Differential Metabolomic Fingerprinting of the Crude Extracts of Three Asteraceae Species with Assessment of Their In Vitro Antioxidant and Enzyme-Inhibitory Activities Supported by In Silico Investigations

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Abstract: The Asteraceae is a large family, rich in ornamental, economical, and medicinally valuable plants. The current study involves the analytical and pharmacological assessment of the methanolic extracts of three less investigated Asteraceae plants, namely Echinops ritro, Centaurea deflexa, and Tripleurospermum decipiens, obtained by three different extraction methodologies viz. maceration (MAC), ultrasound-assisted extraction (UAE), and homogenizer-assisted extraction (HAE). LC-MS-MS analysis of E. ritro, C. deflexa, and T. decipiens extracts led to the identification of ca. 29, 20, and 33 metabolites, respectively, belonging to flavonoids, phenolic acids, and fatty acids/amides. Although there were significant differences in the quantitative metabolite profiles in the extracts of E. ritro and T. decipiens based on the used extraction method, no significant variation was observed in the extracts of C. deflexa in the three implemented extraction techniques. The antioxidant activities of the nine extracts were assessed in vitro using six different assays viz. DPPH, ABTS, CUPRAC, FRAP, PDA, and metal chelation assay (MCA). The HAE/UAEx extracts of E. ritro and the UAE/MAC extracts of C. deflexa displayed the highest antioxidant activity in the DPPH assay, while the UAE extract of T. decipiens showed the strongest antioxidant activity in both the CUPRAC and MCA assays. The enzyme inhibitory activities of the nine extracts were studied in vitro on five different enzymes viz. tyrosinase, α-amylase, α-glucosidase, acetylcholinesterase (AChE), and butyrylcholinesterase (BChE), affecting various pathological diseases. Concerning C. deflexa, its MAC/UAE extracts showed the strongest inhibition on α-amylase, while its UAE/HAE extracts displayed strong inhibitory power on AChE. However, no significant difference was observed on their effects on tyrosinase or BChE. For T. decipiens, its UAE/HAE showed potent inhibition to α-glucosidase, MAC/HAE significantly inhibited AChE and BChE, while UAE could strongly inhibit tyrosinase enzyme. For E. ritro, all extracts equally inhibited α-amylase and α-glucosidase, MAC/HAE strongly affected tyrosinase, HAE/MAC best inhibited BChE, while HAE inhibited AChE to a greater extent. Chemometric analysis using PCA plot was able to discriminate between the plant samples and between the implemented extraction modes. The in vitro enzyme inhibitory activities of the extracts were supported by in silico data, where metabolites, such as the lignan arctin and the flavonoid vicenin-2, dominating the extract of C. deflexa, displayed strong binding to AChE. Similarly, chlorogenic and dicafeoyl quinic acids, which are some of the major metabolites in the extracts of E. ritro and T. decipiens, bound with high affinity to α-glucosidase.
1. Introduction

Oxidative stress has been linked to several complications, such as cancer, diabetes, neurodegenerative, and cardiovascular disorders [1–4]. This is attributed to the imbalance between the production of reactive oxygen/nitrogen radicals and the internal antioxidant defense system [5]. Similarly, the inhibition of some enzymes has been reported to contribute to the healing process in many diseases. For example, α-amylase and glucosidase inhibitors can be used in the management of type 2 diabetes, acetyl cholinesterase inhibitors for the management of Alzheimer symptoms, tyrosine-kinase inhibitors in cancer, and tyrosinase inhibitors for skin whitening and skin diseases [6].

Nature remains a rich source for the discovery and development of new drug entities that can be used in the management of various health conditions [7–12]. Members of family Asteraceae have been reported to display antioxidant effects due to their rich content of polyphenolics, yet many factors affected their antioxidant power; among these are the geographical location and climatic conditions, as well as the extraction procedure and the type of solvent used [13,14]. They have been reported to inhibit the activity of several enzymes, such as acetylcholinesterase, tyrosinase, and α-amylases [15,16]. Many members of Asteraceae have not yet been investigated for their chemical composition and pharmacological values. *Echinops ritro* L. is an Asteraceae plant widely cultivated in Europe, North Africa, and Asia. In China, it is commonly used to stimulate milk secretion in traditional medicine. The plant is rich in quinoline alkaloids, polyacetylenes, sesquiterpenes, polyphenolics, and thiophenes [17]. Few studies were reported on its medicinal value, which focused on its antifungal and antimicrobial properties [18,19]. *Centaurea deflexa*, belonging to the largest genus of the Asteraceae, is mainly distributed in the Mediterranean region and is rich in secondary phytoconstituents, the most characteristic of which are the sesquiterpene lactones responsible for its documented anticancer activity [20–22]. Meanwhile, little is known about its antioxidant activity or its polyphenolic content. *Tripleurospermum* species are distributed in Europe, North America, North Africa, and Asia, with many species predominant in Turkey [23]. Their essential oils were reported to inhibit acetylcholinesterase activity in a concentration-dependent manner [24]; nevertheless, nothing has so far been reported on the pharmacological value of *T. decipiens*.

Therefore, we herein report on the LC-MS-MS metabolic profiling of the methanol extracts of three—little investigated or uninvestigated—plants belonging to the family Asteraceae. In vitro antioxidant activities are intensely studied using seven different assays and correlated with their polyphenolic and flavonoidal content. Moreover, the enzyme-inhibitory capacity of the extracts was evaluated against α-glucosidase, α-amylase, tyrosinase, and acetyl- and butyrylcholinesterases.

2. Materials and Methods

2.1. Plant Materials and Preparation of Extracts

The plant samples were collected in the city of Konya in the 2021 summer season (June) and location information is given below.

*Centaurea deflexa* Wagenitz: between Fetigen and Tosmur location, Taşkent, Konya, 1730 m, Voucher number: EY-3043.

*Echinops ritro* L.: Yazır Location, Konya, 1000 m, Voucher number: EY-3121.

*Tripleurospermum decipiens* (Fisch. & Mey.) Bornm.: Yazır Location, Konya, 1000 m, Voucher number: EY-3088.

The plants were confirmed by one co-author (Dr. Evren Yildiztugay) in Selcuk University and one voucher specimen was deposited in Selcuk University. The plant samples (aerial parts) were dried in the shade at room temperature for approximately one week. The samples were then pulverized using a mill and they were placed in a dark environment.
In the present study, three extraction methods (maceration (MAC), homogenizer-assisted (HAE) and ultrasound-assisted (UAE)) were performed using methanol. The extraction procedures are summarized below. The solid–solvent ratio was 1/20 in all extraction methods.

Maceration (MAC): The plant materials (5 g) were stirred with 100 mL of methanol at room temperature for 24 h in a shaking device.

Homogenizer-assisted extraction (HAE): The plant materials (5 g) were extracted with 100 mL of methanol in one ultra-turrax (6000 × g) for 5 min.

Ultrasound-assisted extraction (UAE): The plant materials (5 g) were extracted with 100 mL of methanol in one ultrasound bath at room temperature for 30 min.

After the extraction procedures, all extracts were filtered using Whatman No.1 filter paper in Büchner flask under vacuum. The solvents were removed using rotary evaporator. All extracts were stored at 4 °C until analysis.

