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Plants of the Family Asteraceae: Evaluation of Biological Properties and Identification of Phenolic Compounds †

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Abstract: The present study focused on the biological analysis of five plants: *Achillea millefolium*, *Arnica montana*, *Calendula officinalis*, *Chamaemelum nobile* and *Taraxacum officinale*. The results indicated that *A. montana* extracts showed the highest content of phenolic compounds. Regarding the biological properties, *A. millefolium* had outstanding antioxidant activity, while *C. officinalis* had the highest rate of antimicrobial and antifungal activity. The anti-inflammatory and cytotoxic activities reflected that *C. nobile* showed the highest effect. In enzyme assays, *C. nobile* and *C. officinalis* extracts showed the highest inhibitory effects on acetylcholinesterase and butyrylcholinesterase enzymes. Overall, this study provides scientific evidence for the evaluation of the potential of medicinal plant extracts for the development of new products.

Keywords: medicinal plants; beneficial effects; biological properties; phenolic compounds

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1. Introduction

Currently, medicinal plants have great relevance due to their reported beneficial health properties. Many studies reflect that their biological properties, such as antioxidant, antitumor and antimicrobial activities, are related to different bioactive compounds, including phenolic compounds. Although some of their mechanisms of action are unknown, in many cases it has been shown that various natural phenolic compounds are related to bioactive properties, and this has aroused the interest of the scientific community [1]. Several medicinal plants are still used for therapeutic purposes, employed in different formulas (decoctions, infusions, ointments, etc.) but, in general, their use has been reduced. However, these plants can be re-valorized for the recovery of bioactive compounds with applications in the food, cosmetic and pharmaceutical industries [2]. In particular, plants from Asteraceae family are promising candidates, due to their beneficial properties and bioactive compounds.

The present study focused on five medicinal plants from the Asteraceae family, namely, *Achillea millefolium* L., *Arnica montana* L., *Calendula officinalis* L., *Chamaemelum nobile* L. and *Taraxacum officinale* (L.) Weber ex F. H. Wigg., all belonging to the Asteraceae family. These plants have been widely used in traditional medicine for the treatment of various disorders, but their use has been reduced. *A. millefolium*, *T. officinale* and *C. officinalis* are the most

studied of these plants, and some of their terpenoids, flavonoids, phenolic acids and carotenoids have been described as bioactive compounds [3,4]. The bioactive compounds of *C. nobile* have not been studied in depth, although this plant is well is known to be especially beneficial for digestive health. Bioactive compounds identified so far include terpenoids, flavonoids, coumarins and other compounds such as esters of angelic and tyglic acids, among others [5]. However, the main bioactive compounds in *A. montana* have been demonstrated to be the so-called sesquiterpene lactones, which are related to its anti-inflammatory effects [6]. On this basis, the study focused on the determination of phenolic compounds and the evaluation of the biological properties of these plants, to deepen knowledge about the bioactive compounds and evaluate their possible use in future bio-based applications.

2. Materials and Methods

2.1. Sample Extraction

The samples were acquired in 2020 from Soria Natural and Pinisan and were received at room temperature, dried and crushed to facilitate and improve the efficiency of the extraction processes. Then, the samples were sieved with a sieve (pore size < 2 mm). The samples were extracted by solid–liquid extraction. A 5 g sample of each species was extracted with 100 mL of methanol–water (60:40 *v*/*v*). Extraction was carried out at 45 °C for 3 h. Then, the extracts were freeze-dried using Telstar LyoAlfa 15 equipment to obtain dry extracts that were used in the subsequent analyses.

2.2. Determination of Phenolic Compounds

The identification of phenolic compounds was carried out using a Dionex UltiMate 3000 UPLC system (Thermo Scientific, San Jose, CA, USA) [7]. The determination was performed using a diode array detector (DAD) and mass spectrometry (MS) (LTQ XL mass spectrometer, Thermo Finnigan, San Jose, CA, USA) working in negative mode. Data acquisition was carried out with an Xcalibur® data system (Thermo Finnigan, San Jose, CA, USA). The phenolic compounds were identified according to their chromatographic characteristics, by their retention, absorption spectra and mass characteristics in comparison to the obtained standard compounds and the literature. For quantitative analysis, calibration curves were prepared with appropriate standards. The results were expressed in mg per g of dry extract. Analyses were performed in triplicate.

