Pharmacological Properties and Chemical Profiles of Passiflora foetida L. Extracts: Novel Insights for Pharmaceuticals and Nutraceuticals

Authors:

Annalisa Chiavaroli, Simonetta Cristina Di Simone, Kouadio Ibrahime Sinan, Maria Chiara Ciferri, Giancarlo Angeles Flores, Gokhan Zengin, Ouattara Katinan Etienne, Gunes Ak, Mohamad Fawzi Mahomoodally, Sharmeen Jugreet, Zoltán Cziáky, József Jek?, Lucia Recinella, Luigi Brunetti, Sheila Leone, Paola Angelini, Roberto Venanzoni, Luigi Menghini, Claudio Ferrante, Giustino Orlando

Date Submitted: 2021-02-22

Keywords: bioinformatics/network pharmacology, skin protection, neuroprotection, anti-oxidant/anti-inflammatory effects, Passiflora foetida, chemical profile

Abstract:

In the present study, Passiflora foetida extracts characterized by different polarities were studied for their phytochemical profile, enzyme inhibitory, and antioxidant potentials. In silico, in vitro and ex vivo studies were also carried out on methanol and water extracts for predicting pharmacokinetics and pharmacodynamics. In this regard, neuronal HypoE22 cells, isolated mouse skin tissues, and pathogen dermatophytes strains were exposed to extracts. Emphasis was given to the preventing effects induced by the extracts on hydrogen peroxide-induced alterations of prostaglandin E2 (PGE2), l-dopa, and serotonin. Chemical analysis revealed the presence of similar compounds in infusion and methanolic extracts. The ex vivo studies also showed protective skin properties by P. foetida water and methanol extracts, as evidenced by the decrease of hydrogen peroxide-induced PGE2 level. Additionally, the blunting effects on hydrogen peroxide-induced l-dopa levels are consistent with the anti-tyrosinase effect exerted by both extracts. In silico studies demonstrated the affinity of extracts' phytochemicals, namely apigenin, chrysoeriol, loliolide, luteolin, quercetin, and vitexin, towards cyclo-oxygenase-2 and tyrosinase. Finally, microbiological tests demonstrated the efficacy of P. foetida methanol and water extracts as anti-mycotic agents against Trichophyton and Arthroderma species, involved in skin inflammation. Hence, P. foetida L. extracts could represent potential sources of pharmaceuticals and nutraceuticals.

Record Type: Published Article

Submitted To: LAPSE (Living Archive for Process Systems Engineering)

Citation (overall record, always the latest version):	LAPSE:2021.0025
Citation (this specific file, latest version):	LAPSE:2021.0025-1
Citation (this specific file, this version):	LAPSE:2021.0025-1v1

DOI of Published Version: https://doi.org/10.3390/pr8091034

processes

MDPI

Article

Pharmacological Properties and Chemical Profiles of *Passiflora foetida* L. Extracts: Novel Insights for Pharmaceuticals and Nutraceuticals

Annalisa Chiavaroli [1], Simonetta Cristina Di Simone [1], Kouadio Ibrahime Sinan [2], Maria Chiara Ciferri [1], Giancarlo Angeles Flores [3], Gokhan Zengin [2,*], Ouattara Katinan Etienne [4], Gunes Ak [2], Mohamad Fawzi Mahomoodally [5,6], Sharmeen Jugreet [6], Zoltán Cziáky [7], József Jekő [7], Lucia Recinella [1], Luigi Brunetti [1], Sheila Leone [1], Paola Angelini [3,*], Roberto Venanzoni [3], Luigi Menghini [1], Claudio Ferrante [1,*] and Giustino Orlando [1]

1 Department of Pharmacy, University "G. d'Annunzio" of Chieti-Pescara, 66100 Chieti, Italy; annalisa.chiavaroli@unich.it (A.C.); disimonesimonetta@gmail.com (S.C.D.S.); mariachiara.ciferri@outlook.it (M.C.C.); lucia.recinella@unich.it (L.R.); luigi.brunetti@unich.it (L.B.); sheila.leone@unich.it (S.L.); luigi.menghini@unich.it (L.M.); giustino.orlando@unich.it (G.O.)
2 Department of Biology, Faculty of Science, Selcuk University, Campus, Konya 42130, Turkey; sinankouadio@gmail.com (K.I.S.); akguneselcuk@gmail.com (G.A.)
3 Department of Chemistry, Biology and Biotechnology, University of Perugia, 06100 Perugia, Italy; giancarlo.angelesflores@studenti.unipg.it (G.A.F.); roberto.venanzoni@unipg.it (R.V.)
4 Laboratoire de Botanique, UFR Biosciences, Université Félix Houphouët-Boigny, Abidjan 00225, Cote D'Ivoire; katinan.etienne@gmail.com
5 Institute of Research and Development, Duy Tan University, Da Nang 550000, Vietnam; mohamad.fawzi.mahomoodally@tdtu.edu.vn
6 Department of Health Sciences, Faculty of Science, University of Mauritius, Réduit 230, Mauritius; sharmeenjugs@gmail.com
7 Agricultural and Molecular Research and Service Institute, University of Nyíregyháza, 4400 Nyíregyháza, Hungary; cziaky.zoltan@nye.hu (Z.C.); jjozsi@gmail.com (J.J.)
* Correspondence: gokhanzengin@selcuk.edu.tr (G.Z.); paola.angelini@unipg.it (P.A.); claudio.ferrante@unich.it (C.F.)