2.2. HPLC-ESI-MS/MS Analysis of the Methanol Extracts of Three Asteraceae Species

The extracts of *Echinops ritro*, *Centaurea deflexa*, and *Tripleurospermum decipiens* obtained by three extraction techniques were analyzed using high-performance liquid chromatography coupled to electrospray ionization mass spectrometry for investigating their metabolic profiles. Analysis was conducted on Shimadzu® 8045 HPLC-ESI-MS/MS using a C18 reversed phase column (Shimpack UPLC—2.7 µm, 2 × 150 mm). Negative and positive ion acquisition modes were implemented using a triple quadrupole mass analyzer, Shimadzu® Corporation. Samples were dissolved in HPLC-grade methanol and filtered using a PTFE membrane (0.2 µm). MS-grade mobile phases were used as follows: A: water with 0.1% formic acid (v/v) and B: methanol with 0.1% formic acid (v/v). The elution profile was 0–2 min, 10% B (isocratic); 2–5 min, 10–30% B in A; 5–15 min, 30–70% B in A; 15–22, 70–80% B in A; 22–26, 80% B in A (isocratic); 29–30 min, 80–10% B in A; 30–35 min, 10% B in A (isocratic) with a flow rate of 0.2 mL/min. Mass detection was performed in a mass range over m/z 100–1200. The temperature of the ion source was adjusted to 200 °C, capillary voltage 3000 eV, desolvation and interface temperatures were set to 526 °C and 300 °C, respectively. Cone gas flow was 50 L/h, while the nebulizing gas flow was 3 L/min. For collision-induced dissociation (CID) MS/MS measurements were performed. The cone voltage for fragmentation was adjusted for each mass peak in a range from 10 to 40 eV. Data were processed using lab solutions software.

2.3. Antioxidant Assays

Antioxidant assays were carried out according to previously reported methodologies [25,26]. The antioxidant potential was expressed as: mg Trolox equivalents (TE)/g extract in 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging, cupric reducing antioxidant capacity (CUPRAC) and ferric reducing antioxidant power (FRAP) tests, mmol TE/g extract in phosphomolybdenum assay (PDA), and mg ethylenediaminetetraacetic acid equivalents (EDTAE)/g extract in metal chelating assay (MCA).

2.4. Enzyme Inhibitory Assays

The enzyme inhibitory assays were carried out according to previously reported methodologies [25,26]. The acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibition were expressed as mg galanthamine equivalents (GALAE)/g extract; tyrosinase inhibition was expressed as mg kojic acid equivalents KAE/g extract; amylase and glucosidase inhibition were expressed as mmol acarbose equivalents (ACAE)/g extract.

2.5. Molecular Docking

Crystal structures of the target proteins were retrieved from the protein data bank (https://www.rcsb.org/, accessed on 3 June 2022) with the following IDs: AChE (PDB ID: 6O52) [27], BuChE (6EQP) [28], tyrosinase (6JU7) [29], amylase (6TP0) [30], and Glucosidase
Water molecules and cocrystal ligand were removed before preparation at physiological pH of 7.4 using Biovia Discovery Studio (DS) (San Diego, CA, USA: Accelrys Software Inc., 2012). During the preparation, the following were performed: correction of atom bond orders, addition of missing side-chain atoms and hydrogen, and energy minimization. The three-dimensional (3D) structure of each ligand was downloaded from the PubChem database (pubchem.ncbi.nlm.nih.gov/ accessed on 3 June 2022). Ligand geometry optimization was performed using the “lig prep” toolkit in Biovia DS.

Docking grid and parameter files were generated using the binding coordinates of each ligand in its respective crystal structure in AutodockTools (https://autodock.scripts.edu, accessed on 3 June 2022) [32]. Autodock 4.2’s Lamarckian genetic algorithm was used to generate distinct ligand conformers and docked to the active site of each protein. Multiple ligand poses with different binding energies were returned and the ligand pose with the lowest binding energy was examined for reasonable binding pose using Biovia DS Visualizer.

2.6. Statistical Analysis

Firstly, for each species, one-way analysis of variance followed by Tukey’s post hoc test were used to assess significant differences between the extracts in terms of their antioxidant and enzyme inhibitory activity ($p<0.05$). In addition, the relationship between bioactive compounds and antioxidant activities as well as enzyme inhibitory activities was assessed by calculating Pearson correlation coefficient. Pearson’s coefficients greater than 0.7 were considered significant. Afterwards, principal component analysis (PCA) following by clustered Image Maps were achieved to compare the biological activities of the three species samples. The statistical analysis was conducted using R software v. 4.1.2

3. Results and Discussion

3.1. ESI-MS-MS Fingerprinting of the Three Asteraceae Crude Extracts under Three Different Extraction Methods

Metabolic profiling of *E. ritro*, *C. deflexa*, and *T. decipens* extracts obtained by three extraction techniques viz. homogenizer-assisted extraction (HAE), maceration (MAC), and ultrasonic-assisted extraction (UAE) was performed using HPLC-ESI-MS/MS. A wide range of diversification was observed between the three species due to their different taxonomical backgrounds as well as the implementation of different extraction techniques. The identification of the compounds was based on their mass data, their characteristic MS² fragments, their UV, and by comparison with previously reported compounds in the literature. As can be noticed in Table 1, twenty compounds with different concentrations were identified in the extracts of *E. ritro*, depending on the extraction method. The majority of these were phenolic acids, fatty acids, and flavonoids. Comparative analysis of the percentage of the detected constituents using the three different extraction methods showed that some techniques improved the extraction of certain metabolites in higher yield compared to others. Caffeic and chlorogenic acids extracted by HAE were almost double and 1.5-times the amount of those extracted by MAC or UAE, respectively. Similarly, quercetin-O-hexoside extracted by HAE had a yield 18-times more than that obtained by UAE or MAC. Fatty acid amides were better extracted by HAE; however, they were never detected in the macerated extract. Ultrasonic-assisted extraction (UAE) was the best methodology for the extraction of dicaffeoylquinic acid, trihydroxy-octadecenoic acid, apigenin-O-hexouronide, and apigenin-p-coumaroyl-hexoside isomer, while MAC was better in the extraction of protocatechuic acid hexoside, shimobashiraside C, betulinic acid, and the amine derivative $N,N',N''$-tris-p-coumaroyl spermidine. Surprisingly, naringenin-coumaroyl hexoside was only detected in *Echinops* extract prepared by MAC and betulinic acid was not observed in the extract obtained by HAE.

The chemical composition of *Centaurea deflexa* is summarized in Table 2. Results revealed the presence of 29 compounds belonging to flavonoids (free or as glycosides), fatty acids and their amides, and organic and phenolic acid derivatives. In contrast to *Echinops,*
there is no significant quantitative variation in the metabolites extracted by the three applied techniques, except for luteolin-\(O\)-hexoside, whose quantity was almost doubled in MAC if compared to HAE. Some compounds could be extracted by one method but not with the other(s). One example is the flavonoid eupatorine, which was only successfully extracted by applying MAC. Similarly, salvigenin was only extracted and in high yield (ca. 9%) by MAC. On the other hand, MAC was not successful in extracting dihydroxyoctadecadienoic acid. Additionally, hispidulin could only be extracted by HAE, as coumaroyl quinic acid and octadecadienoic acid were not detected in the homogenizer-assisted extract. Indeed, the percentage of the major compounds of HAE, MAC, and UAE extracts were, respectively, 10.03, 12.26, and 11.28% for oleamide, 10.28, 9.00, and 10.18% for caffeoyl hexoside, 8.86, 7.90, and 7.77% for arctiin, 5.72, 5.66, and 4.98% for apigenin-di-C-hexoside (vicenin-2), and 5.41, 4.70, and 4.81% for chlorogenic acid/neochlorogenic acid. Our results are in concordance with other published studies, which showed the richness of \textit{C. deflexa} extracts through different phenolics compounds [20].

