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HPLC/MS Phytochemical Profiling with Antioxidant Activities of *Echium humile* Desf. Extracts: ADMET Prediction and Computational Study Targeting Human Peroxiredoxin 5 Receptor

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Abstract: Plant-based antioxidants such as polyphenols have gained significance in primary health care needs, due to their potential and therapeutic use in modern medicine. Thus, the present study aimed to evaluate, for the first time, the effect of solvents on extraction yield, polyphenolics, phytoconstituents and antioxidant properties of various extracts (aqueous, methanol, ethyl acetate, dichloromethane, hexane) of *Echium humile* Desf. (*E. humile* Desf.) through in vitro and in silico studies. Statistically, among the various solvents, both methanol (443.05 ± 0.50 mg GAE/g extract) and aqueous (440.59 ± 0.50 mg GAE/g extract) extracts displayed equipotent and highest total phenolics content (TPC), while dichloromethane extract had the maximum total flavonoid content (TFC) (151.69 ± 0.60 mg QE/g extract), total flavonol content (TFIC) (97.39 ± 0.19 mg QE/g extract) and total carotenoids content (TCC) (537.85 ± 5.06 mg β -CE/g). Meanwhile, the highest total condensed tannin content (TCTC) was recorded together with hexane (131.50 ± 0.1 mg GAE/g extract) and dichloromethane (125.74 ± 5.72 mg GAE/g extract) extracts. The results of antioxidant studies revealed that ethyl acetate extract exhibited a potent scavenging effect through 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) ($IC_{50} = 17.25 \pm 1.76$ μ g/mL) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) (1433.30 ± 1.78 μ g/mL), while dichloromethane extract displayed significant ($p < 0.05$) ferric ion reducing antioxidant power (FRAP) activity (375.00 ± 0.02 μ g/mL) and total antioxidant capacity (TAC) (1999.00 ± 2.05 μ g AAE/g). In addition, the quantification of the polyphenolics content of the most active *E. humile* Desf. extract, ethyl acetate has been assessed by HPLC/MS, leading to the identification of *p*-coumaric, *trans*-ferulic acids and cirsiol as major constituents. Additionally, molecular docking analysis showed that among the selected phytochemicals, naringin (-7.0 kcal/mol) followed by 1,3-di-*O*-caffeoyquinic acid (-6.6 kcal/mol), rosmarinic acid (-6.6 kcal/mol), rutin (-6.5 kcal/mol) and apigenin-7-*O*-glucoside (-6.5 kcal/mol) showed the lowest binding potential. Furthermore, ADME and toxicity parameters justify that identified compounds from ethyl acetate extract are safer to replace the synthetic drugs with side effects. The obtained results can provide

valuable information on the medical and therapeutic potential use of *E. humile* Desf. as a potent antioxidant agent to improve immunity.

Keywords: *Echium humile* Desf.; antioxidant potential; human peroxiredoxin 5 receptor; molecular docking; ADMET

1. Introduction

Traditional medicinal practices based on medicinal and aromatic plants (MAPs) remain the first course of therapy for the treatment of various illnesses in developing countries [1]. It has been estimated that more than 60% of modern pharmaceuticals and commercial drugs are based on MAPs-derived compounds and that about 75% of the world's population used herbal products as foods or to cure various illnesses. Therefore, MAPs are appreciated since their extracts and essential oils along with their bioactive molecules can act as effective therapeutic agents to avoid imbalanced cell redox reactions caused by free radicals [2]. This disorder is responsible for the formation of reactive nitrogen species (RNS) and reactive oxygen species (ROS) inducing damage to proteins, DNA and lipids, which are produced in human tissue cells during chain reactions [3,4]. In addition, free radicals have been largely implicated in cell metabolism, including defense responses of cells, intercellular signaling, phagocytosis and energy production. They are involved in important chronic disorders and have been reported to be associated with various types of diseases such as diabetes mellitus, insulin resistance, asthma, cardiovascular stroke, atherosclerosis, cancer, Alzheimer's and Parkinson's [5–7]. To allow for this balanced metabolic process and to avoid deleterious processes, antioxidant substances can maintain health and protect cells from oxidative and inflammatory damages via scavenging radicals, RNS and ROS, inhibiting different enzymes and chelating metals [6,8]. Their multifunctional role has been related to the diversity of their compositions and mechanisms, which allows them to inhibit different enzymes, chelate metals and scavenge free radicals. For this purpose, the most important natural antioxidants are polyphenols, including hydrolyzed tannin (acid ester polyphenols) and condensed tannin (flavanols polyphenols or proanthocyanins), mainly consumed as secondary metabolites, phytonutrients or dietary bioactive compounds with the role to protect and prevent the body from excessive free radicals and oxidative stress [9–11]. They tend to confer different biological and therapeutic properties [11–14]. Hence, there is a considerable need to identify new potent and effective antioxidant agents from natural sources in order to develop new herbal medicines or nutraceuticals rich in bioactive molecules without adverse effects, unlike synthetic drugs.

The endeavor for drug discovery from plant extracts and their bioactive molecules is of paramount importance to human health, mainly due to their high content of phenolic compounds representing culinary, horticultural and ethnomedicinal benefits [14–16]. It is well known that traditional cures from medicinal plants have emerged as potential sources of antioxidants depending on the complexity of the extracted compounds [17]. They can be used as prescribed medicines with a pivotal role in the development of potent therapeutic drugs. Consequently, extracts from plants can prevent a variety of lifestyle-related diseases and aging as well as the risk of cardiovascular and other chronic diseases. These latter are closely related to active oxygen and lipid peroxidation and to the powerful scavenging effect of their phenolics, flavonoids, carotenoids, anthocyanins and vitamins [7,13–17]. Therefore, due to the lack of natural commercial standards and to counter and revoke oxidative stress, people often still prefer plant-based natural medicines to synthetic medicines [12,13]. Consequently, there is currently a skyrocketing focus on the development of effective, safe and low-cost drugs-based MAPs as additives, with the help of cheminformatic tools, including pharmacokinetics and molecular docking, in order to find a novel pharmacological compound via their ligand–target interactions [7,16].

Among herbs, Saharan plants are a potentially sustainable source of bioactive molecules and are known for their resistance to stress conditions and high content of antioxidants [17]. *E. humile* Desf. plant (Figure 1) belongs to the genus of *Echium* L. (Boraginaceae), which consists of 67 recognized species, and is a small hispid biennial to perennial herb that grows naturally, native to North Africa, mainland Europe and the Macaronesia regions. It has been introduced and distributed to many countries worldwide [18]. The flowers in a corolla in the form of a glowing funnel tube are purple-red at first and then turn blue.



Figure 1. *E. humile* Desf. plant.

The *Echium* L. genus has been previously targeted for its ethnomedicinal properties, showing that *Echium* spp. possess anti-inflammatory, antiviral, antibacterial, sedative, antioxidant and anxiolytic properties [18]. The seed oil of *E. vulcanorum* has been demonstrated for its capacity to be a potential dietetic supplement [19]. Another species named *E. vulgare* has been shown to be able to heal wounds, bruising, pulled muscles, ligaments and sprains in both Turkey and Germany [19,20]. Regarding its phytochemistry, *E. amoenum* has been reported to contain a variety of bioactive phytochemicals such as phenols, flavonoids, terpenoids and naphthoquinones [21].

