluated by plating a loopful of the content of the microwells that did not exhibit coloration in the MIC assay. Different antibiotics were used as negative control (ampicillin (Fisher Scientific, Janssen Pharmaceutics NV, Belgium) and imipenem (Hikma Farmacêutica, S.A., Sintra, Portugal) for Gram-negative bacteria, and vancomycin (Hikma Farmacêutica, S.A., Sintra, Portugal) and ampicillin for Gram-positive bacteria). Culture broth (Muller Hinton Broth; Biolab, Budapest, Hungary) added with 5% dimethylsulfoxide (Merck KgaA, Darmstadt, Germany) inoculated with each bacterium was used as a positive control.

2.6. Statistical Analysis

All analyses were performed in triplicate and the results were presented as mean ± standard deviation (except for antibacterial activity). The results were analyzed by a one-way analysis of variance (ANOVA) followed by a Tukey's HSD test, with $\alpha = 0.05$. A Student's *t*-test was applied when two extracts were compared. SPSS v. 23.0 was used in the analysis.

3. Results and Discussion

3.1. Chemical Composition

The results attained for the proximal composition, free soluble sugars and organic acids are presented in Table 1. The analysis of the macronutrients from *L. canadensis* leaves exposed the carbohydrates as the compounds found in greater quantities (603 g kg^{-1} dw), followed by high amounts of protein (177.5 g kg^{-1} dw) and ashes (166.5 g kg^{-1} dw), with the lipids (53.2 g kg^{-1} dw) being the less abundant. The genus *Lactuca* is known for providing dietary fiber, carbohydrates with lower digestibility and minor caloric content. The number of proteins found in *L. canadensis* is significantly higher in contrast with other vegetables, especially lettuces [17]. In fact, the amount noticed can be compared with other UFP, the superfood *Pereskia aculeata* Miller (ora-pro-nobis), whose protein levels in its dry leaves' present values between 19.6 and 25.5% (*w*/*w*) [18].

Three sugars were identified in *L. canadensis*, namely fructose, trehalose and glucose, with a total free sugars of 86 g kg^{-1} dw (Table 1). In addition to other benefits, these monosaccharides may induce several effects on muscle metabolism, presenting benefits associated with exercise performance [19].

Regarding organic acids, three compounds were found, specifically oxalic, quinic and malic acids, where quinic acid is the most abundant (127.9 g kg^{-1} dw) in our sample (Table 1). Papetti et al. [18] revealed the beneficial properties of this molecule in health, once it presents antioxidant potential and, when conjugated with oxalic, succinic, and shikimic acids, can inhibit the virulence of oral pathogens [20].

Other authors also reported that quinic acid contributes to fruit and vegetables characteristic taste [21]. On the other hand, the leaves of *L. canadensis* also present high content of oxalic acid (64.2 g kg^{-1} dw), which is considered an antinutrient that can lead to adverse health effects, such as irritation of the intestinal mucosa, kidney stones and absorption of calcium [22]. Nevertheless, several wild edible species are consumed in small quantities, such as side or mixed vegetable dishes and, in this sense, there is no risk of high ingestion of oxalic acid [23].

Table 1. Nutritional value and hydrophilic compounds of *L. canadensis* (mean ± SD, n = 3).

Nutritional Value (g kg^{-1} dw)	
Ash	166.5 ± 0.9
Protein	177.5 ± 0.3
Lipids	53.2 ± 0.7
Carbohydrates	603 ± 1
Energy (kcal kg^{-1} dw)	3600 ± 1
Free sugars (g kg^{-1} dw)	
Fructose	13.4 ± 0.4
Glucose	47 ± 4
Trehalose	25.2 ± 0.7
Total	86 ± 5
Organic acids (g kg^{-1} dw)	
Oxalic	64.2 ± 0.2
Quinic	127.9 ± 0.6
Malic	34.4 ± 0.1
Total	226.5 ± 0.6