\textit{Tripleurospermum decipens} extract showed the presence of 33 compounds with predominance in flavonoids, phenolic acids, and fatty acids (Table 3). Different yields of the secondary metabolites were detected using the three extraction methods. Tartaric acid, cirsimaritin, and isorhamnetin-\(O\)-hexouronoide were only detected in UAE, while caffeoyl hexoside, naringenin-coumaroyl-hexoside, and syringic acid were only observed in HAE. Although isorhamnetin-\(O\)-hexouronide was not detected in extracts obtained by MAC or by HAE, its aglycone was detected by applying these techniques. MAC was the only effective method in extracting the triterpene compound butanoyl botulinic acid. MAC, likewise, improved the extraction yield of medioresinol by 1.2-fold compared to HAE and 1.8-fold compared to UAE. Fertaric acid was best extracted by MAC, showing double and triple the yields obtained by HAE and UAE, respectively. Most fatty acids in \textit{T. decipens} were obtained in better yields if extracted by UAE; however, the lowest quantities were observed if HAE was implemented. To the best of our knowledge, our study is the first to report the chemical profile of \textit{T. decipens} extract. However, previously published works studied its volatile compounds, reporting the presence of terpenoids [23,33].

Our results showed the impact of the extraction technique on the chemical composition of the three studied Asteraceae species. The extraction methods can influence the nature of the compounds identified as well as their percentage. In general, HAE and UAE are invasive techniques that result in cellular membrane disruption, thus, showing leakage of the metabolites and, consequently, an improvement in the phytochemical yield. Therefore, many metabolites are better extracted by HAE and UAE. In MAC, the extraction efficacy depends on the passive diffusion of the metabolites outside the plant cell, which might also be affected by the molecule size compared to the pore size between the membrane. For example, quercetin-\(O\)-hexoside yield by HAE and UAE was compared to MAC; however, HAE was better than UAE, which might indicate that the compound underwent chemical degradation after applying ultrasonic waves, hence, decreasing its yield dramatically.
Table 1. Tentative identification of key metabolites in *Echinops ritro* under three different extraction methodologies viz. homogenizer-assisted extraction (HAE), maceration (MAC), and ultrasonic-assisted extraction (UAE).

| Peak No. | Rt | [M−H]/[M+H]* | MS/MS | UV (λ_{max}) | Compound Name | Phytochemical Class | Relative Amount (%) | Ref. |
|---------|----|---------------|-------|-------------|---------------|---------------------|---------------------|------|
| 1       | 0.77 | 377           | 341   | 223, 294    | Caffeic acid derivative | Phenolic acid         | 6.98               | [34] |
| 2       | 1.29 | 315           | 152, 108 | 221        | Protocatechuic acid hexoside | Phenolic acid glycoside | 0.65               | [34] |
| 3       | 1.59 | 353           | 191   | 221, 317    | Neochlorogenic acid or Chlorogenic acid | Phenolic acid         | 2.80               | [34] |
| 4       | 2.59 | 353           | 191   | 221, 317    | Neochlorogenic acid or Chlorogenic acid | Phenolic acid         | 8.36               | [34] |
| 5       | 3.02 | 353           | 191   | 221, 317    | Neochlorogenic acid or Chlorogenic acid | Phenolic acid         | 2.78               | [34] |
| 6       | 6.15 | 197           | 197, 169, 124 | 210, 225 | Syringic acid | Phenolic acid         | 0.94               | [34] |
| 7       | 7.03 | 463/465       | 300, 463, 271, 255, 151 | 210, 317 | Quercetin -O-hexoside or hesperitin hexoside | Flavonoid glycoside | 3.78               | [35–37] |
| 8       | 7.37 | 515           | 353, 173, 179, 135 | 223, 294 | Dicaffeoylquinic acid | Phenolic acid         | 2.63               | [34] |
| 9       | 7.49 | 515           | 353, 173, 179, 135 | 223, 294 | Dicaffeoylquinic acid | Phenolic acid         | 6.92               | [34] |
| 10      | 7.68 | 271           | 204, 325 | 223, 294 | Apigenin-O-hexuronide | Flavonoid glycoside | 0.42               | [38] |
| 11      | 7.70 | 515           | 353, 173, 179, 135 | 223, 294 | Dicaffeoylquinic acid | Phenolic acid         | 9.02               | [34] |
| 12      | 8.73 | 435           | 297, 315, 163, 152, 137, 108 | 217, 324 | Shimobashiraside C | Phenolic acid ester glycoside | 2.20               | [34] |
| 13      | 9.0  | 582/584       | 462, 342, 299, 292, 119 | 222, 289 | N',N',N''-Tris-p-coumaroyl spermidine | Amine derivative      | 1.34               | [39] |
| 14      | 9.47 | 327           | 171, 183, 211, 229, 291, 199 | n.d. | Trihydroxy-octadecadienoic acid | Fatty acid            | 0.71               | [34] |
| 15      | 9.64 | 577           | 269, 145, 431, 117 | 206, 318 | Apigenin-(p-coumaroyl)-hexoside isomer or rhoifolin | Flavonoid glycoside | 0.70               | [34,40] |
| 16      | 9.80 | 579           | 271, 145, 119, 163, 295 | 221, 317 | Naringenin-coumaroyl-hexoside | Flavonoid glycoside | -                 | [34] |
| 17      | 10.02 | 329          | 211, 229, 171, 139, 99, 155 | n.d. | Trihydroxyoctadecadienoic acid or pinelic acid | Fatty acid            | 2.21               | [34,40] |
| 18      | 12.35 | 293          | 265, 275 | n.d. | Octadecadienoic acid | Fatty acid            | 0.43               | [40] |
| 19      | 14.93 | 293          | 265, 275 | n.d. | Octadecadienoic acid | Fatty acid            | 0.44               | [40] |
| 20      | 15.11 | 293          | 265, 275 | n.d. | Octadecadienoic acid | Fatty acid            | 0.24               | [40] |
| 21      | 16.13 | 295          | 277, 171, 195, 183 | n.d. | Hydroxyoctadecadienoic acid | Fatty acid            | 1.68               | [34,40] |
| 22      | 19.64 | 455          | 455   | n.d. | Betulinic acid | Triterpene | -                 | [41] |
| 23      | 21.31 | 256          | 116, 102, 88 | n.d. | Palmitamid | Fatty acid amide | 13.8               | [42,43] |
| 24      | 21.95 | 282          | 97, 69, 149, 163 | n.d. | Oleamide | Fatty acid amide | 49.4               | [42,43] |
| 25      | 22.60 | -/282        | 97, 69, 149, 163 | n.d. | Oleamide | Fatty acid amide | 0.57               | [42,43] |