Therefore, the present study attempts to evaluate for the first time the phytochemistry and antioxidant potential of *E. humile* Desf. aerial parts from hexane, dichloromethane, ethyl acetate, methanol and aqueous extracts. We have also identified the major bioactive compounds of the potent extract(s) by HPLC-MS analysis. Subsequently, molecular docking analysis into the active site of human peroxiredoxin-5 enzyme was carried out to get insight into the potential bioactive compounds for their antioxidant activity. Additionally, drug-likeness profiles have been assessed to evaluate the ability of the potent extract(s) as a suitable candidate for drug discovery and therapeutic assays.

2. Materials and Methods

2.1. Reagents

DPPH, ABTS, -2,6-di-tert-butyl-4-hydroxytoluene (BHT) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma Aldrich (St. Quentin Fallavier, France). Folin–Ciocalteu reagent, sodium carbonate anhydrous (Na_2CO_3), gallic acid, sodium nitrite solution (NaNO_2), sodiumhydroxyde (NaOH), aluminum chloride hexahydrate solution ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$), iron (III) chloride anhydrous (FeCl_3) and catechin

were purchased from Fluka (Buchs, Switzerland). Analytical-grade solvents were used in the study and obtained from Sigma Aldrich (Darmstadt, Germany).

2.2. Plant Material and Extraction

The aerial parts of *E. humile* Desf. were rinsed with distilled aqueous immediately after harvest and left to air dry in a sterile environment in a shade, ground to a fine powder and passed through sieves to provide a homogeneous powder. Five solvents (hexane, dichloromethane, ethyl acetate, methanol and aqueous) were used. Extracts were prepared following the same protocol as described by Bakari et al. [22]. The extracts were obtained by maceration of 200 g of plant material with 600 mL of each solvent for 72 h. Aliquots of extracts were filtered through a 0.22 µm membrane (Millipore Corporation, Bedford, VA, USA) and then injected into the HPLC-MS system

2.3. Phytochemical Profiling

2.3.1. Extraction of TPC

The TPC was assayed using Folin–Ciocalteu reagent. The absorbance was measured at 760 nm using a Bio-Rad Smart Spec Plus spectrophotometer (Bio-Rad, Hercules, CA, USA), and the data were expressed as mg of gallic acid equivalents (GAE) per gram of extract (mg GAE/g). A calibration curve was constructed using gallic acid standard solutions in the range 50–1000 µg/mL ($R^2 = 0.994$).

2.3.2. Extraction of TFC

The TFC was determined using the aluminum chloride colorimetric method and expressed as mg of quercetin equivalents (QE) per gram of extract (mg QE/g). A calibration curve was constructed using quercetin standard solutions in the 50–500 µg/mL range ($R^2 = 0.991$).

2.3.3. Extraction of TFIC

The TFIC was determined according to the method described by Felhi et al. [23], where quercetin was used to make the calibration curve. In our case, 1 mL of 2% aluminum chloride and 3 mL of 5% sodium acetate were added to 1 mL of each extract. The absorbance of samples was measured after 2.5 h at 440 nm. The results are expressed as mg of quercetin equivalents (QE) per gram of extract (mg QE/g). A calibration curve was constructed using gallic acid standard solutions in the 50–1000 µg/mL range ($R^2 = 0.995$).

2.3.4. Extraction of TCTC

The TCTC content was analyzed using the same protocol as Hajlaoui et al. [24]. The absorbance of the solution was learned at 500 nm, and the results were expressed as mg of catechin equivalents (CE) per gram of extract (mg CE/g). A calibration curve was constructed using standard solutions of gallic acid in the 50–500 µg/mL range ($R^2 = 0.993$).

2.3.5. Extraction of TCC

The TCC was determined according to the method described by Bakari et al. [22]. A standard calibration curve was plotted using β-carotene. The results were expressed as β-carotene equivalents (β-carotene E) per gram of extract (µg β-carotene E/g). A calibration curve was constructed using standard gallic acid solutions in the 50–1000 µg/mL range ($R^2 = 0.989$).

2.3.6. HPLC-MS Analysis of Phenolic Compounds

The identification of polyphenolics was done using Shimadzu's HPLC-MS 2020 system. Details of the experiments were the same as those of the reported method of Hajlaoui et al. [24]. The separation of phenolics was performed with a Shimadzu HPLC-MS 2020 system that was equipped with an online degasser (DGU-20A3R), a two-binary pump (LC-20ADXR), an autosampler (SIL-20AXR), a column heater (CTO-20AC)

and a diode array detector (SPD-M20A). Instrument control and data analysis were carried out using Shimadzu lab solution V5.42 SP6 edition (Shimadzu, Kyoto, Japan) through Windows XP. The injection volume was 20 μ L, and the peaks were monitored at 250 nm. Peak identification was obtained by comparing the retention time and the UV spectra of the fraction phenolic chromatogram with those of pure standards, which were purchased from Sigma Aldrich and LGC standards. Mass spectrometric analysis was performed on a Shimadzu mass spectrometer (Shimadzu, Kyoto, Japan). Mass spectra data were recorded on a negative-ionization mode for a mass range of m/z 50–1500. Other mass spectrometer conditions were as follows: nebulizing gas pressure, 40 psi; drying gas flow, 12 L/min; drying gas temperature, 400 $^{\circ}$ C; nebulizing gas flow, 1.5 L/min. The specific negative ionization modes (m/z $[M-H]^{-}$) were used to analyze the compounds.

2.4. Antioxidant Activity

2.4.1. DPPH Free Radical Scavenging Assay

An aliquot of 1 mL of the extract of each extract was added to 0.25 mL of a DPPH methanolic solution. The mixture was shaken vigorously and then left to rest at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 517 nm to determine the values that corresponded to the ability of the extracts to reduce the stable radical DPPH. The scavenging activity was expressed by 50% inhibition concentration values IC_{50} in μ g/mL.

2.4.2. ABTS Free Radical Scavenging Assay

This test was examined by mixing 5 mL of 7 mM ABTS solution and 5 mL of 2.45 mM potassium persulfate solution and then stored in the dark for 16 h. The reaction mixture contained 950 μ L of ABTS solution and 50 μ L of each sample at various concentrations. The absorbance was assessed after 6 min at 734 nm. The activity was expressed as IC_{50} (mg/mL).

2.4.3. FRAP Assay

In this test, 250 μ L of a sample at a concentration of 0.0625 to 1 mg/mL was mixed with a phosphate buffer (500 μ L, 0.2 M, pH 6.6) and potassium ferricyanide (500 μ L, 1%). After incubation and adding 500 μ L of trichloroacetic acid (10%) to each sample, all the mixtures were centrifuged at 1006 μ g for 10 min. Then, 750 μ L of the upper layer was mixed with 750 μ L of distilled water, and 50 μ L of ferric chloride (0.1%) was added. The absorbance was measured at 700 nm and ascorbic acid was used as a positive control. The EC_{50} value (mg/mL) is the effective concentration, giving an absorbance of 0.5 for reducing power.