Free sugars calibration curves: fructose (y = 1.04×, r^2 = 0.999, limit of detection (LOD) = 0.05 mg mL^{-1} and limit of quantitation (LOQ) = 0.18 mg mL^{-1}), glucose (y = 0.935×, r^2 = 0.999, LOD = 0.08 mg mL^{-1} and LOQ = 0.25 mg mL^{-1}) and trehalose (y = 0.991×, r^2 = 0.999, LOD = 0.07 mg mL^{-1} and LOQ = 0.24 mg mL^{-1}). Organic acids calibration curves: oxalic acid (y = 9 × 106 × + 377.946, r^2 = 0.994, LOD = 12.55 μg mL^{-1} and LOQ = 41.82 μg/mL); quinic acid (y = 612.327× + 16.563; r^2 = 1, LOD = 24.18 μg mL^{-1}, LOQ = 80.61 μg mL^{-1}) and malic acid (y = 863.548× + 55.591, r^2 = 0.999, LOD = 35.76 μg mL^{-1} and LOQ = 119.18 μg mL^{-1}).

The fatty acids configuration of *L. canadensis* leaves is presented in Table 2, demonstrating a total of 20 compounds, with C18:3n3 (α-linolenic; 44.4%), followed by C16:0 (palmitic acid; 21.3%) and C18:2n6 (linoleic acid; 20.2%) being the major compounds making, consequently, the polyunsaturated fatty acids (PUFAs) the largest groups (65.3%) over saturated fatty acids (SFA; 28.2%) and monounsaturated fatty acids (MUFA; 6.6%). Another study mentioned a similar fatty acids profile in *L. sativa*, where α-linolenic and linoleic acid were the major compounds found in the mentioned specie, representing about 60 and 20%, respectively, of total fatty acids in this lettuce [6]. These compounds are known to provide health benefits under the prevention of cancer and cardiovascular diseases [24].

Vitamin E is a lipid-soluble compound consisting or four tocopherols (α-, β-, γ- and δ-tocopherol) and tocotrienols, which are related to biological activities, mainly antioxidant action [6]. Vitamin E composition of *L. canadensis* leaves are shown in Table 2, where only α- and γ-tocopherol were detected, with the first being the most abundant (61.2 mg kg^{-1} dw) tocopherol found in our samples. When compared with other lettuces, like *L. sativa*, the

lettuce in study follows the same profile of tocopherols, with the major forms being the α- and γ-tocopherol. However, concerning the total amount of tocopherols, the value for different sub-species of *L. sativa* (green lettuce, butterhead, Batavia, and oak leaf) was 42–152 mg kg^{-1} dw [6], while for the studied species this value was 97 mg kg^{-1} dw. The isoform α-tocopherol represents an important natural antioxidant in plant foods, which can inhibit lipid peroxidation in biological membranes [25].

Table 2. Chemical composition with regard to lipophilic compounds of *L. canadensis* (mean ± SD, *n* = 3).

Fatty Acids	(%)
C6:0	0.180 ± 0.008
C8:0	0.178 ± 0.006
C10:0	0.117 ± 0.001
C12:0	0.125 ± 0.007
C14:0	0.93 ± 0.06
C14:1	0.089 ± 0.002
C15:0	0.49 ± 0.01
C16:0	21.3 ± 0.6
C16:1	2.1 ± 0.1
C17:0	0.268 ± 0.001
C18:0	2.40 ± 0.04
C18:1n9	3.69 ± 0.02
C18:2n6	20.2 ± 0.1
C18:3n3	44.4 ± 0.4
C20:0	1.8 ± 0.1
C21:0	0.27 ± 0.01
C20:3n6	0.30 ± 0.01
C20:4n6	0.409 ± 0.008
C22:0	0.116 ± 0.004
C24:1	0.70 ± 0.02
SFA (%)	28.2 ± 0.4
MUFA (%)	6.6 ± 0.1
PUFA (%)	65.3 ± 0.5
Tocopherols (mg kg^{-1} dw)	
α-tocopherol	61.2 ± 0.7
γ-tocopherol	35.4 ± 0.4
Total	97 ± 1