Received: 4 August 2020; Accepted: 19 August 2020; Published: 24 August 2020

Abstract: In the present study, *Passiflora foetida* extracts characterized by different polarities were studied for their phytochemical profile, enzyme inhibitory, and antioxidant potentials. *In silico*, *in vitro* and *ex vivo* studies were also carried out on methanol and water extracts for predicting pharmacokinetics and pharmacodynamics. In this regard, neuronal HypoE22 cells, isolated mouse skin tissues, and pathogen dermatophytes strains were exposed to extracts. Emphasis was given to the preventing effects induced by the extracts on hydrogen peroxide-induced alterations of prostaglandin E_2 (PGE_2), l-dopa, and serotonin. Chemical analysis revealed the presence of similar compounds in infusion and methanolic extracts. The *ex vivo* studies also showed protective skin properties by *P. foetida* water and methanol extracts, as evidenced by the decrease of hydrogen peroxide-induced PGE_2 level. Additionally, the blunting effects on hydrogen peroxide-induced l-dopa levels are consistent with the anti-tyrosinase effect exerted by both extracts. In silico studies demonstrated the affinity of extracts' phytochemicals, namely apigenin, chrysoeriol, loliolide, luteolin, quercetin, and vitexin, towards cyclo-oxygenase-2 and tyrosinase. Finally, microbiological tests demonstrated the efficacy of *P. foetida* methanol and water extracts as anti-mycotic agents against *Trichophyton* and *Arthroderma* species, involved in skin inflammation. Hence, *P. foetida* L. extracts could represent potential sources of pharmaceuticals and nutraceuticals.

Keywords: *Passiflora foetida*; chemical profile; anti-oxidant/anti-inflammatory effects; neuroprotection; skin protection; bioinformatics/network pharmacology

1. Introduction

The genus *Passiflora* belongs to the Passifloraceae family and the members of this genus have a great interest in their therapeutic properties against anxiety and irritability [1]. Thus, the genus is significantly valuable for the development of novel drugs [2].

The biological activity of many medicinal plants and other natural products is directly related to the bioactive compounds they contain [3]. Consequently, many studies have focused on the analysis of the phytocompounds revealing the presence of alkaloids, phenols, glycosyl flavonoids, and cyanogenic compounds [4]. In earlier studies, the members of this genus contained important phytochemicals such as β-carbolines, harmala alkaloids, coumarins, maltol, phytosterols, and cyanogenic glycosides. Additionally, they have been reported to be rich in phenolic compounds, amino acid α-alanine, and organic acids, including butyric, linoleic, formic, oleic, malic, linolenic, myristic, and palmitic acids [5] as well as d-fructose, d-glucose and raffinose [6].

Passiflora foetida L. popularly known as striking passionflower [7] is a particularly renowned species belonging to the genus *Passiflora*, with tremendous ethnobotanical applications. For instance, the decoction of leaves and fruits of *P. foetida* has been reported to treat asthma and biliousness, while the leaves and root decoction is employed as an emmenagogue and used in hysteria. Additionally, leaf paste is applied to the head for headache and giddiness. Besides, the herb is used in the form of poultices or lotions for erysipelas and skin diseases with inflammation [8]. *P. foetida* has also been described to treat anxiety, insomnia, sexual dysfunction, convulsion, cough as well as cancer [9].

Moreover, studies conducted on *P. foetida* have revealed extracts of the plant to possess numerous promising bioactivities such as antidiarrhoeal, antiulcerogenic, analgesic, antidepressant anti-inflammatory, anti-hypertensive, hepaprotective, anticancer, antibacterial and antinociceptive [8,10–17]. Similarly, several bioactive compounds isolated from *P. foetida*, especially flavonoids, have shown important pharmacological actions, such as luteolin and chrysoeriol that have been found to possess potent anti-inflammatory properties [18].

Although the existence of extensive documentation on the traditional uses of *Passiflora* species associated with a variety of health benefits, many species of the genus have still remained underexplored or have been modestly studied and thus require them to be scientifically validated, with *P. foetida* being one of them. Therefore, phytochemical profile, antioxidant, and enzyme inhibition activities were studied *P. foetida* extracts characterized by different polarities were studied. In silico, *in vitro* and *ex vivo* studies were also carried out on methanol and water extracts in order to predict pharmacokinetics and putative targets underlying traditional and innovative pharmacological applications of *P. foetida*. In this regard, the multidirectional pharmacological approach focused on the activities of the extracts as protective agents, on neuronal HypoE22 cells and isolated skin tissues, and antimicrobials against selected dermatophyte strains deeply involved in skin inflammation. Emphasis was given to the preventing effects induced by the extracts on the alterations of prostaglandin E_2 (PGE_2), l-dopa, and serotonin levels following oxidative stress stimulus (hydrogen peroxide) challenging.

2. Materials and Methods

2.1. Plant Material and Preparation of Extracts

Passiflora foedita materials were collected in the neighborhood of Morofé in the city of Yamoussoukro (Lakes region, Ivory Coast) in 2019 by Kouadio Ibrahime Sinan. The plants were identified by Ouattara Katinan Etienne (Botanist at the Université Félix Houphouet Boigny, Ivory Coast). The plant samples

were deposited in the Department of Biology at Selcuk University. The aerial parts (as mixed) were used. The plant materials were dried in shade for about 10 days and then grounded using a laboratory mill.

Maceration was used for sample preparation with ethyl acetate, methanol, methanol-water (80%), and water as extraction solvents. For this purpose, 5 g of the samples were macerated with the solvents (100 mL) for 24 h at room temperature. After this, the mixtures were filtered and then the solvents were removed via a rotary evaporator. With respect to the infusion extract, 5 g of the plant material were infused with 100 mL boiled water. Thereafter, the water was dried using freeze-drying. All extracts were stored at +4 °C until further studies.

