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Plants of the family Asteraceae: evaluation of biological properties and identification of phenolic compounds

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† Presented at the 1st International Electronic Conference on Chemical Sensors and Analytical Chemistry, 1-15 July, Available online: https://csac2021.sciforum.net/

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 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.

Citation: Lastname, F.; Lastname, F.; Lastname, F. Title. *Chem. Proc.* **2021**, *3*, x. https://doi.org/10.3390/xxxxx

Published: date

1. Introduction

Currently, medicinal plants have a great relevance due to their reported beneficial healthy properties. Many studies reflect that their biological properties, such as antioxidant, antitumor, 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, which have aroused the interest of the scientific community [1]. Medicinal plants are currently used in two main ways. On the one hand, they are used in association with the preservation of traditional knowledge for therapeutic purposes, used in different formulas (decoctions, infusions, ointments, etc.), which have been maintained over time. On the other hand, these plants can be re-valorized for the recovery of bioactive compounds with applications in the food, cosmetic and pharmaceutical industries [2]. In particular, the plants from Asteraceae family are promising candidates for their associated beneficial properties and bioactive compounds.

On this basis, the study focused on the determination of phenolic compounds and the evaluation of the biological properties of five medicinal plants, 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 is traditional medicine for the treatment of various disorders, but its use has been reduced.

2. Materials and Methods

2.1. Sample extraction

First, the samples were dried and crushed to facilitate and improve the efficiency of the extraction processes, then the samples were sieved with a sieve. The conventional extraction method used is a solid-liquid extraction, where 5 g of sample of each species are weighed and 100 mL of solvent (mixture of MetOH and distilled water 60:40 v/v) is added at 45ºC for 3 hours. Then, the extracts were lyophilized to obtain dry-extracts, that were used in the subsequent analysis.

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), following a previous methodology [3]. The determination was performed by 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 Xcalibur® data system (ThermoFinnigan, 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 with literature. For quantitative analysis, calibration curves were prepared with appropriate standards. The results were expressed in mg per g of dry extract (mg/g). Analysis were performed in triplicate.

2.2 Determination of the main biological properties

2.2.1 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 described previously [4]. Using the dose-response values of the results obtained, a parameter that summarizes the potential antioxidant effect of each sample was obtained, i.e., the concentration necessary to produce 50% of the antioxidant response (EC_{50}).

2.2.2 Assessment of antimicrobial activity

The dried extracts were dissolved in distilled H_2O (10 mg/mL) and the procedure described by (Sokovicx́ et al., 2010) [5] was followed. Their activity was studied against five Gram-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). Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration were determined.

2.2.3 Assessment of anti-inflammatory properties

The dried extracts were dissolved in distilled H_2O (8 mg/mL) and serial dilutions (1 - 8 mg/mL) were prepared and then were 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 [6]. The results obtained were expressed as EC_{50} values (μg/mL) and dexamethasone was used as positive control.

2.2.4 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). VERO cell line was used as control. Cytotoxic activity was measured using the sulforhodamine B assay [7]. 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.

2.2.5 Enzymatic

A previously developed colorimetric method was used [8]. It consists of detecting the inhibition of Acetylcholinesterase (AChE) and Butirilcholinesterase (BuChE) activity, by the increase of yellow coloring due to the production of thiocholine. These two enzymes have been reported to be involved in neurological disorders.

3. Results and discussion

3.1. Determination of phenolic compounds

The total phenolic profile Asteraceae showed a great variability, both in content (**Figure 1**) and in identified phenolic compounds. The plant with the highest content of phenolic compounds was *A. montana,* with a concentration of 119 mg/mL, the most representative compound being 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 Luteolin-O-pentosylhexoside. *A. millefolium* extracts achieved a total phenolic content of 81 mg/mL, being the most representative compound the 3-O-Caffeoylquinic acid. *T. officinale* extracts had a phenolic content of 18 mg/mL, being rich in 3-O-Caffeoylquinic acid. Finally, *C. officinalis* extracts had the lowest phenolic content, 14.1 mg/mL, with 3-O-Caffeoylquinic acid as the major compound.

3.2. Antioxidant activity

The extracts of *A. millefolium* showed exceptional activity, with EC_{50} values of 0.013 mg/mL. The extracts of the plants *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 a EC_{50} of 0.035 mg/mL. All these results are presented in **Figure 1**. 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 our knowledge, no study has used TBARS assay to evaluate *A. montana* and *T. officinale*, but their antioxidant properties have been corroborated by different assays.

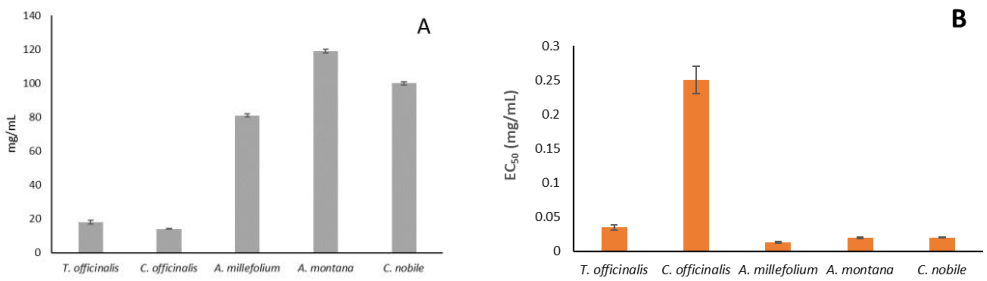

Figure 1. A)Total phenolic content and B) Antioxidant activity in traditional plants of the family Asteraceae: A. *millefolium*, *A. montana*, *C.officinalis*, *C.nobile* and *T. officinale*.