2.3. Determination of the Main Biological Properties

2.3.1. Assessment of Antioxidant Activity

To evaluate the antioxidant activity, the lipid peroxidation inhibition in porcine (*Sus scrofa*) brain homogenates was analyzed, evaluating the decrease in thiobarbituric acid reactive substances (TBARS), as previously described in Pineda et al. [8]. Brain tissue was homogenized in Tris-HCl buffer (20 mM, pH 7.4) and then centrifuged at 3000 *g* for 10 min. An aliquot of the supernatant was incubated with the extracts at different concentrations in the presence of FeSO4 (10 mM) and ascorbic acid (0.1 mM) for 1h at 37 °C. Trichloroacetic acid (28%) and thiobarbituric acid (2%) were added to stop the reaction at 80 °C, and stirred for 20 min. After centrifugation, the color intensity of the malondialdehyde complex in the supernatant was measured via its absorbance at 532 nm. Using the dose–response values of the results obtained, a parameter that summarized the potential antioxidant effect of each sample was obtained, i.e., the concentration necessary to produce 50% of the antioxidant response (EC_{50}) [7].

2.3.2. Assessment of Antimicrobial Activity

The dried extracts were dissolved in distilled water (10 mg/mL) and the procedure described by Soković et al. [9] was followed. The activity was studied against threeGram-negative bacteria: *Escherichia coli*, *Salmonella typhimurium* and *Enterobacter cloacae* and three Gram-positive bacteria: *Bacillus cereus*, *Listeria monocytogenes* and methicillin-resistant

Staphylococcus aureus (MRSA). For antifungal assays, six micromycetes were tested: *Aspergillus fumigatus* (human isolate), *Aspergillus niger* (ATCC 6275), *Aspergillus versicolor* (ATCC11730), *Penicillium funiculosum* (ATCC 36839), *Trichoderma viride* (IAM 5061) and *Penicillium verrucosum var. cyclopium* (food isolate). The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration were determined.

2.3.3. Assessment of Anti-Inflammatory Properties

The dried extracts were dissolved in distilled water (8 mg/mL) and serial dilutions (1–8 mg/mL) were prepared and tested using a RAW 264.7 murine macrophage cell line. Lipopolysaccharide was used to stimulate inflammation and the production of nitric oxide was measured as described previously [10]. The results obtained were expressed as EC_{50} values (µg/mL) and dexamethasone was used as a positive control.

2.3.4. Cytotoxic Properties

Cytotoxicity was assessed using four tumor cell lines: AGS (human gastric adenocarcinoma cell line), CaCo (Caucasian colon adenocarcinoma), MCF-7 (break adenocarcinoma cell line), NCI- H460 (lung cancer). The Vero cell line was used as a control. Cytotoxic activity was measured using the sulforhodamine B assay [11]. The results obtained were expressed as GI_{50} values, i.e., the concentration of extract that inhibited 50% of net cell growth, and ellipticin was used as a positive control.

2.3.5. Enzymatic Activity

A previously developed colorimetric method was used [12]. This consists of detecting the inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activity via the increase in yellow coloring due to the production of thiocholine. These two enzymes have been reported to be involved in neurological disorders. In addition, inhibition of AChe has been recognized as a possible avenue for the symptomatic treatment of Alzheimer's disease [13]. The assay was carried out using three buffers: A with 50 mM Tris–HCl, pH 8; B with 50 mM Tris–HCl, pH 8, 0.1% BSA and C with 50 mM Tris–HCl, pH 8, 0.1 M NaCl and 0.02 M $MgCl_2$. The inhibitory capacity of the extracts was tested at concentrations of 1 and 2 mg/mL.

3. Results and Discussion

The phenolic profile of the selected plants showed great variability, both in quantity and in the identified phenolic compounds (Table 1; Figure 1). The plant with the highest content of phenolic compounds was *A. montana*, with a concentration of 119 mg/mL, where the most representative compound was 5-*O*-caffeolyquinic acid. The extracts of *C. nobile* presented a total phenolic content of 100 mg/mL and, in this case, the major compound was the flavonoid luteolin-*O*-pentosylhexoside. *A. millefolium* extracts achieved a total phenolic content of 81 mg/mL, and the most representative compound was 3-*O*-caffeoylquinic acid. *T. officinale* extracts had a phenolic content of 18 mg/mL and were rich in 3-*O*-caffeoylquinic acid. Finally, *C. officinalis* extracts had the lowest phenolic content, at 14.1 mg/mL, with 3-*O*-caffeoylquinic acid as the major compound. These results coincide with those of other studies, e.g., in the case of the plant *A. montana*, where 5-*O*-caffeoylquinic acid has also previously been reported as the main phenolic acid in the ethanolic extract of this plant [14]. However, it should be noted that the content of phenolic acids can be influenced by external factors such as the type of solvent used in the extraction process, and it is also related to the growing conditions of the plants, as mentioned in previous studies on *A. montana* and other species belonging to the Asteraceae family [15].

Table 1. Total phenolic content and main phenolic compounds identified (mg/mL).