2.2. Profile of Bioactive Compounds

The total phenolic and flavonoid contents in *P. foetida* extracts were determined by common spectrophotometric methods and the details for these methods were described in our previous paper [19]. Gallic acid (GAE, for phenolic, Sigma-Aldrich, St. Louis, MO, USA) and rutin (RE, for flavonoid, Sigma-Aldrich, St. Louis, MO, USA) were used as standards in the assays.

Gradient reversed-phase UHPLC separations with electrospray MS/MS detection (both positive and negative ion modes) were used for the structural characterization of the compounds presenting in different extracts. The UHPLC system consisted of the Dionex Ultimate 3000RS UHPLC (Waltham, MA, USA) instrument coupled to a Thermo Q Exactive Orbitrap mass spectrometer (Waltham, MA, USA). Chromatographic separation was achieved on a reversed-phase column Thermo Accucore C18 (100 mm × 2.1 mm i. d., 2.6 μm, Waltham, MA, USA) [20]. All analytical details were given in the Supplementary Materials.

2.3. Determination of Antioxidant and Enzyme Inhibitory Effects

Antioxidant properties of *P. foetida* extracts were tested by different methods. These methods included metal chelating, reducing power, and radical quenching assays. In these assays, standards were trolox (TE) and ethylenediaminetetraacetic acid (EDTA) [21]. Regarding the enzyme inhibition effects, we selected some enzymes including tyrosinase (EC1.14.18.1, from mushroom), cholinesterase (electric eel acetylcholinesterase (AChE) (type-VI-S), EC 3.1.1.7; horse serum butyrylcholinesterase (BChE) (EC 3.1.1.8)), α–amylase (EC. 3.2.1.1, from porcine pancreas), and α-glucosidase (EC. 3.2.1.20, from *Saccharomyces cerevisiae*). In enzyme inhibitory assays, standards were kojic acid (KAE), galantamine (GALAE), and acarbose (ACAE). Both antioxidant and enzyme inhibitory results were expressed as standard equivalents. All reagents, enzymes and standards were purchased from Sigma-Aldrich, St. Louis, MO, USA

2.4. Brine Shrimp (Artemia salina) Lethality Test

Brine shrimp (*Artemia salina*) cysts were cultured in oxygenated artificial water, as previously reported [22]. After 24 h, brine shrimp larvae were gently transferred with a pipette in 6 well plates containing 2 mL of *P. foetida* extracts at different concentrations (0.1–20 mg/mL) in artificial seawater. Ten larvae per well were incubated at 25–28 °C for 24 h. After 24 h, the number of living nauplii was counted under a light microscope and compared to the control untreated group. Results were expressed as a percentage of mortality calculated as: $((T-S)/T) \times 100$. T is the total number of incubated larvae and S is the number of survival nauplii. Living nauplii were considered those exhibiting light activating movements during 10 s of observation. For each experimental condition, two replicates per plate were performed and experimental triplicates were performed in separate plates. Experiments were conducted in triplicates. Results of the eco-toxicological test were expressed as percentage lethality.

2.5. Cell Culture

The anti-proliferative effect of *P. foetida* extracts was evaluated in rat hypothalamus HypoE22 cells and cultured in DMEM supplemented with 10% (*v*/*v*) heat-inactivated fetal bovine serum and penicillin-streptomycin (100 μg/mL) (all from EuroClone SpA Life-Sciences-Division, Milano,

Italy) [22]. The Hypo-E22 cell line (Catalogue Number: CLU213) was purchased from Cedarlane Corporation (Burlington, ON, Canada). Cells were grown at 37 °C in a humified atmosphere of 5% CO_2. When indicated, the cells were treated with H_2O_2 300 µM for 3 h and different concentrations of *P. foetida* extracts (10–500 µg/mL). The cell viability was evaluated after 24 h of culture by MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl tetrazolium bromide) growth assay (Sigma-Aldrich, St. Louis, MO, USA), based on the capability of viable cells to reduce MTT into a colored formazan product. The cells were seeded into 96-well plates at 5 × 103 cells/well. At the established time point, the medium was replaced with a fresh one containing 0.5 mg/mL MTT, and the cells were incubated for 3 h at 37 °C. After a further incubation of the samples in DMSO for 30 min at 37 °C, the absorbance at 570 nm was measured using a Multiscan GO microplate spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The values obtained in the absence of cells were considered as background and subtracted from the optical density values of the samples. Three independent experiments were performed under the same experimental conditions. The wound healing test was employed to determine the role of the extracts on spontaneous cell migration. The detailed paradigm is included in our previous paper [22].

2.6. Ex Vivo Model of LPS-Induced Toxicity in Isolated Mouse Skin Tissue

Skin specimens were collected from male adult C57BL6 mice sacrificed by CO_2 inhalation. The experimental paradigm obtained the approval from the Italian Ministry of Health (Authorization Number: F4738.N.5QP, released on 8 February 2020). Skin specimens were stimulated with water and methanol *P. foetida* extracts (10–500 µg/mL). PGE_2 level (pg/mg wet tissue) was measured by radioimmunoassay (RIA) [23] in tissue supernatants.

2.7. HPLC Analysis

Cell and tissue supernatants were analyzed through high-performance liquid chromatography (HPLC) coupled to electrochemical detection for the determination of 5-HT and l-dopa levels respectively. The detailed protocol related to l-dopa and 5-HT identification and quantification is included in a previous article of ours [24].