Rt: retention time recorded for each compound; λ_{max}: wavelength of maximum UV absorptio; HAE: homogenizer-assisted extraction; MAC: maceration; UAE: ultrasonic-assisted extraction.
| Peak No. | R<sub>t</sub> | ([M−H]/[M+H])<sup>+</sup> | MS/MS | UV (λ<sub>max</sub>) | Compound Name | Phytochemical Class | Relative Amount (%) | Ref. |
|---------|-------------|----------------|---------|-----------------|----------------|-----------------|---------------------|-----|
| 1.      | 0.75        | 191            | -       | 265             | Quinic acid    | Organic acid    | 4.74                | [44] |
| 2.      | 1.65        | 343            | 267, 203, 177, 135 | 275, 330       | Eupatorin      | Flavonoid       | 3.96                | -   |
| 3.      | 2.05        | 417            | 285, 249, 199, 144 | 265, 360       | Kaempferol pentoside | Phenolic acid | 1.95                | -   |
| 4.      | 2.45        | 353            | 191      | 339             | Chlorogenic acid/ Neochlorogenic acid | Phenolic acid | 5.41                | -   |
| 5.      | 3.40        | 341            | 193, 175 | 265             | Caffeoyl hexoside | Phenolic acid | 10.28               | -   |
| 6.      | 4.44        | 337            | 191, 163 | 265             | Coumaroyl quinic acid | Phenolic acid | -                   | -   |
| 7.      | 5.25        | 593            | 271, 333 | 271, 333        | Apigenin-di-C-hexoside (Vicenin-2) | Flavonoid glycoside | 5.72                | -   |
| 8.      | 5.75        | 579            | 459, 599, 369 | 271, 330        | Naringonin-O-neohesperidoside (Naringin) | Flavonoid glycoside | 2.13                | -   |
| 9.      | 6.02        | 197            | 169, 124 | 271             | Syringic acid  | Phenolic acid | 4.71                | -   |
| 10.     | 6.09        | 337            | 191, 163 | 265             | Coumaroylquinic acid | Phenolic acid | 3.23                | -   |
| 11.     | 6.20        | 563            | 503, 473, 443, 383, 353 | 271, 333 | Apigenin-C-hexoside-C-pentoside (Schaftoside) | Flavonoid glycoside | 3.23                | -   |
| 12.     | 7.42        | 515            | 285, 191, 179, 135 | 234, 294        | Dicaffeoylquinic acid | Phenolic acid | 2.73                | -   |
| 13.     | 7.52        | 447            | 285      | 252, 340        | Luteolin-O-hexoside | Flavonoid glycoside | 1.42                | -   |
| 14.     | 8.35        | 579            | 371      | 255, 278        | Arctin         | Lignan         | 8.86                | 7.90 |
| 15.     | 8.67        | 285            | -        | 252, 340        | Luteolin       | Flavonoid      | 2.56                | 1.91 |
| 16.     | 9.40        | 327            | 229, 211, 171, 139 | n.d.           | Trihydroxyoctadecadienoic acid | Fatty acid | 1.46                | 1.70 |
| 17.     | 9.57        | 783            | 639, 545, 399 | 269, 327        | Jaceosidin di-O-hexoside-deoxyhexoside | Flavonoid glycoside | 2.06                | 1.24 |
| 18.     | 9.61        | 290            | 299, 284, 256 | 269, 327        | Trihydroxyoctadecenoic acid | Flavonoid | 2.06                | -   |
| 19.     | 9.97        | 329            | 229, 211, 183, 171 | n.d.           | Trihydroxyoctadecenoic acid | Fatty acid | 2.47                | 2.23 |
| 20.     | 13.78       | 313            | 255, 225 | 276, 331        | Dihydroxydimethoxyflavone (Cirsimaritin) | Flavonoid | 0.27                | 0.40 |
| 21.     | 15.00       | 293            | 275, 211, 183, 171 | n.d.           | Octadecadienoic acid | Fatty acid | 2.63                | 1.25 |
| 22.     | 16.10       | 295/297        | 277, 171 | n.d.           | Hydroxyoctadecadienoic acid | Fatty acid | 3.01                | 4.15 |
| 23.     | 16.60       | 311            | 293, 183, 171, 153, 137, 131 | n.d.           | Dihydroxyoctadecadienoic acid | Fatty acid | 1.09                | -   |
| 24.     | 17.43       | 293            | 275, 211, 183, 171 | n.d.           | Octadecadienoic acid | Fatty acid | -                   | 1.58 |
| 25.     | 20.77       | 271            | 225      | n.d.           | Hydroxyhexadecanoic acid | Fatty acid | 2.50                | 2.51 |
| 26.     | 21.28       | 256.25         | 116, 102, 88, 71 | n.d.           | Palmitamide    | Fatty acid amide | 3.39                | 3.27 |
| 27.     | 21.90       | 282.30         | 265, 247, 149, 135, 121, 111, 97, 83 | n.d.           | Oleamide       | Fatty acid amide | 10.03               | 12.26 |
| 28.     | 21.83       | 343            | 315, 299, 285, 253, 225 | 276, 331        | Dihydroxytrimethoxyflavone | Flavonoid | 2.08                | 1.47 |
| 29.     | 24.80       | 327            | -        | 270, 331        | Hydroxytrimethoxyflavone (Salvigenin) | Flavonoid | 2.08                | 1.47 |

R<sub>t</sub>: retention time recorded for each compound; λ<sub>max</sub>: wavelength of maximum UV absorptio; HAE: homogenizer-assisted extraction; MAC: maceration; UAE: ultrasonic-assisted extraction.
Table 3. Tentative identification of key metabolites in *Tripleurospermum decipiens* under three different extraction methodologies viz. homogenizer-assisted extraction (HAE), maceration (MAC), and ultrasonic-assisted extraction (UAE).