Caproic acid (C6:0); caprylic acid (C8:0); capric acid (C10:0); lauric acid (C12:0); myristic acid (C14:0); myristoleic acid (C14:1); pentadecanoic acid (C15:0); palmitic acid (C16:0); palmitoleic acid (C16:1); heptadecanoic acid (C17:0); stearic acid (C18:0); oleic acid (C18:1n9); linoleic acid (C18:2n6); α-linolenic acid (C18:3n3); arachidic acid (C20:0); heneicosanoic acid (C21:0); arachidonic acid (C20:4n6); *cis*-11,14,17-eicosatrienoic acid (C20:3n3); behenic acid (C22:0); lignoceric acid (C24:0); SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. Tocopherols calibration curves: α-tocopherol (y = 1.295×, r^2 = 0.991, LOD = 18.06 ng mL^{-1} and LOQ = 60.20 ng mL^{-1}); γ-tocopherol (y = 0.567×, r^2 = 0.991, LOD = 14.79 ng mL^{-1}, LOQ = 49.32 ng mL^{-1}).

3.2. Phenolic Compounds

The tentative identification of the phenolic compounds found in the infusion formulations and hydroethanolic extracts of *L. canadensis*, as well as the retention times (Rt), maximum absorbance in the visible region (λ max), pseudomolecular ion ($[M-H]^-$), and the main ion fragments (MS^2) information achieved by HPLC-DAD-ESI/MS^n, are presented in Table 3.

The effort to identify the distinct phenolic compounds was performed in existing published data and, whenever possible, in comparison to the available standard compounds. Overall, eight phenolic compounds were tentatively identified according to its mass and fragmentation described in the literature, being five phenolic acids and three flavonoids. Regarding phenolic acids, peaks 1 and 2 ($[M-H]^-$ at m/z 353) and 3 ($[M-H]^-$ at m/z 353) were tentatively identified by comparing their retention times and UV spectrum with avail-

able commercial standards, being therefore identified as *cis* and *trans* 3-*O*-caffeoylquinic acid, and 5-*O*-caffeoylquinic acid, respectively. Peaks 4 and 5 ($[M - H]^-$ at m/z 473) were also tentatively identified as *cis* and *trans* chicoric acid, respectively, by comparison with the previously described by Petropoulos et al. [26] in *Cichorium spinosum* L.

Regarding flavonoid compounds, peak 6 presented a pseudomolecular ion $[M - H]^-$ at m/z 461 and a unique MS^2 fragment at m/z 285 (loss of 176 u), being therefore tentatively identified as luteolin-*O*-glucuronide. Peak 8 ($[M - H]^-$ at m/z 445) also presented a unique MS^2 fragment at m/z 269 (loss of 176 u), being tentatively identified as apigenin-*O*-glucuronide. Finally, peak 7 ($[M - H]^-$ at m/z 549) additionally to the main MS^2 fragment at m/z 301 (quercetin aglycone and loss of 176 u), also presented significant fragments at m/z 505 (loss of 44 u) and m/z 463 (loss of 42 u) which correspond to the loss of a malonyl group followed by glucuronyl, being tentatively identified as quercetin-*O*-malonyl-hexoside and apigenin-*O*-glucuronide.

Some of the tentatively identified phenolic compounds in our study have been previously reported in other plants of the *Lactuca* genus, namely in *L. sativa*. These compounds include quercetin derivatives, chicoric and caffeoylquinic acids [27], which is in accordance with the reported herein. Also, other study by Llorach et al. [28] have tentatively identified luteolin derivatives in additional lettuce varieties. Once again, as far as our research group was able to ascertain, this is the first study concerning the phenolic composition of *Lactuca canadensis*, and therefore it is not possible to make a greater and better comparison of results with other previous studies.

The results of the quantification of the phenolic compounds present in the analyzed infusion and hydroethanolic extracts are presented in Table 4. In both preparations, luteolin-*O*-glucuronide was found to be the major phenolic compound (5.46 and 4.6 mg g^{-1} of extract, respectively). However, the phenolic compounds found in minor amounts differ in both extracts, with 5-*O*-caffeoylquinic acid (0.450 mg g^{-1} of extract) being the smallest in the hydroethanolic extract, and *cis* 3-*O*-caffeoylquinic acid (2.148 mg g^{-1} of extract) in the infusion. Even though, individually, the luteolin-*O*-glucuronide compounds were found in greater quantities, the group of phenolic acids was predominant in both of the studied extracts in *L. canadensis*.

Table 3. Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, and tentative identification of the phenolic compounds present in the infusion preparations and hydroethanolic extracts of *L. canadensis*.