2.8. Antifungal Activity of the Extract

The antifungal activity of *P. foetida* extracts was evaluated against multiple dermatophytes species, namely *Trichophyton rubrum* (CCF 4933), *T. rubrum* (CCF 4879), *T. rubrum* (CCF 4834), *T. mentagrophytes* (CCF 4823), *T. mentagrophytes* (CCF 5930), *Arthroderma crocatum* (CCF 5300), *A. curreyi* (CCF 5207), *A. gypseum* (CCF 6261), *A. insingulare* (CCF 5417), *A. quadrifidum* (CCF 5792). The experimental procedure was reported in our previous paper [22].

2.9. Bioinformatics and Docking Studies

The bioinformatics study was carried out according to the protocol published by Gu et al. [25]. The canonical SMILES were run by the SwissTargetPrediction and SwissADME platforms, for *in silico* prediction of putative targets and pharmacokinetic profile. Additionally, the prediction of phytochemicals' toxicity was conducted through the "Toxicity Estimation Software Tool" (T.E.S.T.) developed by the United States Environmental Protection Agency (EPA). The identification of target proteins was confirmed by the UniProt database (https://www.uniprot.org/). Subsequently, a components-targets analysis was conducted through Cytoscape software (3.7.2 version). Docking calculations were carried out as previously reported [26]. The Protein Data Bank (PDB) numbers of each docked proteins were: 5M8P for the tyrosinase; 1CX2 for the cyclo-oxygenase (COX)-2 enzyme; 2Z5X for the monoamine oxygenase-A (MAO-A) enzyme; 1GQR for the acetylcholinesterase (AchE) enzyme.

2.10. Statistical Analysis

Data were means ± S.D. of three experiments conducted in triplicate and analyzed by analysis of variance (ANOVA) coupled to Tukey post hoc test. Statistical significance was set at $p < 0.05$, and GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA, USA).

3. Results and Discussion

In the present study, the quantitative analysis indicated the total phenolic and flavonoid contents of the tested extracts ranging from 22.28–36.91 and 3.22–50.11 mg/g respectively. While the ethyl acetate extract contained the highest phenolic content (39.61 ± 0.26 mg/g), the methanolic extract (80%) was found to yield the highest flavonoid content (50.11 ± 0.78 mg/g) compared to the other extracts, thus indicating that ethyl acetate and methanolic (80%) as effective solvents to extract phenolics and flavonoids from *P. foetida*, respectively. On the other hand, water maceration and infusion extracts contained the lowest TFC (3.22 ± 0.45 and 4.27 ± 0.45 mg/g, respectively) (Table 1). Polar solvents are frequently used for recovering polyphenols from plant matrices. The most suitable solvents are aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate [23]. This is consistent, albeit partially, with the present findings. In fact, it is recognized that one of the most crucial factors affecting the extraction efficiency of bioactive compounds from plant materials and their resulting health benefits is the extraction solvent [27].

Table 1. Total phenolic and flavonoid contents of the tested extracts.

Extracts	TPC (mg GAE/g)	TFC (mg RE/g)
EA	39.61 ± 0.26 ^{a}	14.50 ± 0.54 ^{b}
MeOH	24.59 ± 0.24 ^{b}	10.52 ± 0.59 ^{c}
MeOH (80%)	21.30 ± 0.20 ^{d}	50.11 ± 0.78 ^{a}
Water maceration	22.18 ± 0.16 ^{c}	3.22 ± 0.45 ^{d}
Infusion	24.24 ± 0.51 ^{b}	4.27 ± 0.45 ^{d}

Values are reported as mean ± S.D. EA: Ethyl acetate; MeOH: methanolic; TPC: Total phenolic content; TFC: Total flavonoid content; GAE: Gallic acid equivalent; RE: Rutin equivalent. Different letters (a, b, c and d) indicate significant differences in the extracts ($p < 0.05$).

The total flavonoid and phenolic contents of *P. foetida* extracts have also been studied [28–30]. Notably, in one study, it was found that the flavonoid and phenolic contents were higher in aqueous and ethanolic extracts of *P. foetida* leaf and root, whereas lesser amounts were present in petroleum ether extracts [28].

Remarkably, the phytochemical analysis of methanolic and infusion extracts revealed the same 47 compounds to be present in both extracts (Tables 2 and 3). However, methanolic extract was found to contain an additional compound, namely isorhamnetin-O-hexoside. The extracts were found to contain miscellaneous mixtures of compounds composed of flavones, flavonols, and their derivatives including flavones glycosides and hexosides, amongst others. In fact, flavonoids, especially their glycosides, are the most vital phytochemicals in diets and are of great general interest owing to their diverse bioactivity. Natural flavonoids mostly exist as their O-glycoside or C-glycoside forms in plants [31]. Some examples of flavonoid O-glycosides identified in the extracts herein were isoquercitrin and isorhamnetin-3-O-glucoside. However, the presence of flavonoid C-glycosides was mostly dominant in the extracts. Examples included vitexin, isovitexin, orientin, and isoorientin, among others. Other *Passiflora* species have also been reported to be good sources of bioactive flavonoid C-glycosides [32,33]. In the study of Zucolotto et al. [32], the C-glycosyl flavonoids profile of leaf and pericarp extracts of South American *Passiflora* species were investigated. Although the different species and varieties showed different major constituents, the C-glycosyl flavonoids identified more frequently were orientin, isoorientin, vitexin, and isovitexin. This is in accordance with the findings of the present study. Paradoxically, it was noted that the chemical composition identified in the methanolic extract of *P. foetida* herein differed significantly from the one obtained in the study of Sisin et al. [29].