2.4.4. TAC Assay

This test was assessed according to the same experiments as described by Bakari et al. [22]. Here, a mixture of 0.2 mL of extract and 2 mL of reagent solution (0.6 M of H_2SO_4 , 28 mM Na_3PO_4 and 4 mM $(NH_4)_2MoO_4$) was prepared. Then, the absorbance was measured at 695 nm after incubation for 90 min in boiling water. The TAC activity was expressed as mg AAE/g (AAE, ascorbic acid equivalent).

All of the measurements were performed in triplicate.

2.5. Molecular Docking Approach

Interactions between the selected identified bioactive phytochemicals and the receptor human peroxiredoxin 5 were assessed by in silico molecular docking in order to explore the preferred orientation of the ligands in the binding site of receptors. We used the following protocol as described previously by our team [7]. Polar hydrogens and Gasteiger charges were assigned with AutoDockTools1.5.2 (ADT), and the PDBQT file format was prepared [25]. The same software was used to select a docking grid. In human peroxiredoxin 5 enzyme (PDB: 1HD2), the grid box site was established at 7.611, 43.828

and 29.921 Å (x, y, and z, respectively) using a grid of 70, 58 and 58 points (x, y and z, respectively) and including spacing of 0.375 Å.

The structures of the natural compounds were minimized using a conjugate gradient AMMP incorporated in VEGA ZZ. The conversion of the file from PDB to PDBQT was done using AutoDockTools1.5.2. We applied AutoDock Vina software with an exhaustiveness parameter of 32 to perform the docking simulations. ADT was used for the docking conformation analysis. Receptor ligand interactions were visualized by Discovery Studio Visualizer (Dassault Systems BIOVIA, San Diego, CA, USA, 2015).

2.6. ADMET Analysis

The ADMET (absorption, distribution, metabolism, excretion, toxicity) profiling of the selected compounds was estimated using SwissADME online server and pkCSM online tools [26–28].

2.7. Statistical Analysis

All assays were done in triplicate and results are reported as mean \pm standard error. An analysis of variance was performed on all the variables using the General Linear Model using the Statistic 8.0 for Windows (Analytical Software, New York, NY, USA) to assess the correlations between DPPH, FRAP, ABTS and TAC and phenolic contents according to Hajlaoui et al. [24].

3. Results and Discussion

3.1. Effect of Solvent on Extraction Yield

Extraction is the first crucial step in the preparation of plant formulations. The polarity of the used solvents for extraction plays an essential role in both the profitability and the pharmacological activities of the extract. Solvent selection is based on their polarity index. Results (Table 1) showed that the strongest extraction yield was obtained in methanol (7.25%) followed by aqueous (3.11%), hexane (1.58%) dichloromethane (0.90%) and ethyl acetate (0.64%), respectively. As shown, the extractability power of the tested solvents was not mainly related to their polarities index. Thus, the extraction yields strongly depended on the solubility of phytoconstituents that encompassed the whole extract [23]. The high yield of methanol may be attributed to its capacity to extract hydrophilic and lipophilic molecules [29]. Additionally, it may be explained by the higher solubility of the extractable bioactive components in methanol as well as its strong ability to solubilize most secondary metabolites [30,31].

Table 1. Yields, TPC, TFC, TFIC, TCTC and TCC in different extracts of *E. humile* Desf.

Extracts	Polarity Index	Yields (%)	TPC (mg GAE/g)	TFC (mg QE/g)	TFIC (mg QE/g)	TCTC (mg CE/g)	TCC (mg β -CE/g)
Hexane	0	1.58	76.72 \pm 0.68 ^c	58.42 \pm 0.03 ^c	18.95 \pm 0.19 ^c	131.50 \pm 0.16 ^a	175.73 \pm 7.40 ^b
Dichloromethane	3.7	0.90	389.81 \pm 2.74 ^b	151.69 \pm 0.60 ^a	97.39 \pm 0.19 ^a	125.74 \pm 5.72 ^a	537.85 \pm 5.06 ^c
Ethyl acetate	4.4	0.64	390.32 \pm 5.09 ^b	97.04 \pm 0.27 ^b	56.96 \pm 0.45 ^b	55.59 \pm 0.29 ^b	-
Methanol	6.6	7.25	443.05 \pm 0.50 ^a	14.48 \pm 0.16 ^d	11.36 \pm 0.06 ^d	47.97 \pm 0.19 ^b	-
Aqueous	9	3.11	440.59 \pm 0.50 ^a	14.96 \pm 0.62 ^d	9.66 \pm 0.06 ^e	6.89 \pm 0.17 ^c	40.92 \pm 0.21 ^a

TPC, total phenolic content; TFC, total flavonoid content; TFIC, Total flavonoid contents; TCTC, total condensed tannin content; TCC, Total carotenoid contents; GAE, Gallic acid equivalent/g dry extract; QE, quercetine equivalent/g dry extract; CE, catechine equivalent/g dry extract; QE, quercetine equivalent/g dry extract; β -CE, β -carotene equivalent/g dry extract. Each data point represents mean \pm sd of three independent replicates. ^{a, b, c, d and e}: different letters within same columns differ significantly ($p < 0.05$).

3.2. Effect of Solvent Extraction TPC, TFC, TFIC, TCTC and TCC

The optimization of extraction conditions is a very important task that significantly affects the phytochemistry as well as the medicinal values of extracts used for various therapeutic purposes.

Concerning the TPC (Table 1), the depicted results outlined that methanolic *E. humile* Desf. Extract exhibited the maximum TPC (443.05 ± 0.50 mg GAE/g), which is insignificantly ($p > 0.05$) different from aqueous extract (440.59 ± 0.50 mg GAE/g). These two extracts were significantly different ($p < 0.05$) from ethyl acetate and dichloromethane extracts (390.32 ± 5.09 and 389.81 ± 2.74 mg GAE/g, respectively), whereas the hexane extract presented the minimum content (76.72 ± 0.68 mg GAE/g). These results suggest the presence of more polar and water-soluble components in the extract. Moreover, the highest TPC in both methanolic and aqueous extracts is due to their capacity to solubilize more flavonoids components containing polar phenolic hydroxyl groups [32].

Flavonoids are a principal index for nutritional assessment in food ingredients and pharmaceuticals investigation, since they are powerful antioxidants. The TFC values reported in this study (Table 1) outlined a significant difference ($p < 0.05$) in total flavonoid contents. The greatest content was recorded for dichloromethane extract (151.69 ± 0.60 mg QE/g) followed successively by ethyl acetate (97.04 ± 0.27 mg QE/g) and hexane extracts (58.42 ± 0.03 mg QE/g); however, methanol and aqueous extracts showed the lowest amounts (14.48 ± 0.16 and 14.96 ± 0.62 mg QE/g, respectively). Our results justify the richness of extracts in aglycone flavonoids. The effectiveness of dichloromethane suggests the abundance of less polar flavonoids.

As can be seen in Table 1, results for flavonols are similar to those of flavonoids, and the best amount was found in dichloromethane extract (97.39 ± 0.19 mg QE/g), which is statistically significantly different from the other extracts ($p < 0.05$).