3.3. Antimicrobial activity

Regarding antimicrobial activity, all the plant extracts displayed significant antimicrobial effects, being *C. officinalis* the most remarkable. This plant presented MIC ranging from 0.25 to 0.5 mg/mL for all the tested bacteria and fungi. MBC and MFC ranged between 0.5-1 mg/mL. The most susceptible bacteria were the gram-positive species, while *T. viride* was the most susceptible fungi. *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. Regarding *C. officinalis*, the results the disc diffusion method of a study using petal extracts also reported significant comparable antibacterial effects against Gram-positive and Gram-negative bacteria [9]. The results found in the literature are very similar to those obtained experimentally and therefore corroborate the hypothesis of the possible use of *C. officinalis* plant as a possible source of antimicrobial compounds.

3.4. Anti-inflammatory and cytotoxic activities

According to the results (**Figura 2**), *C. nobile* extracts showed the greatest effect in both assays, with a EC_{50} values of 15.2±0.1 µg/mL for the anti-inflammatory activity, and GI_{50} values between 54 and 10.3 µg/mL, in the case of cytotoxic activity. *A. millefolium* also showed considerable results, with and EI_{50} of 30 µg/mL for the anti-inflammatory activity and GI_{50} values ranging between 42 and 125 µg/mL. The anti-inflammatory and cytotoxic properties against different tumor cell lines of these species have been previously evaluated [10–12].

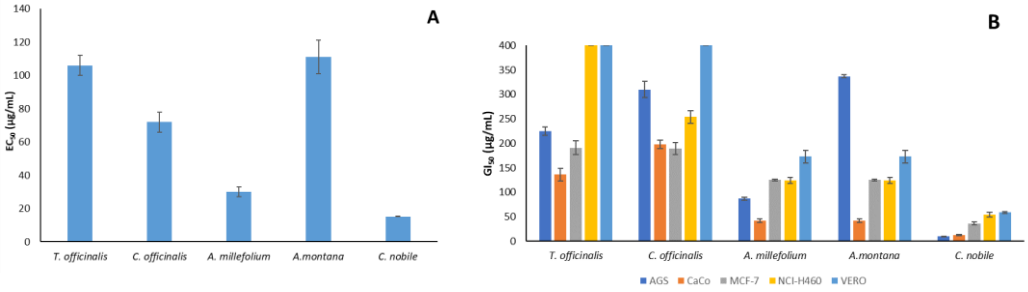

Figura 2. ***A)***Anti-inflammatory and *B)* cytotoxic activity of *A. millefolium, A. montana, C. officinalis, C. nobile* and *T. officinale*.

3.5. Enzymatic activity

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* causes an inhibition > 50% in both concentrations tested. *C. nobile* also showed a remarkable inhibitory effect agains this enzyme, with an inhibition >40% at 2 mg/mL and >20% at 1 mg/mL. The enzymatic activity of some of the selected plants has been tested in previous studies, reporting similar results [13].

4. Conclusions

All the plants studied had diverse phenolic composition and biological activities. Regarding phenolic compounds, *A. montana* extracts showed the highest content. *A. millefolium* showed high antioxidant activity, *C. officinalis* had the highest rate of antimicrobial and antifungal activities. In the case of anti-inflammatory and cytotoxic activities, *C. nobile* extracts showed the highest anti-inflammatory and cytotoxic activity. Finally in the enzyme assays, both *C. nobile* and *C. officinalis* extracts showed the highest inhibitory effects. Therefore, this study provides scientific evidence for the evaluation of the potential of medicinal plant extracts for the development of new products.

Author Contributions: Conceptualization, M. B.-M, P. G.-O, L.B and M.A.P, methodology M. B.-M, B. N.-E, A.S, F.B, M.N, M.S, T.C.-F, R.C and I.C.F.R.-F, software M. B.-M, B. N.-E and A.S 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, writing – review and editing M. B.-M and P. G, visualization P. G, 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.

Funding: The JU receives support from the European Union's Horizon 2020 research and innovation program and the Bio Based Industries Consortium. The project SYSTEMIC Knowledge hub on Nutrition and Food Security, has received funding from national research funding parties in Belgium (FWO), France (INRA), Germany (BLE), Italy (MIPAAF), Latvia (IZM), Norway (RCN), Portugal (FCT), and Spain (AEI) in a joint action of JPI HDHL, JPI-OCEANS and FACCE-JPI launched in 2019 under the ERA-NET ERA-HDHL (n° 696295).

Acknowledgments: The research leading to these results was supported by MICINN supporting the Ramón y Cajal grant for M.A. Prieto (RYC-2017-22891); by Xunta de Galicia for supporting the program EXCELENCIA-ED431F 2020/12 and the pre-doctoral grant of P. García-Oliveira (ED481A-2019/295); by EcoChestnut Project (Erasmus+ KA202) that supports the work of B. Nuñez-Estevez, the program Grupos de Referencia Competitiva (GRUPO AA1-GRC 2018) that supports the work M. Barral-Martínez; by the Bio Based Industries Joint Undertaking (JU) under grant agreement No 888003 UP4HEALTH Project (H2020-BBI-JTI-2019) that supports the work of P. Garcia-Perez and by the Ibero-American Program on Science and Technology (CYTED—AQUA-CIBUS, P317RT0003). The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES to CIMO (UIDB/00690/2020); and to the national funding by FCT, P.I., through the institutional scientific employment program-contract for L. Barros contract.

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

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