| Peak No. | Rt (min) | [M−H]/[M+H]+ MS/MS | UV ($\lambda_{max}$) | Compound Name | Phytochemical Class | Relative Amount (%) | Ref. |
|----------|----------|----------------------|----------------------|-----------------|---------------------|---------------------|------|
|          |          |                      |                      |                 | HAE                 | MAC                 | UAE  |
| 1.       | 0.76     | 149 - 225            |                      | Tartaric acid   | Organic acid        | -                   | 3.63 | [58] |
| 2.       | 1.21     | 191 - 265            |                      | Quinic acid     | Organic acid        | 1.55                | 2.04 | 0.96 | [44] |
| 3.       | 1.52     | 353 - 325            |                      | Chlorogenic acid | Phenolic acid       | 0.38                | 0.43 | 7.35 | [34] |
| 4.       | 2.38     | 353 - 325            |                      | Phenolic acid   | Phenolic acid       | 7.78                | 9.66 | -    | [34] |
| 5.       | 2.73     | 353 - 325            |                      | Phenolic acid   | Phenolic acid       | 1.47                | -    | -    | [34] |
| 6.       | 3.46     | 193, 161             |                      | Caffeoyl hexoside | Phenolic acid glycoside | 1.22 | -    | -    | [47] |
| 7.       | 3.97     | 207, 163, 119, 89    | n.d.                | Medioresinol    | Lignan              | 8.80                | 11.19| 6.23 | [59] |
| 8.       | 4.83     | 179, 149, 135        |                      | Caffeic acid    | Phenolic acid       | 4.25                | 5.61 | 3.46 | [60,61] |
| 9.       | 5.63     | 207, 209, 179, 135, 97/99 |                      | 2-benzoylbenzoic acid | Phenolic acid | 2.10                | 2.13 | 2.58 | [62] |
| 10.      | 6.01     | 169, 124             |                      | Syringic acid   | Phenolic acid       | 0.99                | -    | -    | [52] |
| 11.      | 6.28     | 316 - 371            |                      | Myricetin-O-hexoside | Flavonoid glycoside | 1.02                | 0.84 | 0.77 | [63] |
| 12.      | 6.52     | 353, 191, 173, 179   |                      | Fertaric acid   | Phenolic acid       | 0.98                | 1.99 | 0.62 | [65] |
| 13.      | 7.06     | 360 - 324            |                      | Patuletin-O-hexoside | Flavonoid glycoside | 1.32                | 1.05 | 1.03 | [66,67] |
| 14.      | 7.45     | 353, 191, 173, 179   |                      | Dicaffeoylquinic acid | Phenolic acid | 8.54                | 8.25 | 6.09 | [68] |
| 15.      | 7.66     | 191, 169, 149, 134   |                      | Myricetin-O-hexoside | Flavonoid glycoside | 9.99                | 10.13| 7.67 | [68] |
| 16.      | 7.98     | 207, 163, 119, 89    |                      | Medioresinol    | Lignan              | 8.80                | 11.19| 6.23 | [59] |
| 17.      | 8.33     | 271, 145, 119, 163, 295 |                      | Naringenin-coumaroyl-O-hexoside | Flavonoid glycoside | 1.63                | -    | -    | [34] |
| 18.      | 8.66     | 130, 151             |                      | Isorhamnetin-O-hexoside | Flavonoid glycoside | 2.33                | 2.52 | 2.16 | [68] |
| 19.      | 9.00     | 330, 315, 287        |                      | Quercetin-dimethyl ether | Flavonoid | 0.64                | 0.91 | 1.23 | [66] |
| 20.      | 9.43     | 291, 229, 211, 183, 171, 147 | n.d.            | Trifluoro-octadecanoic acid | Fatty acid | 0.40                | 0.58 | 0.96 | [44] |
| 21.      | 9.60     | 665, 45, 399         |                      | Acetylsin-O-hexoside | Flavonoid glycoside | 1.48                | 2.13 | 3.56 | [55] |
| 22.      | 9.81     | 300, 151             |                      | Isorhamnetin     | Flavonoid glycoside | 3.64                | 4.98 | 5.71 | [68] |
| 23.      | 9.98     | 299, 229, 211, 171   | n.d.                | Trifluoro-octadecanoic acid | Fatty acid | 1.57                | 2.45 | 3.15 | [55,56] |
| 24.      | 11.46    | 217, 185, 99         | n.d.                | Eicosadienoic acid | Fatty acid | 0.68                | 1.56 | 1.98 | [70] |
| 25.      | 12.19    | 207                 | n.d.                | Hexadecadienoic acid | Fatty acid | 0.43                | 1.63 | 2.24 | [55,56] |
| 26.      | 13.50    | 255, 325             |                      | Dihydroxydimethoxyflavone (Cirsimaritin) | Flavonoid | -                   | -    | 0.13 | [57] |
| 27.      | 13.78    | 255, 325             |                      | Dihydroxydimethoxyflavone (Cirsimaritin) | Flavonoid | -                   | -    | 0.20 | [57] |
| 28.      | 14.92    | 293                 | n.d.                | Octadecadienoic acid | Fatty acid | 1.73                | 3.99 | 2.93 | [55,56] |
| 29.      | 15.08    | 293                 | n.d.                | Octadecadienoic acid | Fatty acid | -                   | -    | 1.54 | [55,56] |
| 30.      | 15.44    | 293                 | n.d.                | Octadecadienoic acid | Fatty acid | 0.75                | -    | 1.08 | [70] |
| 31.      | 16.04    | 293                 | n.d.                | Hydroxystearic acid | Fatty acid | -                   | 3.37 | 3.76 | [55,56] |
| 32.      | 16.08    | 293                 | n.d.                | Hydroxystearic acid | Fatty acid | 2.04                | -    | -    | [55,56] |
| 33.      | 16.41    | 311                 | n.d.                | Hydroxystearic acid | Fatty acid | 0.38                | -    | 0.31 | [55,56] |
| 34.      | 16.59    | 183, 171, 133, 137, 131 | n.d.           | Hydroxystearic acid | Fatty acid | 1.33                | -    | 1.33 | [55,56] |
| 35.      | 16.88    | 293                 | n.d.                | Octadecadienoic acid | Fatty acid | -                   | -    | 0.19 | [55,56] |
| 36.      | 17.20    | 293                 | n.d.                | Octadecadienoic acid | Fatty acid | -                   | -    | 0.23 | [55,56] |
| 37.      | 18.16    | 295                 | n.d.                | Hydroxyoctadecenoic acid | Fatty acid | 1.32                | 2.21 | 3.74 | [55,56] |
| 38.      | 18.83    | 297                 | n.d.                | Hydroxyoctadecenoic acid | Fatty acid | 1.70                | 1.49 | 1.91 | [55,56] |
| 39.      | 20.22    | 297                 | n.d.                | Hydroxyoctadecenoic acid | Fatty acid | 0.89                | -    | 0.29 | [55,56] |
| 40.      | 20.72    | 271                 | n.d.                | Hydroxyhexadecanoic acid | Fatty acid | -                   | -    | -    | -    |

Rt: retention time recorded for each compound; $\lambda_{max}$: wavelength of maximum UV absorptio; HAE: homogenizer-assisted extraction; MAC: maceration; UAE: ultrasonic-assisted extraction.
3.2. In Vitro Assessment of the Antioxidant Activities in the Extracts

With the aim of highlighting the antioxidant properties in the extracts of three plants, six antioxidant tests were carried out in vitro. Firstly, the antioxidant activities in each Asteraceae species were discussed individually. As shown in Figure 1, all extracts showed significant antioxidant effects with variability related to the nature of the extracts.

*Tripleurospermum* decipiens: MAC and UAE revealed the strongest antioxidant activity for the CUPRAC test, with values of 170.57 ± 4.33 and 173.45 ± 2.11 mg TE/g for the MAC and UAE samples, respectively. In addition, HAE and UAE demonstrated the strongest antioxidant effects for the DPPH test (HAE = 48.62 ± 0.14; UAE = 48.46 ± 0.04 mg TE/g). Further, the sample of UAE had the highest FRAP (101.54 ± 0.46 mg TE/g) and PBD (1.42 ± 0.03 mg TE/g) activity. At last, no significant difference was obtained between the extracts for antioxidant capacity using the MCA and ABTS tests.

*Echinops nitro*: No significant difference was observed between the extracts for antioxidant capacity using the MCA and PBD tests. The extract obtained with HAE displayed strong antioxidant capacity with ABTS (173.09 ± 2.73 mg TE/g), FRAP (144.61 ± 1.75 mg TE/g), and CUPRAC (273.27 ± 4.32 mg TE/g), while both extracts derived from HAE and UAE exhibited the highest antioxidant effect with DPPH (HAE= 160.83 ± 1.28; UAE = 159.21 ± 1.18 mg TE/g) (Table 4).

| Species                  | Extraction Method | DPPH (mg TE/g) | ABTS (mg TE/g) | CUPRAC (mg TE/g) | FRAP (mg TE/g) | MCA (mg EDTAE/g) | PBD (mmol TE/g) |
|--------------------------|------------------|----------------|----------------|------------------|----------------|------------------|---------------|
| *Echinops nitro*         | HAE              | 160.83 ± 1.28  | 173.09 ± 2.73  | 273.27 ± 4.32 a  | 144.61 ± 1.75 a| 16.43 ± 1.49 a   | 1.14 ± 0.12 a   |
|                          | MAC              | 148.68 ± 1.25 b| 159.62 ± 2.10 c| 262.04 ± 2.08 b  | 134.32 ± 1.03 b| 15.97 ± 0.29 a   | 1.22 ± 0.10 a   |
|                          | UAE              | 159.21 ± 1.18 a| 165.36 ± 0.59 b| 254.98 ± 4.50 b  | 132.92 ± 3.15 b| 17.38 ± 0.12 a   | 1.07 ± 0.07 a   |
| *Centaurea deflexa*      | HAE              | 48.62 ± 0.14 a | 101.30 ± 0.06 a| 144.50 ± 2.23 a  | 97.34 ± 0.77 a | 19.01 ± 0.46 a   | 1.02 ± 0.05 a   |
|                          | MAC              | 48.11 ± 0.04 b | 101.40 ± 0.07 a| 170.57 ± 4.33 a  | 99.39 ± 0.66 b | 19.40 ± 0.30 a   | 1.19 ± 0.04 b   |
|                          | UAE              | 48.46 ± 0.04 a | 101.39 ± 0.18 a| 173.45 ± 2.11 a  | 101.54 ± 0.46 a| 19.82 ± 0.14 a   | 1.42 ± 0.03 a   |
| *Tripleurospermum decipiens* | HAE            | 48.83 ± 0.06 a | 101.34 ± 0.09 a| 197.81 ± 0.12 b  | 107.73 ± 0.33 a| 18.41 ± 0.29 b   | 1.03 ± 0.02 b   |
|                          | MAC              | 48.43 ± 0.03 b | 101.20 ± 0.09 a| 195.22 ± 2.17 b  | 103.81 ± 0.89 b| 17.68 ± 0.15 c   | 1.34 ± 0.16 a   |
|                          | UAE              | 48.32 ± 0.05 c | 101.23 ± 0.17 a| 200.72 ± 2.79 a  | 144.61 ± 1.75 a| 19.69 ± 0.21 a   | 1.30 ± 0.05 a   |

Values are reported as mean ± SD of three parallel measurements. TE: Trolox equivalent; EDTAE: EDTA equivalent. HAE: homogenizer-assisted extraction; MAC: maceration; UAE: ultrasonic-assisted extraction. Different letters ("a" indicates the strongest ability) indicated significant differences for each species extracts (p < 0.05).