Table 2. Chemical profile of infusion extract.

No.	Name	Formula	Rt	$(M + H)^+$	$(M - H)^-$	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Relative Ratio (%)	Literature
1	Pantothenic acid	C9H17NO5	7.24	220.11850		202.1077	184.0972	116.0346	90.0555	72.0451	2.16	
2	Kynurenic acid	C10H7NO3	14.18	190.05042		162.0551	144.0448	116.0501	89.0393		0.40	[34]
3	Dihydroxycoumarin	C9H6O4	14.92	179.03444		147.0442	135.0443	133.0287	123.0443	105.0338	0.20	
4	Naringenin-6,8-di-C-glucoside	C27H32O15	17.38		595.16630	577.1575	505.1364	475.1267	385.0935	355.0831	0.08	
5	Luteolin-di-C-hexoside	C27H30O16	18.36	611.16121		593.1509	575.1404	473.1085	341.0658	311.0552	0.19	
6	Phaselic acid (2-O-Caffeoylmalic acid)	C13H12O8	18.73		295.04540	179.0342	135.0441	133.0131	115.0024	71.0123	0.37	
7	Isololiolide	C11H16O3	18.78	197.11777		179.1069	161.0963	135.1171	133.1015	107.0861	0.82	
8	Vicenin-2 (Apigenin-6,8-di-C-glucoside)	C27H30O15	19.36	595.16630		559.1451	541.1334	457.1133	325.0706	295.0601	7.26	
9	Apigenin-C-hexoside-C-pentoside isomer 1	C26H28O14	19.78	565.15574		547.1455	529.1345	379.0815	325.0708	295.0605	0.19	
10	Luteolin-C-hexoside-C-pentoside	C26H28O15	20.03	581.15065		563.1394	545.1295	395.0762	341.0656	311.0549	0.09	
11	Loliolide	C11H16O3	20.08	197.11777		179.1069	161.0964	135.1172	133.1015	107.0860	4.46	
12	Apigenin-C-hexoside-C-pentoside isomer 2	C26H28O14	20.46	565.15574		529.1345	511.1239	427.1031	409.0923	295.0605	0.96	
13	Apigenin-C-hexoside-C-pentoside isomer 3	C26H28O14	20.73	565.15574		529.1350	511.1242	427.1031	409.0921	295.0603	1.04	
14	Orientin (Luteolin-8-C-glucoside)	C21H20O11	20.86	449.10839		431.0979	413.0873	353.0660	329.0658	299.0555	0.29	[18]
15	Apigenin-C-hexoside-C-pentoside isomer 4	C26H28O14	21.11	565.15574		529.1350	469.1136	379.0816	325.0710	295.0603	6.32	
16	Isoorientin (Luteolin-6-C-glucoside)	C21H20O11	21.19	449.10839		431.0973	413.0872	353.0659	329.0659	299.0553	1.59	
17	Luteolin-C-hexoside	C21H20O11	21.56	449.10839		431.0974	413.0871	353.0658	329.0657	299.0553	0.10	
18 [1]	Vitexin (Apigenin-8-C-glucoside)	C21H20O10	21.84	433.11347		415.1031	397.0921	379.0812	313.0709	283.0605	11.58	[18]
19	Apigenin-C-hexoside-C-pentoside isomer 5	C26H28O14	22.11	565.15574		529.1349	397.0924	379.0814	325.0710	295.0605	0.38	
20 [1]	Vitexin-2″-O-rhamnoside	C27H30O14	22.18	579.17138		433.1133	415.1025	397.0922	313.0708	283.0603	5.69	
21	Apigenin-C-hexoside-C-pentoside isomer 6	C26H28O14	22.39	565.15574		529.1348	397.0922	379.0815	325.0708	295.0605	0.49	
22	Methoxy-tetrahydroxy(iso)flavone-O-hexoside isomer 1	C22H22O12	22.47		477.10331	462.0813	315.0519	300.0281	299.0200	271.0254	0.06	
23	Apigenin-C-hexoside-C-pentoside isomer 7	C26H28O14	22.61	565.15574		529.1346	379.0814	337.0710	325.0706	295.0603	0.36	
24	Isovitexin (Apigenin-6-C-glucoside)	C21H20O10	22.74	433.11347		415.1024	397.0921	379.0812	313.0708	283.0602	30.95	
25	Isoscoparin or Scoparin	C22H22O11	23.17	463.12404		445.1131	427.1027	367.0813	343.0813	313.0707	0.20	
26	Methoxy-tetrahydroxy(iso)flavone-O-hexoside isomer 2	C22H22O12	23.35		477.10331	462.0819	315.0518	301.0360	299.0202	271.0253	0.02	
27 [1]	Isoquercitrin (Quercetin-3-O-glucoside)	C21H20O12	23.47		463.08765	301.0359	300.0280	271.0254	255.0301	178.9978	0.19	
28 [1]	Isorhamnetin-3-O-glucoside	C22H22O12	25.48		477.10330	315.0521	314.0440	285.0411	271.0252	243.0299	0.23	
29	Abscisic acid	C15H20O4	25.81		263.12834	219.1388	204.1151	201.1280	152.0832	151.0754	0.14	
30	Dihydroxy(iso)flavone-C-hexoside	C21H20O9	26.18	417.11856		399.1079	351.0865	321.0759	297.0759	267.0653	0.16	
31	Methoxy-pentahydroxy(iso)flavone isomer 1	C16H12O8	26.34		331.04540	316.0229	287.0204	271.0254	270.0171	259.0248	0.24	
32	Methoxy-tetrahydroxy(iso)flavone-O-hexoside isomer 3	C22H22O12	27.18		477.10331	462.0805	315.0517	300.0281	299.0202	271.0254	0.05	
33	Methoxy-pentahydroxy(iso)flavone isomer 2	C16H12O8	27.53		331.04540	316.0229	287.0203	271.0248	270.0172	259.0243	0.13	
34 [1]	Quercetin (3,3′,4′,5,7-Pentahydroxyflavone)	C15H10O7	27.57		301.03483	273.0402	178.9978	151.0026	121.0282	107.0125	0.14	
35 [1]	Naringenin (4′,5,7-Trihydroxyflavanone)	C15H12O5	27.75		271.06065	227.0706	177.0186	151.0026	119.0489	107.0125	0.15	
36	Methoxy-pentahydroxy(iso)flavone isomer 3	C16H12O8	27.78		331.04540	316.0227	299.0202	287.0216	271.0253	259.0256	0.55	
37	Trihydroxy-trimethoxy(iso)flavone-O hexoside	C24H26O13	27.95		521.12952	506.1082	359.0779	358.0700	344.0544	329.0309	0.44	
38 [1]	Luteolin (3′,4′,5,7-Tetrahydroxyflavone)	C15H10O6	28.44		285.03991	217.0500	199.0397	175.0394	151.0026	133.0283	1.91	[18]
39	Quercetin-4′-O-methyl ether	C16H12O7	28.79		315.05048	300.0280	271.0252	255.0301	243.0298	227.0344	6.71	[18]
40	Apigenin (4′,5,7-Trihydroxyflavone)	C15H10O5	30.28		269.04500	227.0352	225.0555	151.0027	149.0232	117.0332	1.38	[18]
41	Chrysoeriol (3′-Methoxy-4′,5,7-trihydroxyflavone)	C16H12O6	30.57		299.05556	284.0331	256.0379	227.0347	151.0025	107.0123	0.60	[18]
42	Dimethoxy-trihydroxy(iso)flavone isomer 1	C17H14O7	31.11		329.06613	314.0439	299.0202	285.0413	271.0254	243.0300	5.51	
43	Dihydroxy-dimethoxy(iso)flavone isomer 1	C17H14O6	32.37		313.07122	298.0484	297.0409	283.0251	269.0465	255.0298	0.01	[35]
44	Dihydroxy-dimethoxy(iso)flavone isomer 2	C17H14O6	32.92		313.07122	298.0483	297.0409	283.0252	269.0467	255.0291	0.03	[35]
45	Dimethoxy-trihydroxy(iso)flavone isomer 2	C17H14O7	33.29		329.06613	314.0439	299.0202	285.0402	271.0254	243.0306	5.12	
46	Dihydroxy-dimethoxy(iso)flavone isomer 3	C17H14O6	34.80		313.07122	298.0487	283.0252	270.0541	269.0455	255.0300	0.03	
47	Dihydroxy-dimethoxy(iso)flavone isomer 4	C17H14O6	35.48		313.07122	298.0487	283.0253	269.0460	255.0301	242.0219	0.06	[35]