Condensed tannins are a class of oligomeric and polymeric phenolic compounds consisting of oligomers and polymers of the flavan-3-ol monomer units with relatively high molecular weight. The results depicted in Table 1 outlined that the greatest amount of TCTC was recorded to hexane (131.50 ± 0.16 mg CE/g) followed by dichloromethane (125.74 ± 5.72 mg CE/g), ethyl acetate (55.59 ± 0.29 mg CE/g) and methanol (47.97 ± 0.19 mg CE/g) extracts. The lowest content was recorded for aqueous extract (6.89 ± 0.17 mg CE/g) which is significantly different ($p < 0.05$) from other extracts. The lowest amount of tannins in aqueous solvent was explained by their weaker stability as well as by their limited solubility in a polar organic solvent with only the presence of fewer quantity of hydrolyable tannins. In contrast, their highest amount in dichloromethane and hexane is due to the presence of excessive levels of hydrolysable tannins.

Carotenoids belong to the isoprenoid group of pigments, which have been extensively studied for their health benefits. As shown in Table 1, there was a significant variation in TCC among the samples. The data show that dichloromethane extract had the highest ($p < 0.05$) level of carotenoids (537.85 ± 5.06 mg β -CE/g) followed by hexane extract (175.73 ± 7.40 mg β -CE/g) and that aqueous extract possesses the lowest content of carotenoids (40.92 ± 0.21 mg β -CE/g). However, both ethyl acetate and methanol extracts are devoid of carotenoids, showing that they are lacking in polar carotenoids. Our results are entirely consistent with the rule indicating that low polar compounds were more soluble in solvents with lower polarity.

A literature survey revealed the existence of phenolic acids, flavonoids, tannins, lignans and naphthoquinones in other *Echium* genera. Bazzaz et al. [33] listed the presence of saponins and tannins in the aerial organs of *E. italicum*. In addition, Chaouche et al. [34] enumerated the richness of both *E. pycnanthum* leaves and roots in saponins, sterols, triterpenes, flavonoids and tannins. The petals of *E. amoenum* mainly contain anthocyanidin; however, flavonoid aglycons and volatile oil are in lower amounts [24]. Rosmarinic acid, a phenolic acid, is widely distributed in *Echium* species such as *E. amoenum*, *E. russicum*, and *E. vulgare* [18]. Moreover, aqueous *E. russicum* is dominated by salivianolic acid, followed by radosiin, lithospermic acid and eritrinchin [35]. Additionally, flavonoids are commonly distributed in other *Echium* genus. *E. arenarium* extracts have been shown to contain four flavonoids (luteolin-7-O-glucoside, myricitrin, myricetin and quercetin) [36]. Furthermore, some steroids have been also identified in *Echium*; for example, stigmast-4-ene-3,6-dione and β -sitosterol were identified in *E. vulgare*. Other studies

on *Echium* species describe shikonin derivatives, phenolic acids, flavonoids, pyrrolizidine alkaloids and fatty acids as major chemical constituents [37]. Radwan et al. [38] reported the presence of four flavonoids, including luteolin-7-*O*-rutinoside, apigenin, apigenin-7-*O*-rhamnoside and quercetin-3-*O*-rhamnoside in *E. sericeum* (*E. creticum*). The compound 3,3-dimethylacrylshikonin, was found in *E. gaditanum* in high levels [39]. 3,3-dimethylacrylshikonin and acetylshikonin are the essential compounds of *E. plantagineum* [40].

3.3. Phytochemical Determinations

Identification and quantification of phenolics from natural herbs is becoming increasingly important due to their potential applications in combatting many diseases. The results so far obtained by the Folin–Ciocalteu assay are well supported. According to our present knowledge, no studies have been reported regarding phenolic compounds *E. humile* Desf. extracts. In this study, we identified the phenolic acids of the most active *E. humile* Desf. extract, ethyl acetate, through the HPLC-MS technique. In total, we have successfully identified 21 phenolic compounds for ethyl acetate extract with their identities, retention times (Rt), pseudomolecular ions $[M-H]^-$ and levels, including nine phenolic acids—quinic acid, protocatechuic acid, caffeic acid, 1,3-di-*O*-caffeoyquinic acid, *p*-coumaric acid, *trans*-ferulic acid, rosmarinic acid, salvianic acid and 4,5-di-*O*-caffeoyquinic acid; 10 flavonoids: naringin, hyperoside (quercetin-3-*O*-galactoside), rutin, apigenin-7-*O*-glucoside, quercetin (quercetin-3-*O*-rhamnoside), naringenin, luteolin, cirsiolol, apigenin and acacetin; and 2 flavonols: (+)-Catechin and epicatechin (Table 2).

Table 2. HPLC–MS analysis of ethyl acetate extract of *E. humile* Desf. aerial parts.

Peak	Retention Time (min)	MS[M–H] [−] <i>m/z</i>	Compounds	Concentration (μg/g)
1	2.130	191.00	Quinic acid	11.178 ± 0.42
2	7.385	153.00	Protocatechuic acid	15.88 ± 0.54
3	9.189	289.00	(+)-Catechin	37.7 ± 1.45
4	13.795	289.00	Epicatechin	18.56 ± 2.14
5	12.993	179.00	Caffeic acid	71.01 ± 1.32
6	14.960	515.00	1,3-di- <i>O</i> -caffeoyquinic acid	8.191 ± 0.36
7	17.087	163.00	<i>p</i>-Coumaric acid	2052 ± 7.34
8	18.744	193.00	<i>trans</i>-Ferulic acid	323.4 ± 1.96
9	21.634	579.00	Naringin	2.507 ± 0.09
10	22.209	359.00	Rosmarinic acid	16.25 ± 0.98
11	22.910	463.00	Hyperoside (quercetin-3- <i>O</i> -galactoside)	5.135 ± 0.71
12	22.888	609.00	Rutin	4.781 ± 0.12
13	23.754	717.00	Salviolinic acid	29.29 ± 0.6
14	23.754	515.00	4,5-di-<i>O</i>-caffeoyquinic acid	228.1 ± 1.75
15	24.302	431.00	Apigenin-7- <i>O</i> -glucoside	4.02 ± 0.61
16	25.112	447.00	Quercetrin (quercetin-3- <i>O</i> -rhamnoside)	15.23 ± 0.77
17	26.977	271.00	Naringenin	1.56 ± 0.12
18	29.763	285.00	Luteolin	6.302 ± 1.60
19	23.451	329.00	Cirsiliol	583.5 ± 5.18
20	31.852	269.00	Apigenin	7.427 ± 2.14
21	37.061	283.00	Acacetin	69.5 ± 1.89

The major identified compounds in ethyl acetate *E. humile* Desf. extract were *p*-coumaric acid (Rt = 17.087 min) obtained at *m/z* = 163 with a level 2052 ± 7.34 μg/g, representing about 58% of the whole extract, followed by cirsiolol (Rt = 23.451 min) obtained at *m/z* = 329 with an amount of 583.5 ± 5.18 μg/g, giving it a percentage of 16%. Both *trans*-ferulic acid and 4,5-di-*O*-caffeoyquinic acid were found in lower amounts, representing about 9% and 6%, respectively. Figure 2 illustrates some identified compounds from *E. humile* Desf. ethyl acetate extract.