*Tripleurospermum decipiens*: Considering this species, UAE sample showed the strongest antioxidant activity for the MCA and CUPRAC tests, with antioxidant values of 19.69 ± 0.21 mg EDTAE/g and 200.72 ± 2.79 mg TE/g, respectively. In contrast, the HAE sample was the most effective DPPH scavenger (48.83 ± 0.06 mg TE/g). Moreover, both UAE and HAE samples exhibited the strongest FRAP activity, with the value of 109.58 ± 1.50 and 107.73 ± 0.33 mg TE/g, respectively. The highest PBD activity was demonstrated by UAE (1.30 ± 0.05 mg TE/g) and MAC (1.34 ± 0.16 mg TE/g) samples.

A previous study showed that the antioxidant activities in *E. nitro* were reported by AYDIN, et al. [71], who obtained important results. However, both of the other plants (*T. decipiens* and *C. deflexa*) have not yet been investigated for their antioxidant effects. Nevertheless, antioxidant properties of other species of *Centaurea* and *Tripleurospermum* genus have been demonstrated by different studies [72–74]. The antioxidant activities of plant extracts can be attributed to their major bioactive compounds. Indeed, for *E. nitro*, DPPH showed positive and significant correlation with En23 and En24 (Figure 2). Similarly, a strong positive correlation was found between ABTS, CUPRAC, and FRAP and En4, En5, En7, En11, and En25. ABTS activity was also bound to En23 and En24. In addition, MCA was significantly linked to En6, En10, En14, En17, En20, and En21, whereas PBD was positively correlated with En16 and En22. Regarding *C. deflexa*, a positive significant correlation was observed between DPPH and Cd1, Cd3, Cd4, Cd5, Cd9, Cd12, Cd15, Cd18, Cd19, Cd21, Cd23, and Cd28. Furthermore, a significant positive Pearson coefficient was obtained between ABTS and CUPRAC, FRAP, MCA, and PBD and Cd6, Cd13, and Cd20.
In addition, ABTS and CUPRAC were positively and significantly correlated with Cd16, Cd22, Cd24, and Cd27 while FRAP, MCA, and PBD were linked to Cd26. In *T. decipiens*, both ABTS and DPPH was positively and significantly bound to Td5, Td6, Td10, Td11, Td14, Td18, Td33, and Td40. Similarly, a positive and significant correlation was found between CUPRAC, FRAP, and MCA and Td1, Td3, Td9, Td17, Td27, Td28, Td30, Td31, Td35, Td36, Td37, and Td39. Furthermore, PBD was positively and significantly correlated with Td20, Td23, Td24, Td25, Td26, Td29, Td32, and Td38.

Main identified substances, such as oleamide (found in *E. ritro* and *C. deflexa* extracts), showed antioxidant properties according to some reported pharmacological studies [75,76]. Moreover, chlorogenic acid, present in plant extracts, also demonstrated interesting remarkable in vitro and in vivo activities by several investigations [77,78].
Figure 1. Antioxidant properties of the tested extracts. TE: Trolox equivalent; EDTAE: EDTA equivalent. HAE: homogenizer-assisted extraction; MAC: maceration; UAE: ultrasonic-assisted extraction. The statistical evaluation was performed by ANOVA (the letters (a, b, and c) indicate significant differences in the extraction methods for same species, by Tukey’s test, \( p < 0.05 \)).
was observed between the tyrosinase inhibition potentiality of the extract obtained from (Figure 3). Regarding Centaurea deflexa plant extracts was carried out by the inhibitory effect on tyrosinase, an enzyme activating
\[\alpha\]-value (62.19 ± 0.38 and 62.28 ± 0.59 mg KAE/g) of tyrosinase. No significant difference was observed between the tyrosinase inhibition potentiality of the extract obtained from Centaurea deflexa (Figure 3). Regarding Tripleurospermum decipens, the extract obtained from UAE showed the highest anti-tyrosinase effect, followed by the extract obtained from MAC and HAE.

3.3. Enzyme Inhibitory Effects

Inhibition of carbohydrate and glyceride-hydrolyzing enzymes is a promising therapeutic strategy in the management of type 2 diabetes mellitus (T2DM). In our case, we evaluated, in vitro, the inhibitory capacity of EOs, obtained from different phenological stages, on the catalytic activity of \(\alpha\)-glucosidase and \(\alpha\)-amylase. The results showed that extracts obtained from C. deflexa using MAC and UAE methods showed important inhibition of \(\alpha\)-amylase, with inhibitory values of 0.30 ± 0.01 and 0.28 ± 0.01 mmol ACAE/g for MAC and UAE, respectively. However, the extract obtained from T. decipens using the UAE and HAE methods revealed the highest inhibitory value of \(\alpha\)-glucosidase (0.98 ± 0.01 and 0.91 ± 0.01 mmol ACAE/g). Interestingly, all the extracts of E. nitro showed the same inhibitory effects on \(\alpha\)-amylase and \(\alpha\)-glucosidase. Similarly, T. decipens samples exhibited the inhibitory effects on \(\alpha\)-amylase, while those of C. deflexa had the same inhibitory effects on \(\alpha\)-glucosidase.

On the other hand, skin aging is a natural process related to endogenous (metabolic, cellular, and hormonal processes) and exogenous (chronic exposure to pollutants, toxic chemicals, ionizing radiation, etc.) factors that cumulatively damage skin appearance and physiology [79,80]. In our study, the evaluation of the dermatoprotective activity of plant extracts was carried out by the inhibitory effect on tyrosinase, an enzyme activating the oxidation of tyrosine, leading to melanin secretion. As can been seen in Table 5, the extract obtained by MAC and HAE methods from E. nitro showed the important inhibitory value (62.19 ± 0.38 and 62.28 ± 0.59 mg KAE/g) of tyrosinase. No significant difference was observed between the tyrosinase inhibition potentiality of the extract obtained from C. deflexa (Figure 3). Regarding T. decipens, the extract obtained from UAE showed the highest anti-tyrosinase effect, followed by the extract obtained from MAC and HAE.
Table 5. Enzyme inhibitory abilities of the methanol extracts of the three Asteraceae species.