[1] Confirmed by standard.

Table 3. Chemical profile of methanolic extract.

No.	Name	Formula	Rt	$(M + H)^+$	$(M - H)^-$	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Relative Ratio (%)	Literature
1	Pantothenic acid	C9H17NO5	7.39	220.11850		202.1077	184.0973	116.0347	90.0556	72.0452	1.72	
2	Kynurenic acid	C10H7NO3	14.21	190.05042		162.0551	144.0447	116.0497	89.0392		0.36	[34]
3	Dihydroxycoumarin	C9H6O4	14.92	179.03444		147.0442	135.0444	133.0287	123.0444	105.0338	0.18	
4	Naringenin-6,8-di-C-glucoside	C27H32O15	17.38		595.16630	577.1563	505.1341	475.1252	385.0934	355.0828	0.07	
5	Luteolin-di-C-hexoside	C27H30O16	18.39	611.16121		593.1504	575.1391	473.1087	341.0659	311.0553	0.11	
6	Phaselic acid (2-O-Caffeoylmalic acid)	C13H12O8	18.74		295.04540	179.0342	135.0440	133.0131	115.0024	71.0123	0.14	
7	Isololiolide	C11H16O3	18.80	197.11777		179.1070	161.0962	135.1171	133.1015	107.0860	0.83	
8	Vicenin-2 (Apigenin-6,8-di-C-glucoside)	C27H30O15	19.38	595.16630		559.1458	541.1343	457.1138	325.0709	295.0604	4.91	
9	Apigenin-C-hexoside-C-pentoside isomer 1	C26H28O14	19.79	565.15574		547.1466	529.1346	379.0814	325.0710	295.0606	0.14	
10	Luteolin-C-hexoside-C-pentoside	C26H28O15	20.05	581.15065		563.1425	545.1283	395.0771	341.0663	311.0553	0.05	
11	Loliolide	C11H16O3	20.09	197.11777		179.1070	161.0963	135.1172	133.1015	107.0860	4.84	
12	Apigenin-C-hexoside-C-pentoside isomer 2	C26H28O14	20.48	565.15574		529.1347	511.1249	427.1035	409.0924	295.0607	0.73	
13	Apigenin-C-hexoside-C-pentoside isomer 3	C26H28O14	20.75	565.15574		529.1352	511.1248	427.1032	409.0924	295.0602	0.73	
14	Orientin (Luteolin-8-C-glucoside)	C21H20O11	20.87	449.10839		431.0981	413.0876	353.0660	329.0660	299.0553	0.26	[18]
15	Apigenin-C-hexoside-C-pentoside isomer 4	C26H28O14	21.12	565.15574		529.1350	469.1142	379.0815	325.0710	295.0605	4.32	
16	Isoorientin (Luteolin-6-C-glucoside)	C21H20O11	21.21	449.10839		431.0982	413.0872	353.0660	329.0660	299.0553	1.52	
17	Luteolin-C-hexoside	C21H20O11	21.57	449.10839		431.0982	413.0872	353.0661	329.0660	299.0554	0.33	
18 [1]	Vitexin (Apigenin-8-C-glucoside)	C21H20O10	21.84	433.11347		415.1030	397.0922	379.0818	313.0710	283.0605	10.27	[18]
19	Apigenin-C-hexoside-C-pentoside isomer 5	C26H28O14	22.13	565.15574		529.1351	397.0923	379.0818	325.0710	295.0606	0.29	
20 [1]	Vitexin-2″-O-rhamnoside	C27H30O14	22.21	579.17138		433.1135	415.1028	397.0924	313.0709	283.0604	5.06	
21	Apigenin-C-hexoside-C-pentoside isomer 6	C26H28O14	22.39	565.15574		529.1352	397.0924	379.0815	325.0710	295.0604	0.35	
22	Methoxy-tetrahydroxy(iso)flavone-O-hexoside isomer 1	C22H22O12	22.47		477.10331	462.0812	315.0516	300.0280	299.0205	271.0252	0.07	
23	Apigenin-C-hexoside-C-pentoside isomer 7	C26H28O14	22.61	565.15574		529.1351	379.0818	337.0707	325.0710	295.0603	0.22	
24	Isovitexin (Apigenin-6-C-glucoside)	C21H20O10	22.73	433.11347		415.1032	397.0920	379.0815	313.0709	283.0604	26.73	