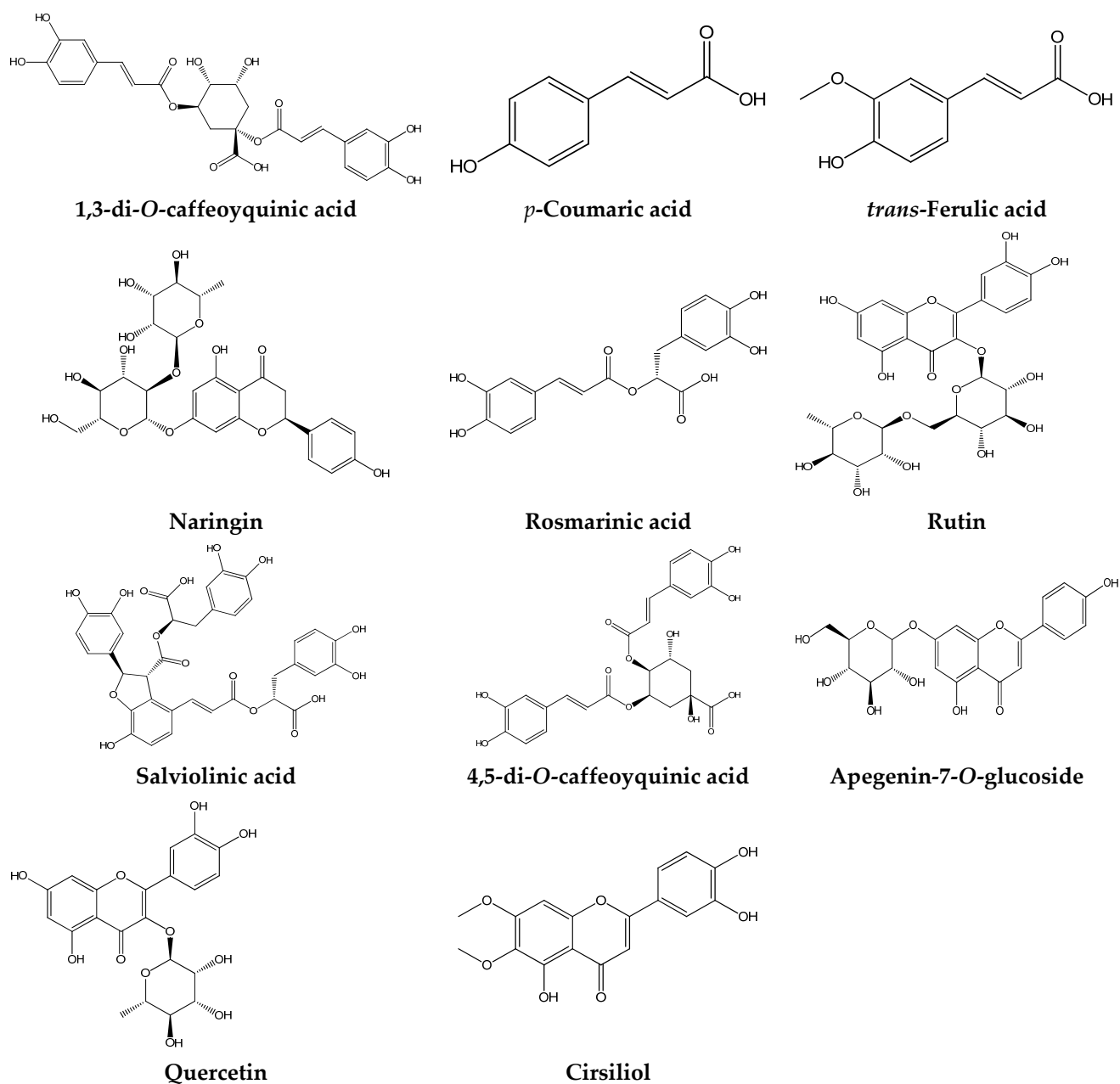


Figure 2. Structures of some phytochemicals from *E. humile* Desf. ethyl acetate extract.

Structurally, these secondary metabolites exert powerful antioxidant activity, due to the hydrogen donating properties of phenols, subsequently quenching free radical-induced lipid peroxidation and enhancing the ability to scavenge free radicals.

3.4. Antioxidant Activities

Polyphenolics, which are widely distributed in plants, are aromatic compounds bearing one or more hydroxyl groups that play a major role in antioxidant activity by acting as singlet oxygen quenchers, reducing agents and metal chelators as well as electron or hydrogen donors. Thus, they are excellent scavengers of ROS with the ability to control antioxidant activity. In this study, different *E. humile* Desf. extracts (hexane, dichloromethane, ethyl acetate, methanol and aqueous) were screened for their antioxidant activity using four spectrophotometric methods (DPPH, ABTS, FRAP and CAT).

3.4.1. DPPH Activity

DPPH is a stable organic nitrogen-free radical and is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate the antioxidant capacity. According to our literature survey, the antioxidant activity of *E. humile* Desf. extracts has not been previously reported elsewhere. The DPPH results are expressed in IC₅₀ values which represent the concentration of extracts necessary to inhibit 50% of free radicals. As shown in Table 3, the IC₅₀ values ranged from 17.25 ± 1.76 to 1002.5 ± 3.53 µg/mL with ethyl acetate extract revealed the potent ($p < 0.05$) activity in DPPH radical scavenging assay (6.2-fold higher than ascorbic acid as a positive control) followed by methanol extract (64.00 ± 1.65 µg/mL). The high DPPH scavenging ability in *E. humile* Desf. ethyl acetate extract compared to the methanolic was explained by their different amounts of TFC (7 times higher) and TFIC (5 times higher) since they have close TPC and CTC levels.

Table 3. Antioxidant activity: DPPH, FRAP, ABTS and TAC in different extracts of *E. humile* Desf.

Extracts	DPPH IC ₅₀ (µg/mL)	ABTS IC ₅₀ (µg/mL)	FRAP CE ₅₀ (µg/mL)	TAC (µg AAE/g)
Hexane	—	—	3875.00 ± 0.02 ^a	827.93 ± 2.60 ^a
Dichloromethane	1002.5 ± 3.53 ^a	1505.00 ± 0.54 ^{b,c}	375.00 ± 0.02 ^e	1999.00 ± 2.05 ^b
Ethylacetate	17.25 ± 1.76 ^c	1433.30 ± 1.78 ^c	617.33 ± 0.04 ^d	1219.50 ± 6.09 ^c
Methanol	64.00 ± 1.65 ^b	1583.33 ± 1.45 ^b	1372.30 ± 0.01 ^c	362.30 ± 2.53 ^d
Aqueous	—	1708.30 ± 0.27 ^a	2187.16 ± 0.61 ^b	251.21 ± 2.65 ^d
Ascorbicacid	108.11 ± 0.06 ^d	—	31.02 ± 0.48 ^f	—
Trolox	—	71.00 ± 0.04 ^d	—	—

a, b, c, d and e: different letters within same columns differ significantly ($p < 0.05$).