| Species            | Extraction Methods | \( \text{AChE (mg GALAE/g)} \) | \( \text{BChE (mg GALAE/g)} \) | \( \text{Tyrosinase (mg KAE/g)} \) | \( \text{Amylase (mmol ACAE/g)} \) | \( \text{Glucosidase (mmol ACAE/g)} \) |
|--------------------|--------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| \( \text{Echinops ritro} \) | HAE                | 2.41 ± 0.04 \( ^a \)            | 0.80 ± 0.10 \( ^a \)           | 62.28 ± 0.59 \( ^a \)          | 0.29 ± 0.01 \( ^a \)           | 1.01 ± 0.03 \( ^a \)          |
|                    | MAC                | 2.31 ± 0.02 \( ^b \)            | 0.87 ± 0.11 \( ^a \)           | 62.19 ± 0.38 \( ^a \)          | 0.29 ± 0.01 \( ^a \)           | 1.06 ± 0.01 \( ^a \)          |
|                    | UAE                | 2.27 ± 0.01 \( ^b \)            | 0.36 ± 0.03 \( ^b \)           | 60.64 ± 0.48 \( ^b \)          | 0.29 ± 0.01 \( ^a \)           | 0.97 ± 0.10 \( ^a \)          |
| \( \text{Centaurea deflexa} \) | HAE                | 2.27 ± 0.01 \( ^a \)            | 1.49 ± 0.01 \( ^a \)           | 62.29 ± 0.99 \( ^a \)          | 0.26 ± 0.01 \( ^b \)           | 0.99 ± 0.04 \( ^a \)          |
|                    | MAC                | 2.13 ± 0.05 \( ^b \)            | 1.31 ± 0.06 \( ^a \)           | 62.32 ± 0.03 \( ^a \)          | 0.30 ± 0.01 \( ^a \)           | 0.98 ± 0.08 \( ^a \)          |
|                    | UAE                | 2.25 ± 0.02 \( ^a \)            | 1.34 ± 0.13 \( ^a \)           | 62.80 ± 0.31 \( ^a \)          | 0.28 ± 0.01 \( ^a \)           | 0.90 ± 0.07 \( ^a \)          |
| \( \text{Tripleurospermum decipens} \) | HAE                | 2.46 ± 0.01 \( ^a \)            | 1.69 ± 0.11 \( ^a \)           | 62.56 ± 0.79 \( ^b \)          | 0.29 ± 0.01 \( ^a \)           | 0.91 ± 0.04 \( ^a \)          |
|                    | MAC                | 2.22 ± 0.02 \( ^a \)            | 1.96 ± 0.18 \( ^a \)           | 63.86 ± 0.21 \( ^a \)          | 0.30 ± 0.01 \( ^a \)           | 0.81 ± 0.01 \( ^b \)          |
|                    | UAE                | 2.27 ± 0.02 \( ^b \)            | 1.52 ± 0.03 \( ^b \)           | 64.30 ± 0.41 \( ^a \)          | 0.31 ± 0.01 \( ^a \)           | 0.98 ± 0.04 \( ^a \)          |

Values are reported as mean ± SD of three parallel measurements. GALAE: Galantamine; KAE: Kojic acid; ACAE: Acarbose equivalent; HAE: homogenizer-assisted extraction; MAC: maceration; UAE: ultrasonic-assisted extraction. Different letters ("a" indicates the strongest ability) indicated significant differences for each species extracts \( (p < 0.05) \).

Figure 3. Enzyme inhibitory of the tested extracts. GALAE: Galantamine; KAE: Kojic acid; ACAE: Acarbose equivalent; HAE: homogenizer-assisted extraction; MAC: maceration; UAE: ultrasonic-assisted extraction. The statistical evaluation was performed by ANOVA (by Tukey’s test, \( p < 0.05 \)).

On the other hand, hyperactivities of acetylcholinesterase enzymes significantly impact memory functions and can lead, with other risk factors, to Alzheimer’s disease. In this regard, natural inhibitors that can reduce or minimize catalytic activities of acetylcholinesterase lead to an increase in acetylcholine levels in the synapses and may, therefore, improve memory function in Alzheimer’s patients. In our study, the HAE extract from \( E. ritro \) suppressed AChE levels at inhibitory values of 2.41 ± 0.04 mg GALAE/g. In addition, HAE and MAC extracts from \( E. ritro \) demonstrated stronger galantamine equivalent values against BChE (HAE = 0.80 ± 0.10 and MAC = 0.87 ± 0.11 mg GALAE/g). Concerning \( C. deflexa \), UAE and HAE extracts revealed the highest anti-AChE activity (UAE = 2.25 ± 0.02; HAE = 2.27 ± 0.01 mg GALAE/g); nonetheless, all extracts exhibited the same anti-BChE activity. As for \( T. decipens \), among the three extracts, HAE and MAC had the greatest capacity to inhibit AChE and BChE, respectively, with a value of 2.46 ± 0.01 and 1.96 ± 0.18 mg GALAE/g, respectively (Figure 3). Furthermore, the observed bioactivity may be...
correlated to the high levels of the numerous bioactive compounds present in the extracts. Indeed, examination of Pearson correlation coefficient in Figure 4 highlighted the presence of significant correlation between the evaluated bioactivities and the bioactive compounds. As an example, concerning *E. nitro*, significant correlation was found between AChE and En4, En5, En7, En11, and En25. Similarly, amylase was bound to En4, En5, En7, En11, En23, En24, and En25, while glucosidase was correlated to En16 and En22. Regarding *C. deflexa*, a strong positive correlation was found between AChE and Cd1, Cd3, Cd4, Cd5, Cd9, Cd12, Cd15, Cd19, Cd21, Cd23, and Cd28. BChE was positively linked to Cd1, Cd4, Cd12, Cd14, Cd18, Cd21, and Cd23. Further, anti-tyrosinase activity was significantly correlated to Cd19, Cd26, and Cd28. In addition, significant positive correlation was obtained between α-amylase inhibition and Cd2, Cd6, Cd13, Cd16, Cd20, Cd22, Cd24, Cd27, Cd29, and between α-glucosidase and Cd7, Cd8, Cd10, Cd11, Cd17, and Cd25.

Figure 4. Relationship between secondary metabolites and enzyme inhibitory activity (*p* < 0.05). (A) *Echinops nitro*. (B) *Centaurea deflexa*. (C) *Tripleurospermum decipens*. For compound numbers refer to Tables 1–3.

To our knowledge, there are few published data concerning the enzymatic inhibitory effects of the studied plants, which makes our investigation the first original work. It was reported in the literature that enzyme inhibitory effects of *T. decipiens* were investigated over a long time by Göger, et al. [81]. However, other species in *Echinops*, *Centaurea*, and *Tripleurospermum* genus exhibited different biological and pharmacological activities, including antidiabetic and dermatoprotective effects, as well as neuroprotective properties [82,83]. Different studies have proved the biological effects of chlorogenic acid, including its antidiabetic effects [77,84].

3.4. Comparison of the Biological Activities of the Three Species Samples

The principal component analysis was conducted to compare the extracts of the three *Asteraceae* species in terms of their antioxidant and enzyme inhibitory activities. A summary
of variability and eigenvalues obtained from PCA is provided in Figure 5. The first three components captured approximatively 82% of the variability and were the only components for which the eigenvalues were higher than 1. These three components were linked with a variable degree covariance to the following group of bioactivities: (1) DPPH, ABTS, FRAP, CUPRAC, BChE, and MCA, (2) Amylase, PBD, and tyrosinase, (3) AChE. Figure 5 depicts the distribution of samples on the three scatter plots, derived from PC1, PC2, and PC3. In the first two scatter plots, the samples of *E. nitro* was separated from those of *C. deflexa* and *T. decipiens*, along the first component. In addition, in the first scatter plot, the extracts obtained from *C. deflexa* using HAE were separated from the other samples. Similarly, in the second scatter plot, the extracts derived from *T. decipens* using HAE were removed from the remaining samples. These two tendencies were also observed in the third scatter plot. These observations suggested the existence of two main clusters as well as some subgroups in one of the two main groups. For better visualization of the clusters, a heatmap was produced for the result of the PCA, in consideration of the retained components. As can be seen in Figure 6, the extracts were split into two main clusters. Cluster A comprised the extracts obtained from *E. nitro* using HAE and UAE. Both samples showed the highest antioxidant and anti-BChE activities. Cluster B, enclosing the remaining samples, can be divided into four sub-clusters, namely B1, B2, B3, and B5. Cluster B1 contained *T. decipens*-HAE and was characterized by the strongest anti-AChE activity. Cluster B4 comprised *T. decipens*-UAE and *T. decipens*-MCA extracts. Both exhibited remarkably anti-tyrosinase, anti-BChE, and anti-amylase activities, compared to other samples.