25	Isoscoparin or Scoparin	C22H22O11	23.17	463.12404		445.1141	427.1030	367.0817	343.0816	313.0711	0.18	
26	Methoxy-tetrahydroxy(iso)flavone-O-hexoside isomer 2	C22H22O12	23.36		477.10331	462.0811	315.0516	300.0280	299.0201	271.0252	0.42	
27 [1]	Isoquercitrin (Quercetin-3-O-glucoside)	C21H20O12	23.47		463.08765	301.0358	300.0280	271.0252	255.0300	178.9980	0.20	
28 [1]	Isorhamnetin-3-O-glucoside	C22H22O12	25.49		477.10330	315.0515	314.0437	285.0410	271.0252	243.0298	0.27	
29	Isorhamnetin-O-hexoside	C22H22O12	25.71		477.10330	315.0517	314.0436	300.0281	271.0253	243.0298	0.19	
30	Abscisic acid	C15H20O4	25.81		263.12834	219.1388	204.1152	201.1280	152.0832	151.0754	0.09	
31	Dihydroxy(iso)flavone-C-hexoside	C21H20O9	26.19	417.11856		399.1074	351.0864	321.0760	297.0761	267.0653	0.18	
32	Methoxy-pentahydroxy(iso)flavone isomer 1	C16H12O8	26.35		331.04540	316.0228	287.0203	271.0253	270.0175	259.0247	0.74	
33	Methoxy-tetrahydroxy(iso)flavone-O-hexoside isomer 3	C22H22O12	27.20		477.10331	462.0810	315.0516	300.0280	299.0205	271.0252	0.13	
34	Methoxy-pentahydroxy(iso)flavone isomer 2	C16H12O8	27.54		331.04540	316.0228	287.0192	271.0265	270.0181	259.0237	0.40	
35 [1]	Quercetin (3,3′,4′,5,7-Pentahydroxyflavone)	C15H10O7	27.57		301.03483	273.0410	178.9978	151.0026	121.0282	107.0126	0.46	
36 [1]	Naringenin (4′,5,7-Trihydroxyflavanone)	C15H12O5	27.76		271.06065	227.0713	177.0184	151.0026	119.0489	107.0126	0.19	
37	Methoxy-pentahydroxy(iso)flavone isomer 3	C16H12O8	27.77		331.04540	316.0229	299.0206	287.0214	271.0254	259.0259	1.26	
38	Trihydroxy-trimethoxy(iso)flavone-O hexoside	C24H26O13	27.95		521.12952	506.1118	359.0777	358.0700	344.0542	329.0309	0.55	
39 [1]	Luteolin (3′,4′,5,7-Tetrahydroxyflavone)	C15H10O6	28.44		285.03991	217.0506	199.0390	175.0392	151.0027	133.0282	2.88	[18]
40	Quercetin-4′-O-methyl ether	C16H12O7	28.80		315.05048	300.0280	271.0252	255.0300	243.0297	227.0341	8.52	[18]
41	Apigenin (4′,5,7-Trihydroxyflavone)	C15H10O5	30.28		269.04500	227.0346	225.0553	151.0026	149.0234	117.0331	1.51	[18]
42	Chrysoeriol (3′-Methoxy-4′,5,7-trihydroxyflavone)	C16H12O6	30.59		299.05556	284.0330	256.0380	227.0334	151.0025	107.0125	1.03	[18]
43	Dimethoxy-trihydroxy(iso)flavone isomer 1	C17H14O7	31.11		329.06613	314.0438	299.0201	285.0410	271.0252	243.0298	8.04	
44	Dihydroxy-dimethoxy(iso)flavone isomer 1	C17H14O6	32.37		313.07122	298.0487	297.0409	283.0252	269.0457	255.0299	0.04	[35]
45	Dihydroxy-dimethoxy(iso)flavone isomer 2	C17H14O6	32.93		313.07122	298.0487	297.0408	283.0253	269.0455	255.0301	0.07	[35]
46	Dimethoxy-trihydroxy(iso)flavone isomer 2	C17H14O7	33.29		329.06613	314.0437	299.0201	285.0407	271.0252	243.0294	8.11	
47	Dihydroxy-dimethoxy(iso)flavone isomer 3	C17H14O6	34.82		313.07122	298.0487	283.0255	270.0537	269.0461	255.0300	0.11	[35]
48	Dihydroxy-dimethoxy(iso)flavone isomer 4	C17H14O6	35.50		313.07122	298.0488	283.0254	269.0465	255.0301	242.0224	0.22	[35]