3.4.2. ABTS Activity

ABTS radical scavengers such as DPPH measure the antioxidant capacity using a single-electron transfer mechanism. The results of ABTS radical scavenging activity (Table 3) demonstrate that *E. humile* Desf. extracts had promising scavenging ability, where ethyl acetate extract was found to be most active (1433.30 ± 1.78 mg/mL), followed by dichloromethane extract (1505.00 ± 0.54 mg/mL). In contrast, the weaker scavenging effect of aqueous extract might be attributed to its lower levels in TFC and CTC. The high scavenging effect of dichloromethane extract as compared to aqueous, methanol and hexane extracts might be explained by the significant difference in TFC, TFIC, CTC and TCC.

3.4.3. FRAP Activity

FRAP assay is an interesting method to measure the reductive ability of antioxidants based on electron transfer. In the present study, moderate to weak reducing power over all extracts varied significantly ($p < 0.05$) from 375.00 ± 0.02 to 3875.00 ± 0.02 µg/mL, with the strongest activity (375.00 ± 0.02 µg/mL) pertaining to the dichloromethane extract, which was especially rich in TFC, TFIC, TCT and TCC.

3.4.4. TAC Activity

The results summarized in Table 3 shows that the TAC of extracts was significantly different ($p < 0.05$) in a concentration-dependent manner. The order of TAC scavenging activity of the extracts was found to be as follows, dichloromethane > ethyl acetate > hexane > methanol > aqueous extracts. Hence, the highest total antioxidant capacity of dichloromethane extract (1999.00 ± 2.05) may be mainly due to the presence of phenolic contents and carotenoids, which act as reducing agents by donating the electrons and reacting with free radicals [27]. In addition, the TAC of the aqueous extract was found to be the lowest (251.21 ± 2.65), and this may be due to its lower level in TFC, CTC and TCC.

The presence of a high amount of phenolic compounds, which act as reducing agents and hydrogen donors, and are capable of scavenging free radicals, considerably increases the antioxidant activity.

3.5. Correlation between DPPH, ABTS, FRAP and TAC Assays: Pearson Correlation

Several studies have investigated the relationship between the antioxidant activity and the content of polyphenol compounds in herbs. Table 4 outlines a Pearson correlation between antioxidant assays DPPH, ABTS, FRAP and TAC (Table 4). Significant correlations were found between the various methods used to determine the antioxidant potential, especially between TAC and other assays, ($R = 0.794$, and $R = 0.631$), with DPPH and FRAP, respectively. In addition, a moderate and negative correlation was found between FRAP and ABTS ($R = -0.478$) and between FRAP and DPPH ($R = -0.515$).

Table 4. Pearson's Correlation.

	ABTS	DPPH	FRAP	TAC	TCC	TFC	TFIC	TPC
DPPH	0.037							
FRAP	−0.478 *	−0.515 *						
TAC	−0.222	0.794 **	0.631 **					
TCC	−0.169	0.957 ***	−0.278	0.769 **				
TFC	−0.220	0.756 **	−0.618 **	0.995 ***	0.740 **			
TFIC	−0.077	0.810 ***	−0.709 **	0.987 ***	0.758 **	0.987 ***		
TPC	0.984 ***	−0.023	−0.479 *	−0.282	−0.249	−0.285	0.140	
CTC	−0.636	0.688 **	−0.233	0.797 **	0.738 **	0.758 **	0.706 **	−0.645 **

*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

3.6. Correlation between Antioxidant Activities and Polyphenols Content

Pearson correlation analyses between DPPH, ABTS, FRAP and TAC and TPC, TFC, TFIC, TCTC and TCC are illustrated in (Table 4). First of all, TPC correlated strongly with ABTS assay ($R = 0.984$), which was highly significant ($p = 0.000$), and a moderate negative correlation was shown with FRAP assay ($R = -0.479$). On the other hand, flavonoids and flavonols showed high and significant positive correlation with TAC ($R = 0.995$ and $R = 0.987$, $p = 0.000$) and DPPH ($R = 0.756$ and $R = 0.810$) and good and negative correlation with FRAP. The high correlation between TFIC and TFC ($R = 0.987$, $p = 0.000$) is natural because flavonols are a subclass of flavonoid.

High positive correlations between antioxidant properties evaluated by all the assays and phenolic compounds are important contributors to antioxidant potential of *E. humile* Desf. Meanwhile, carotenoid content demonstrated negative relationships with ABTS and FRAP assays; however, a high and significant correlation was found with DPPH and TAC antioxidant assays, implying the important contribution of carotenoids towards the observed antioxidant activities. Furthermore, correlation studies between carotenoids and antioxidant activities are scarce. Some studies are in agreement with our study, showing positive correlations [41,42].

Research on *Echium* antioxidant activity from other species remains very scanty so far. The ethanol extracts from herbs and roots of *E. italicum*, *E. vulgare*, *E. angustifolium* and *E. parviflorum* showed that *E. italicum* root extract displayed high scavenging activity, followed by *E. angustifolium* and *E. vulgare* root extract; however, *E. parviflorum* herb extract presents weaker activity [37]. Additionally, shikonin and pyrrolizidine alkaloids from *E. italicum* root have been proved for their antioxidant activity [43]. *E. sericeum* (*E. creticum*), revealed potent antioxidant activity towards DPPH, iron-reducing power, ABST, iron chelation and β -carotene assays [38]. Chaouche et al. [34] reported in their study the antioxidant activity of hydromethanolic root extracts of *E. pycnanthum* collected in southern Algeria. *E. vulgare* and *E. rubrum* have been tested for their antioxidant activity via metal-chelating (Fe^{2+}), FRAP, TAC, OH radical, DPPH and ABTS radical scavenging assays. Results showed high potency of *E. vulgare*, due to its high TPC and TFC values [44].

Moreover, Kefi et al. [36] demonstrated that ethyl acetate extracts of the aerial parts of *E. arenarium* collected from Tunisia exhibited high antioxidant activity given by DPPH and β -carotene bleaching inhibitions with the highest TPC when compared to those obtained from aqueous, hydroethanolic and cyclohexane extracts.

3.7. Structure Antioxidant Properties Relationships

In order to modulate the contribution of the identified compounds on the antioxidant activity based upon on the aforementioned results, we showed that ethyl acetate extract exhibited the strongest scavenging capacity in DPPH and ABTS assays; however, in the FRAP test, dichloromethane was the most effective, and in TAC assay, aqueous and methanol extracts were the potent extractable solvent. The antioxidant potency of ethyl acetate *E. humile* Desf. extract was related to its bioactive compounds. In a similar study, Scherer and Godoy [42] mentioned that the antioxidant activity of phenolic acids depends on the degree of hydroxylation, as is the case with protocatechuic acid (dihydroxylated) and gallic acid (trihydroxylated) displaying higher antioxidant activity when compared to caffeic acid and ferulic acid (replacement of the hydroxyl group on the aromatic ring with a methoxyl group). In the case of flavonoids, both the position of the hydroxyl groups and the degree of hydroxylation affect the scavenging ability of their free radicals. For example, quercetin can be hydrophilic and lipophilic: depending on the type of substituents in the molecule, it was found to possess a higher potency than rutin in which the hydroxyl group is replaced by rutinose. The glycosylation of at least one hydroxyl group of quercetin derivatives enhanced its hydrophilicity [45]. The same authors reported that both ferulic and chlorogenic acids are good contributors to the DPPH scavenging ability. As a major metabolite of antioxidant polyphenols, the phenolic acid protocatechuic acid was demonstrated to be a good contributor of antioxidant activity [46]. Additionally, it should be pointed out that for flavonoids, the B-ring hydroxylation pattern governed the DPPH scavenging effect. Those bearing an ortho 3',4'-dihydroxy moieties in the B-ring (i.e., luteolin) displayed much higher antioxidant activity than those with only one free hydroxyl group at C-3' or C-4' position (i.e., apigenin), due to their capacity to more delocalize the unpaired electron in the aroxyl radical structure [47].