Peak	Rt (min)	λ_{max} (nm)	$[M - H]^-$ (m/z)	MS^2 (m/z)	Tentative Identification
1	6.24	325	353	191(100), 179(10), 173(5), 135(5)	cis 3-*O*-Caffeoylquinic acid
2	6.76	324	353	191(100), 179(13), 173(6), 135(5)	trans 3-*O*-Caffeoylquinic acid
3	9.14	324	353	191(100), 179(25), 173(4), 135(7)	5-*O*-Caffeoylquinic acid
4	12.09	328	473	311(100), 293(98),179(57),149(76), 135(5)	*cis* Chicoric acid
5	13.98	328	473	311(100), 293(95), 179(52), 149(53), 135(5)	*trans* Chicoric acid
6	18.16	346	461	285(100)	Luteolin-*O*-glucuronide
7	19.65	352	549	505(43), 463(32), 301(100)	Quercetin-*O*-malonyl-hexoside
8	22.44	329	445	269(100)	Apigenin-*O*-glucuronide

Table 4. Quantification (mg g^{-1} of extract) of the phenolic compounds present in the infusion preparations and hydroethanolic extracts of *L. canadensis* (mean ± SD, *n* = 3).

Peaks	Infusion	Hydroethanolic	*t*-Students Test *p*-Value
1	2.15 ± 0.04	1.961 ± 0.004	<0.001
2	2.7 ± 0.2	2.31 ± 0.07	<0.001
3	2.54 ± 0.09	0.45 ± 0.03	<0.001

Table 4. *Cont.*

Peaks	Infusion	Hydroethanolic	*t*-Students Test *p*-Value
4	4.67 ± 0.03	4.326 ± 0.025	0.992
5	5.08 ± 0.03	3.9 ± 0.2	<0.001
6	5.46 ± 0.09	4.6 ± 0.1	0.289
7	3.58 ± 0.09	3.40 ± 0.07	0.429
8	2.81 ± 0.08	2.725 ± 0.004	<0.001
TPA	17.1 ± 0.1	12.9 ± 0.2	0.218
TF	11.9 ± 0.3	10.8 ± 0.2	0.474
TPC	29.0 ± 0.4	23.7 ± 0.4	0.877

TPA—Total Phenolic Acids; TF—Total Flavonoids; TPC—Total phenolic compounds. Standard calibration curves used for quantification: chlorogenic acid (y = 312503x − 199432, r^2 = 0.9999, LOD = 1.20 μg mL^{-1} and LOQ = 3.62 μg mL^{-1}, peaks 1–3); caffeic acid (y = 388345x + 406369, r^2 = 0.999, LOD = 0.78 μg mL^{-1} and LOQ = 1.97 μg mL^{-1}, peaks 4 and 5) and quercetin-3-*O*-rutinoside (y = 13.343x + 76.751, r^2 = 0.9998, LOD = 0.14 μg mL^{-1} and LOQ= 0.45 μg mL^{-1}, peaks 6–8). Significant differences ($p < 0.001$) between extracts were assessed by a Student's *t*-test.

3.3. Bioactive Properties

L. canadensis leaves were used to prepare infusion and hydroethanolic extracts in order to assess their capacity to prevent lipid peroxidation in porcine brain cell homogenates and the oxidative haemolysis of sheep red blood cells. In both assays (Table 5), the infusion extract presented a better performance when compared to the hydroethanolic, with statistically significant differences ($p < 0.05$) between them. Regarding the TBARS assay, the infusion extract presented an EC_{50} value of 0.69 mg mL^{-1} over 1.17 mg mL^{-1} from the hydroethanolic extract. As for the OxHLIA assay, for a 120 min Δt, the IC_{50} value of the infusion extract was 211 μg mL^{-1} against 297 μg mL^{-1} of the hydroethanolic. These results show that the antioxidant compounds act by distinct mechanisms, each with its targets in the reaction system [29].