Plants	Main Phenolic Compounds	Quantification	TPC
Achillea millefolium	3-*O*-Caffeoylquinic acid	18.85 ± 0.03	81
Arnica montana	5-*O*-Caffeolyquinic acid	23.9 ± 0.3	119
Calendula officinalis	3-*O*-Caffeoylquinic acid	9.8 ± 0.2	14.1
Chamaemelum nobile	Luteolin-*O*-pentosylhexoside	49.6 ± 0.5	100
Taraxacum officinale	3-*O*-Caffeoylquinic acid	6.74 ± 0.4	18

TPC: total phenolic compounds.

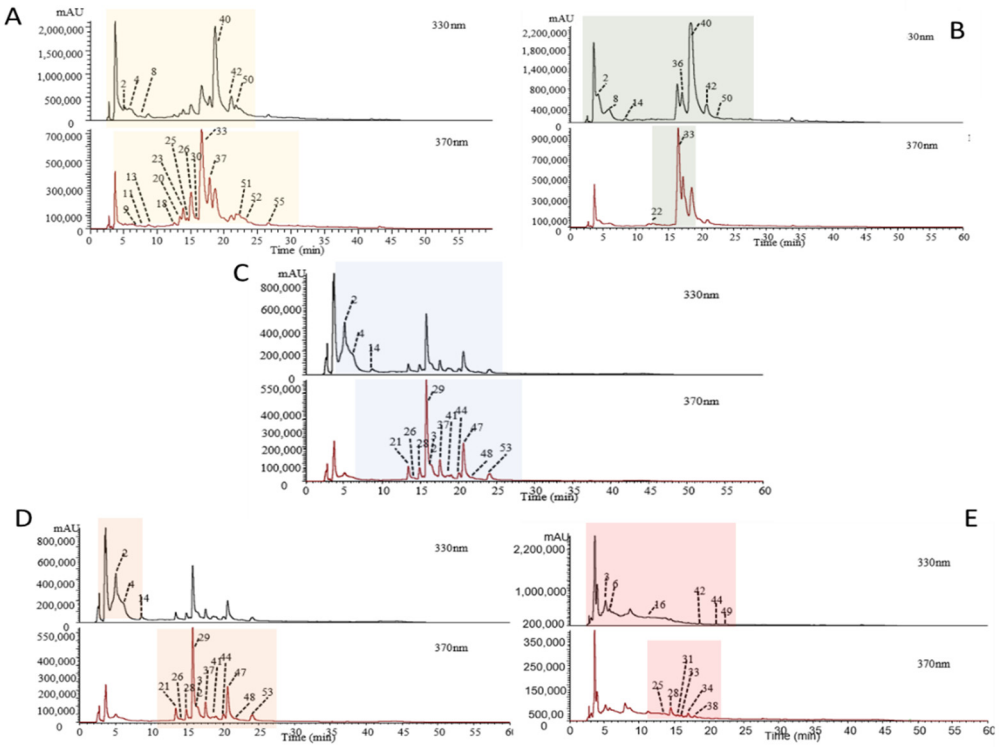

Figure 1. Representative chromatogram of the phenolic compounds identified: (**A**) *A. millefolium*; (**B**) *A. montana*; (**C**) *C. officinalis*; (**D**) *C. nobile*; (**E**) *T. officinale*.

Regarding antioxidant activity, the extracts of *A. millefolium* showed exceptional activity, with an EC_{50} value of 0.013 mg/mL. The extracts of *A. montana*, *C. nobile* and *C. officinalis* showed similar EC_{50} values (0.2, 0.2 and 0.25 mg/mL, respectively). Finally, the extracts of *T. officinale* showed the lowest antioxidant activity with an EC_{50} of 0.035 mg/mL. These results are presented in Figure 2. In previous studies, this assay has been employed to evaluate the antioxidant activity of *A. millefolium*, *C. officinalis* and *C. nobile*, reporting significant results. To the best of our knowledge, no study has used the TBARS assay to evaluate *A. montana* and *T. officinale*, but their antioxidant properties have been corroborated by various studies showing positive results [16–18].

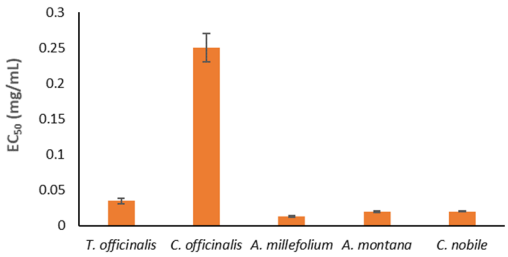

Figure 2. Antioxidant activity in traditional plants of the family Asteraceae: *A. millefolium*, *A. montana*, *C. officinalis*, *C. nobile* and *T. officinale*.