It has been reported that hyperoside, luteolin, and 4,5-di-O-caffeoylquinic acid show similar DPPH and ABTS radical scavenging capacity, but they are higher than that of apigenin, apigenin-7-O-glucoside and acacetin [48]. Additionally, the same authors indicated that 3,4-di-O-caffeoylquinic acid shows great FRAP reduction ability. Flavonoids show relatively weak FRAP reduction activity in the following order: hyperoside > luteolin > apigenin > apigenin-7-O-glucoside > acacetin [49].

Rashmi et al. [47] have reported that naringenin possesses a DPPH-scavenging effect following the neutralization of hydrogen peroxide, superoxide, hydroxyl radicals and nitric oxide radical, DPPH and lipid peroxidation. Nishimura et al. [50] showed the high inhibition of ROS by quercetin than naringenin. Both naringin and naringenin were found to possess greater effectiveness in the protection against oxidative and DNA damage and that naringenin exhibited higher antioxidant capacity and hydroxyl and superoxide radical scavenger efficiency than naringin [51]. Nile et al. [52] have demonstrated that flavonoids prevent the propagation reactions of free radicals and their formation by inhibiting enzymes such as xanthine oxidase that are involved in the initiation reaction or by chelating transition metals. The antioxidant activity of flavonoids is also reported to be due to their capacity to chelate metal catalysts, transfer electrons free radicals, activate antioxidant enzymes, reduce alpha-tocopherol radicals and inhibit oxidases [53]. It has been reported that *p*-coumaric acid (4-hydroxycinnamic acid), with multiple health benefits, exhibited potent antioxidant effects, with the ability to scavenge ROS and free radicals [54]. Guleria et al. [55] stated that some phenolic acids have anti-radical and antioxidant potential measured by DPPH \bullet and FRAP assays in the order caffeic acid > ferulic acid > chlorogenic acid > *p*-coumaric acid. The high potency of caffeic acid as compared to *p*-coumaric acid as observed in the present study (Table 3) may be due to the

presence of 3,4-position of dihydroxylation on the phenolic ring in caffeic acid and may be explained also by the presence of additional conjugation in the propionic side chain that facilitates the electron delocalization, by resonance, between the aromatic ring and the propionic group [55]. Cirsiliol (5,3,4-trihydroxy-6,7-dimethoxyflavone) as the most potent inhibitor of arachidonate 5-lipoxygenase, an enzyme responsible for leukotriene biosynthesis have been reported to display potent antioxidant activity [56]. The antioxidant activity of rutin has been also proved and structurally is due to the presence of an aromatic ring linked to a hydroxyl group [57]. Rosmarinic acid is an acid ester of caffeic acid and 3(3,4-dihydroxyphenyl)lactic acid and has been demonstrated to have antioxidant activity [18]. In another work, higher DPPH ability has been observed for gallic acid and caffeic acid than protocatechuic acid and rosmarinic acid. The catechol-type O-diphenols, such as protocatechuic acid and caffeic acid, possess powerful scavenging capacity [58]. The antioxidant activity of salvianolic acid has been reported and was reported to reduce intracellular as well as intravascular oxidative stress [59].

3.8. Molecular Docking Study

The molecular docking of the selected ligands to the catalytic pocket of human peroxiredoxin 5 enzyme (PDB: 1HD2), a thioredoxin peroxidase that acts mainly by reducing alkyl hydroperoxides and peroxyxynitrite via cytosolic or mitochondrial thioredoxins and permits the reduction of hydrogen peroxide and alkyl peroxide with the help of thiol-containing donor molecules, has been carried out in order to confirm and reinforce the in vitro antioxidant results. The results of the binding energies between the twenty-one identified phytochemicals and the targeted enzyme, human peroxiredoxin 5 (1HD2), were found to vary from −7.0 to −4.4 kcal/mol (Table 5).

Table 5. Binding energy of the identified constituents from *E. humile* Desf. ethyl extract complexed with human peroxiredoxin 5 enzyme.

N°	Entry	Binding Energy (kcal/mol)
1	Quinic acid	−4.6
2	Protocatechuic acid	−4.4
3	(+)-Catechin	−5.4
4	Epicatechin	−5.5
5	Caffeic acid	−4.6
6	1,3-di-O-caffeoyquinic acid	−6.6
7	<i>p</i> -Coumaric acid	−4.4
8	<i>Trans</i> -Ferulic acid	−4.7
9	Naringin	−7.0
10	Rosmarinic acid	−6.6
11	Hyperoside (quercetin-3-O-galactoside)	−5.8
12	Rutin	−6.5
13	Salviolinic acid	−6.3
14	4,5-di-O-caffeoyquinic acid	−5.5
15	Apigenin-7-O-glucoside	−6.5
16	Quercetrin (quercetin-3-O-rhamnoside)	−6.3
17	Naringenin	−5.7
18	Luteolin	−5.7
19	Cirsiliol	−5.5
20	Apigenin	−5.8
21	Acacetin	−5.8

Receptor–Ligand Complex Interactions

The ligand-based drug designing approaches using molecular docking analysis have been assessed to find the best fit orientation of the selected phytochemicals and the human peroxiredoxin 5 enzyme. The detailed results present in Table S1 and Figure S1

justify the high binding potential of the selected bioactive compounds (Table S1). From the summarized docking results in Table 5 and Table S1 and Figure S1, we conclude that the top selected phytochemicals, based upon their lowest binding energies values (≤ -6.60 kcal/mol) with the top major ones (Table 5), are entirely consistent with the previous work of Declercq et al. [60,61]. The authors, by analyzing the crystal structure of human peroxiredoxin 5 (1HD2), revealed that in the active site of the protein, the residue Cys47 located at the N-terminal part of the kinked helix $\alpha 2$, inside a small cavity, was directly involved in peroxide reductase activity by forming an intramolecular disulfide intermediate in the oxidized enzyme. Additionally, the residue Arg127, which is bounded with the S atom of Cys47 at a distance of 3.3 Å, enhanced the positively charged active site pocket. Additionally, they confirmed the presence of Thr44, in which the O atom interacts with the S atom of the catalytic cysteine residue (Cys47) at a distance of 3.0 Å. Moreover, they indicate that one side of the active site pocket contains several hydrophobic residues, including Leu116, Ile119 and Phe120, whose side-chains are located near the benzoate aromatic ring, which can act as hydroxyl radical scavenger (via its benzoate ion).