Figure 5. Principal component analysis. (A) Scatter plot showing the distribution of the samples in the factorial plan derived from the three retained principal components. (B) Contribution of biological activities on the principal components of PCA.
Figure 6. Clustered image map (red color: high bioactivity. Blue color: low bioactivity) on biological activities dataset.

3.5. Molecular Docking

Compounds accounting for \( \geq 5\% \) of the total bioactive compound content were further studied using molecular docking to estimate their binding strength and to predict their binding mode to each of the five enzymes (AChE, BChE, tyrosinase, amylase, and glucosidase). These compounds show the potential to bind to all enzymes, as suggested by their binding energy scores (Table 6). Furthermore, the majority of compounds displayed binding preference for AChE, BChE, and glucosidase. For instance, apigenin-di-C-hexoside (Vicenin-2) and arctiin bound strongly to the AChE and, to a lesser extent, to BChE, moderately to the amylase and glucosidase, but modestly to the tyrosinase. On the other hand, chlorogenic acid and dicaffeoylquinic acid preferentially bound to glucosidase. Hence, we visualized protein–ligand interaction details for these compounds to examine the interaction patterns.

The major contributors to the interaction in all the docking complexes are H-bonds and \( \pi-\pi \) interactions formed between hydroxyl groups and aromatic rings on the ligands and the residues in the active site of the target enzymes (Figure 7). In addition, a few hydrophobic contacts and several van der Waals interactions increased the binding strength. Apigenin-di-C-hexoside (Vicenin-2) spanned the cavity of AChE by forming multiple H-bonds with polar amino acid residues at the entrance to and deep inside the tunnel (Figure 7A). Arctiin bound strongly to BChE, mainly via \( \pi-\pi \) interactions and a couple of van der Waals interactions (Figure 7B). Amylase formed multiple H-bonds, a couple of \( \pi-\pi \) interactions, and van der Waals interactions throughout the amylase catalytic channel (Figure 7C). Similarly, the major interactions between glucosidase and chlorogenic acid are H-bonds formed throughout the glucosidase active site (Figure 7D). Therefore, the biological activities displayed by these compounds are likely due to the inhibition of these enzymes.
Table 6. Binding energy scores of key metabolites in Asteraceae species extracts obtained by different extraction methods. Scores highlighted in yellow may result in good inhibition.

| Compound                        | AChE  | BChE  | Tyrosinase (Kcal/mol) | Amylase | Glucosidase |
|---------------------------------|-------|-------|-----------------------|---------|-------------|
| Caffeic acid derivative         | −6.77 | −5.56 | −4.54                 | −4.08   | −5.03       |
| Neochlorogenic acid             | −10.83| −8.61 | −4.62                 | −6.02   | −5.21       |
| Chlorogenic acid                | −11.30| −7.54 | −4.08                 | −5.78   | −12.29      |
| Dicaffeoylquinic acid           | −10.44| −10.23| −4.34                 | −6.53   | −11.31      |
| Palmitamide                     | −6.02 | −5.68 | −3.45                 | −2.84   | −3.17       |
| Oleamide                        | −7.61 | −5.86 | −2.14                 | −2.89   | −3.16       |
| Quinic acid                     | −7.35 | −6.33 | −2.67                 | −4.56   | −6.46       |
| Caffeoyl hexoside               | −8.50 | −7.50 | −5.11                 | −8.36   | −10.38      |
| Apigenin-di-C-hexoside (Vicenin-2)| −16.15| −12.02| −5.21                 | −9.47   | −8.43       |
| Arctiin                         | −15.10| −8.77 | −5.14                 | −8.02   | −7.34       |
| Medioresinol                    | −11.22| −5.99 | −4.12                 | −4.86   | −7.27       |
| Caftaric acid                   | −11.81| −8.96 | −5.40                 | −7.24   | −11.63      |

**Figure 7.** Interaction between some selected key metabolites in Asteraceae species extracts obtained by different extraction methods and target enzymes: (A) AChE and apigenin-di-C-hexoside (vicenin-2), (B) BChE and arctiin, (C) amylase and caffeoyl hexoside, and (D) glucosidase and chlorogenic acid.
3.6. ADMET Prediction

ADMET (Absorption–Distribution–Metabolism–Excretion–Toxicity) properties in key metabolites in Asteraceae species extracts obtained by different extraction methods were predicted using Biovia DS. Further, 95 and 99% of a compound with high gastrointestinal absorption is expected to fall in ellipses colored in red and green, respectively. Moreover, 95 and 99% of a compound with blood–brain permeability is expected to be in ellipses colored in magenta and aqua, respectively (Figure 8). Palmitamide and caffeic acid were predicted to have high gastrointestinal (GI) absorption and blood-barrier penetration probability. Oleamide was predicted to have low probability of crossing the blood–brain barrier and low GI absorption due to its high polarity. Similarly, neochlorogenic acid, chlorogenic acid, dicafeoylquinic acid, palmitamide, quinic acid, caffeoyl hexoside, apigenin-di-C-hexoside (vicenin-2), arctiin, mediioresinol, and caftaric acid were found to have low GI absorption and low blood–brain barrier penetration probability. Nonetheless, all the compounds are not likely to be associated with any toxicities.

![ADMET plot](image)

Figure 8. ADMET plot of logarithm of logP (octanol-water partition coefficient) against topological polar surface area (PSA); 95 and 99% of a compound with high gastrointestinal absorption is expected to fall in ellipses colored in red and green, respectively; 95 and 99% of a compound with blood–brain permeability is expected to be in ellipses colored in magenta and aqua, respectively.

4. Conclusions

Species belonging to the Asteraceae are rich in secondary metabolites. LC-MS-MS-guided profiling of the crude extracts of three Asteraceae plant samples, each obtained by three different extraction methods, namely HAE, MAC, and UAE, revealed the presence of a wide array of phytoconstituents. E. ritro extracts are predominately rich in oleamide,
representing ca. 50% of the whole chromatogram as well as the phenolic acids 5caffeoyl quinic acid and chlorogenic acid and the fatty acid amide palmitamide. Similarly, the major peaks in T. decipiens extract are the simple phenolic acids chlorogenic, 5caffeoyl quinic, and caftaric acids, as well as the lignan compound medioresinol. On the other hand, C. deflexa showed predominance in the flavonoids, such as vicenin-2 and lignans as arctin, but also with significant quantities of oleamide, chlorogenic acid, and caffeoyl hexoside. In addition to the chemical profiles, the extracts were tested for antioxidant and enzyme inhibitory properties. The biological activities depended on the used extraction solvents for each species and, in general, E. nitro exhibited stronger antioxidant ability as compared to other species. With regard to the enzyme inhibitory effects, all tested extracts showed inhibitory potentials. Our results could provide valuable insights to produce functional applications using the Asteraceae species and they could be considered as important sources of health-promoting compounds. However, further studies, such as toxicity and bioavailability, need to understand the full functional pictures of the tested species.

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