Regarding antimicrobial activity, all the plant extracts displayed significant antimicrobial effects, with *C. officinalis* being the most remarkable. This plant presented MIC values ranging from 0.25 to 0.5 mg/mL for all the tested bacteria and fungi. MBC and MFC values ranged between 0.5 and 1 mg/mL. The most susceptible bacteria were the Gram-positive species, while *T. viride* was the most susceptible fungus. *T. officinale* also showed relevant antibacterial potential, while *C. nobile* was also effective against fungi species. The antimicrobial potential of these species has been previously confirmed. Focusing on *C. officinalis*, a study reported that petal extracts of this plant showed comparable antibacterial effects against Gram-positive and Gram-negative bacteria using the disk diffusion method [19]. The results found in the literature are very similar to those obtained experimentally and therefore corroborate the hypothesis that the *C. officinalis* plant could be used as a possible source of antimicrobial compounds.

According to the results (Figure 3), *C. nobile* extracts showed the greatest effects in both assays, with EC_{50} values of 15.21 µg/mL for anti-inflammatory activity and GI_{50} values between 54 and 10.3 µg/mL in the case of cytotoxic activity. *A. millefolium* also showed significant results, with an EI_{50} of 30 µg/mL for anti-inflammatory activity and GI_{50} values ranging between 42 and 125 µg/mL. Considering the *C. nobile* results, the anti-inflammatory and the cytotoxic properties of this plant have been reported previously, showing positive results [20,21], and therefore this plant could be a promising source of anti-inflammatory and cytotoxic extracts.

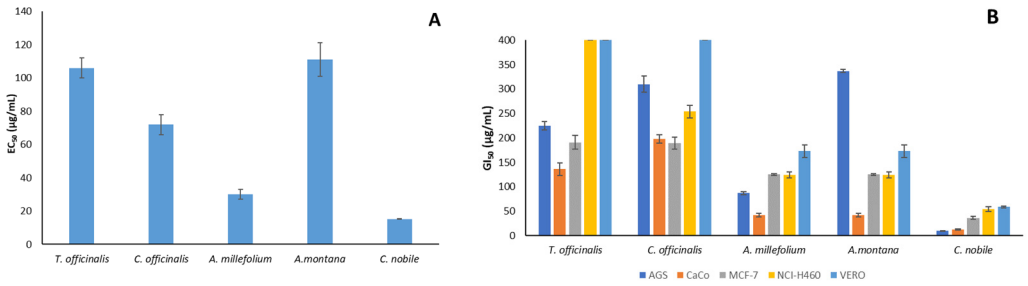

Figure 3. (**A**) Anti-inflammatory and (**B**) cytotoxic activity of *A. millefolium*, *A. montana*, *C. officinalis*, *C. nobile* and *T. officinale*.

Finally, *C. nobile* showed the highest inhibitory effects on AChE activity for the extract concentrations tested (1 and 2 mg/mL), causing an inhibition of >35% and >60%, respectively. In the case of the BuChE enzyme, *C. officinalis* caused an inhibition of >50% in both concentrations tested. *C. nobile* also showed a remarkable inhibitory effect against this enzyme, with an inhibition of >40% at 2 mg/mL and >20% at 1 mg/mL. To the best of our knowledge, no previous studies have evaluated the enzymatic activity of the selected plants.

4. Conclusions

All the plants studied had diverse phenolic compositions and biological activities. Regarding phenolic compounds, *A. montana* extracts showed the highest content. Regarding bioactivities, *A. millefolium* showed high antioxidant activity and *C. officinalis* showed the best antimicrobial and antifungal activities. In the case of anti-inflammatory and cytotoxic activities, *C. nobile* extracts achieved the best results. Finally in the enzyme assays, both *C. nobile* and *C. officinalis* extracts showed the highest inhibitory effects. Therefore, this study provides scientific evidence of the potential of medicinal plants as a source of extracts and bioactive compounds that may be considered for the development of new products.

Supplementary Materials: The poster presentation is available online at: https://www.mdpi.com/article/10.3390/CSAC2021-10486/s1.

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validation, F.B., I.C.F.R.F., M.A.P. and L.B.; formal analysis, A.S., F.B., M.N., M.S., T.C.F., R.C. and I.C.F.R.F.; investigation, M.B.-M., P.G.-O., A.S. and F.B.; writing—original draft preparation, M.B.-M. and P.G.-O.; writing—review and editing, M.B.-M. and P.G.-O.; visualization, P.G.-O., T.C.F. and L.B.; supervision, M.A.P., L.B. and J.S.-G. All authors have read and agreed to the published version of the manuscript.

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

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