Regarding our results, the highest stability of naringin-1HD2 complex (-7.0 kcal/mol) is mainly due to the highest established interaction number of H bonds with Ala42 (1.97 Å), Phe43 (2.57 Å), Asn76 (2.15 Å), Arg124 (2.11 Å) (2.36 Å) and Thr147 (2.60 Å) residues. 1,3-di-*O*-caffeoyquinic acid (-6.60 kcal/mol) and rosmarinic acid (-6.60 kcal/mol) were implicated in the antioxidant activity via the forming of H bonds with the remarkable residues Cys47 (2.55 Å) and Arg127 (2.51 Å) for 1,3-di-*O*-caffeoyquinic acid and Cys47 (2.76 Å) and Arg127 (2.37 Å) for rosmarinic acid. In contrast, *p*-coumaric acid, representing about 58% of the whole extract, interacts with human peroxiredoxin 5 (1HD2) to form a less stable complex (-4.40 kcal/mol) in which the main residue in the active site was implicated via hydrophobic interactions such as C-H bonds with Thr44 (2.76 Å), Cys47 (3.25 Å), Arg127 (2.33 Å). For more details, see Table 5 and Figure S1.

3.9. Pharmacokinetics and Toxicity Profiling

To minimize failures in the drug discovery process and to predict their fate in organisms, rapid ADMET evaluation based on computational strategies is urgently needed and is sought by medicinal chemists [61–63]. Hence, we are focused on the three selected top major identified phytochemicals (*p*-coumaric acid, *trans*-ferulic acid, cirsiolol), representing about 84% of the extracts that have been screened for their ADMET properties, to determine whether they fulfill the drug-likeness conditions. The outcomes summarized in Table 6 showed that *p*-coumaric acid, *trans*-ferulic acid and cirsiolol obeyed Lipinski's rule of five (RO5) and were well absorbed in the gastrointestinal tract, exhibiting good skin permeation ($\text{LogK}_p \approx -6.41$ to -6.25). Bioavailability score (55–85%) predicted that they were not a P-gp (an ATP dependent drug efflux pump) substrate, which gives them the property of being a good candidate against multidrug-resistant cancer cells, overexpressing this drug transporter.

Cirsiolol was found to be non-permeant, meaning that it was unable to cross the blood–brain barrier (BBB) to the brain where it binds to specific receptors. Their distribution in the tissues rather than in plasma may be assessed by the distribution volume logVD_{ss} with cirsiolol and was found to be more distributed. The CNS permeability parameter measuring the blood–brain permeability surface area product indicates that they are moderately permeant. Their effects on cytochrome P450 isoforms CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4, which are the main players in Phase I metabolism, have been assessed. CYP3A4, representing 28% of the whole enzyme system, acts on lipophilic substrates. *p*-Coumaric acid and *trans*-ferulic acid were not inhibitors of any predicted CYP450 isoforms, but, cirsiolol was only inhibited by CYP2C19 and CYP2C9 isoenzymes.

The bioavailability radar of the major compounds (84% of the whole extract) represented by the pink area suggests that they predict good drug-likeness properties. The results of the BOILED-Egg model (Figure S2) showed that both *p*-coumaric acid and *trans*-ferulic acid appeared with red points in the yellow ellipse with high probability of

brain penetration and are non-substrate of P-gp (PGP[−]); however, cirsiol appeared with a red point in the white area, meaning it had the possibility of being highly absorbed by the gastrointestinal and also was PGP[−].

The toxicity profile (Table 6) of *p*-coumaric acid, *trans*-ferulic acid and cirsiol given by their AMES toxicity/hepatotoxicity/skin sensitization/hERG I/II inhibitors' parameters have been assessed and the data, which indicate that the selected compounds do not present any toxicity by these assays.

Table 6. Pharmacokinetics, drug-likeness and toxicity prediction of the major compounds of *E. humile* Desf. ethyl acetate extract.

Entry	<i>p</i> -Coumaric Acid	<i>Trans</i> -Ferulic Acid	Cirsiol
Pharmacokinetics/Drug-likeness			
GI absorption	High	High	High
BBB permeant	Yes	Yes	No
P-gp substrate	No	No	No
CYP1A2 inhibitor	No	No	Yes
CYP2C19 inhibitor	No	No	No
CYP2C9 inhibitor	No	No	No
CYP2D6 inhibitor	No	No	Yes
CYP3A4 inhibitor	No	No	Yes
Log Kp (cm/s)	−6.26	−6.41	−6.25
Lipinski	Yes	Yes	Yes
Bioavailability Score	0.85	0.85	0.55
log VDss (human)	−1.151	−1.367	1.153
CNS permeability	−2.418	−2.612	−2.251
Toxicity			
AMES toxicity	No	No	No
Hepatotoxicity	No	No	No
hERG I/II inhibitors	No	No	No
Skin Sensitization	No	No	No

In silico pharmacokinetic and toxicological studies indicated that *E. humile* extract, ethyl extract and their bioactive compounds could be used for further development and drug design.

4. Conclusions

The results of the present study showed that the yield, TPC, TFC, TFIC, CTC and TCT of *E. humile* Desf. extracts and their antioxidants were significantly influenced by the solvent used in extraction. The highest levels of polyphenol contents were obtained with ethyl acetate extract and methanol. HPLC-MS analysis of the most active extract (ethyl acetate) revealed that *p*-coumaric acid is the most prominent phenolic compound, representing about 58% of the whole extract, followed by cirsiol. Correlation between phytochemical contents and DPPH, ABTS, FRAP and TAC assays have been investigated, suggesting their high contribution to the antioxidant potential of *E. humile* Desf. extracts. Additionally, molecular docking approach of the major phytochemical compounds and those exhibiting the lowest binding energies showed that they had good binding modes with the human peroxiredoxin 5 active sites. A computational ADME study on the abundant compounds allowed us to forecast good pharmacokinetics and drug-like characters of the extract. The evidence presented can later sharpen further in vivo investigation of the potential application of *E. humile* Desf. extracts as a powerful source of antioxidant agents.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11112165/s1>, Table S1. Major phytochemicals with lowest binding energies and their interaction residues with human peroxiredoxin 5. Figure S1. Interactions of human peroxiredoxin 5 receptor (PDB: 1HD2) with the selected major phytochemicals of *E. humile* Desf. ethyl extract with

the lowest binding energies. Figure S2. Bioavailability radar (A) and BOILED-Egg model (B) of the major constituents.

Author Contributions: Conceptualization S.A., H.H., K.A. and S.G.; methodology, H.H., S.A., A.K. and M.S.; formal analysis, H.H., K.A. and S.A.; software, M.S. and A.K.; investigation, A.K., S.A., H.H., M.S., K.A. and S.G.; writing—original draft preparation, H.H., K.A., A.K., S.G. and M.S.; writing—review and editing, H.H., A.K., S.G. and M.S.; supervision, A.K. and M.S.; funding acquisition, K.A.; project administration, K.A. All authors have read and agreed to the published version of the manuscript.

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