The results of antimicrobial properties of *L. canadensis* leaves extracts against Gram-negative and Gram-positive bacteria are presented in Table 6. The results present that, in the established concentrations (20 to 0.156 mg mL^{-1}), both extracts were ineffective against *P. mirabilis* and *L. monocytogenes*, though being active or discreetly active against the lasting bacteria. In general, both extracts show similar ranges of antimicrobial activities with exception of infusion extract against *E. coli*, which is more effective than the hydroethanolic (10 and 20 mg mL^{-1}, respectively). The lowest minimum inhibitory concentration (MIC) values were obtained against Gram-negative *M. morganii* (2.5 mg mL^{-1}) in both extracts. Antimicrobial activity of *L. sativa var. longifolia* extracts were also found by Edziri et al. [30] against different Gram-negative (*E. coli, K. pneumoniae, Serratia marcescens, Acinetobacter baumannii, Enterobacter cloacae*) and Gram-positive bacteria (*Bacillus subtills, S. aureus, E. faecium, E. faecalis*, and *Corynebacterium* spp.). In this study, methanolic extract had better results when compared with the aqueous one, which may be due to the highest total of phenolic compounds found in methanolic extract.

Table 5. Antioxidant activity of *L. canadensis* infusion preparations and hydroethanolic extracts (mean ± SD, $n = 3$).

Extract	TBARS (EC_{50} Values, mg mL^{-1})	OxHLIA (IC_{50} Values, μg mL^{-1})	
		Δt = 60 min	Δt = 120 min
Infusion	0.69 ± 0.02 ^{b}	135 ± 2 ^{b}	211 ± 2 ^{b}
Hydroethanolic	1.17 ± 0.06 ^{c}	192 ± 3 ^{c}	297 ± 4 ^{c}
Trolox	0.0058 ± 0.0006 ^{a}	19 ± 1 ^{a}	41 ± 4 ^{a}

EC_{50}: extract concentration corresponding to 50% of antioxidant activity (TBARS assay) and IC_{50} values translate the extract concentration required to keep 50% of the erythrocyte population intact for 60 and 120 min (OxHLIA assay). In each column, different letters indicate significant differences ($p < 0.05$) among extracts.

Table 6. Antimicrobial activity (MIC and MBC, mg mL^{-1}) of *L. canadensis* infusion preparations and hydroethanolic extracts.

	Infusion		**Hydroethanolic**		**Ampicillin (20 mg mL^{-1})**		**Imipenem (1 mg mL^{-1})**		**Vancomycin (1 mg mL^{-1})**	
Bacteria	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Gram (−)										
Escherichia coli	10	>20	20	>20	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.
Klebsiella pneumoniae	5	>20	5	>20	10	20	<0.0078	<0.0078	n.t.	n.t.
Morganella morganii	2.5	>20	2.5	>20	20	>20	<0.0078	<0.0078	n.t.	n.t.
Proteus mirabilis	>20	>20	>20	>20	<015	<0.15	<0.0078	<0.0078	n.t.	n.t.
Pseudomonas aeruginosa	20	>20	20	>20	>20	>20	0.5	1	n.t.	n.t.
Gram (+)										
Enterococcus faecalis	5	>20	10	>20	<0.15	<0.15	n.t.	n.t.	<0.0078	<0.0078
Listeria monocytogenes	>20	>20	>20	>20	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.
MRSA	5	>20	5	>20	<0.15	<0.15	n.t.	n.t.	0.25	0.5

MIC—minimum inhibitory concentration; MBC—minimum bactericidal concentrations; MRSA—Methicillin-resistant *S. aureus*; n.t.—not tested.

4. Conclusions

This work aimed at a detailed characterization of *L. canadensis*, an edible wild plant for which there is a great lack of data. The present study provides a report of the nutritional and chemical composition of *L. canadensis* leaves, as well as their bioactive properties, for which detailed data are missing from literature. Its characterization allowed us to settle that this UFP can contribute to a balanced diet as a source of nutrients and bioactive compounds. The infusion extract showed to be more effective in terms of antioxidant activity, while, regarding antimicrobial activity, both extracts showed similar effects against the tried microorganisms.

Thus, this study validates *L. canadensis* as a suitable edible plant for inclusion in a balanced and diversified diet, as a source of nutrients and bioactive compounds that promote well-being. These data also contribute to the knowledge of this underexploited food plant, which could be used as a new food ingredient with potential applicability as a source of functional compounds.

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