[1] Confirmed by standard.

Among the compounds, luteolin and apigenin and several of their derivatives could be identified. Indeed, apigenin has been reported to possess various beneficial health effects such as antioxidant, anti-inflammatory, and chemopreventive effects [36]. Similarly, luteolin and its glycosides are widely distributed in the plant kingdom and preclinical studies have demonstrated this flavone to exhibit a range of pharmacological actions, including antimicrobial, anticancer, antioxidant and anti-inflammatory [37]. Naringenin was also detected in the extracts. Interestingly, naringenin is endowed with a wide range of biological effects on human health, including antidiabetic, antiviral, cardioprotective, antioxidant, and anti-hyperlipidemic properties, amongst others [38]. Nonetheless, quercetin, also identified in the studied extracts, is a flavonoid found in fruits and vegetables, having unique biological properties that may improve both mental and physical performance and reduce the risk of infections [39]. Epidemiological evidence suggests that flavonoids may play a vital function in the decreased risk of chronic diseases associated with a diet rich in plant-derived foods [37]. Hence, the chemical profiles of extracts of *P. foetida* identified in the present study suggest that the plant can be a good source of flavonoids and therefore can be considered for the potential development of nutraceuticals.

Assays based upon the use of DPPH and ABTS radicals are among the most popular spectrophotometric methods for determining the antioxidant capacity of various samples, including plant extracts [40]. In the present study, all tested extracts were found to be fairly good radical scavengers. However, in DPPH assay, the methanolic (80%) extract showed the highest scavenging potential, while in ABTS assay, the ethyl acetate extract was the most potent (68.98 ± 2.80 mg/g), followed by infusion and water maceration extracts (62.08 ± 1.11 and 61.81 ± 1.44 mg/g respectively).

Likewise, ethyl acetate extracts displayed the highest reducing activity in both CUPRAC and FRAP assays (162.83 ± 0.24 and 65.35 ± 0.57 mg/g respectively), followed by methanolic extract (CUPRAC: 103.00 ± 1.93 mg/g and FRAP: 35.76 ± 1.43 mg/g). Nonetheless, reducing activity was also reasonably displayed by the other extracts ranging from 54.46–66.83 mg/g in CUPRAC assay and 30.36–32.38 mg/g in FRAP assay. Moreover, all extracts acted as metal chelators (14.10–19.21 mg/g), although the highest metal chelating effects were achieved by methanolic and infusion extracts (19.21 ± 0.09 and 18.00 ± 0.12 mg/g respectively). Regarding the phosphomolybdenum assay, the extracts' antioxidant capacity ranged from 0.66–2.68 mM/g, with ethyl acetate extract showing the highest whilst methanolic (80%) extract, the lowest activity (Table 4).

Table 4. Antioxidant abilities of the tested extracts.

Extracts	DPPH	ABTS	CUPRAC	FRAP	Metal Chelating	PBD
	(mg TE/g)				(mg EDTAE/g)	(mM TE/g)
EA	26.69 ± 1.85 ^{b}	68.98 ± 2.80 ^{a}	162.83 ± 0.24 ^{a}	65.35 ± 0.57 ^{a}	15.60 ± 0.26 ^{c}	2.68 ± 0.19 ^{a}
MeOH	28.57 ± 0.36 ^{b}	50.99 ± 0.89 ^{c}	103.00 ± 1.93 ^{b}	35.76 ± 1.43 ^{b}	19.21 ± 0.09 ^{a}	2.28 ± 0.11 ^{b}
MeOH (80%)	31.74 ± 0.90 ^{a}	55.06 ± 3.32 ^{c}	66.83 ± 0.21 ^{c}	30.36 ± 0.48 ^{d}	15.24 ± 0.58 ^{c}	0.66 ± 0.10 ^{d}
Water maceration	20.77 ± 0.18 ^{c}	61.81 ± 1.44 ^{b}	54.46 ± 1.44 ^{e}	31.59 ± 0.28 ^{c,d}	14.10 ± 0.40 ^{d}	1.01 ± 0.01 ^{c}
Infusion	21.81 ± 0.37 ^{c}	62.08 ± 1.11 ^{b}	61.33 ± 1.70 ^{d}	32.38 ± 0.08^{c}	18.00 ± 0.12 ^{b}	1.02 ± 0.02 ^{c}

Values are reported as mean ± S.D. EA: Ethyl acetate; MeOH: methanolic; TE: Trolox equivalent; EDTAE: EDTA equivalent; PBD: Phosphomolybdenum. Different letters (a, b, c, d, and e) indicate significant differences in the extracts ($p < 0.05$).

Other studies have also attested to the antioxidant potential of *P. foetida* extract by the radical scavenging mechanism. For instance, Ajane and Patil [30] reported *P. foetida* extract to show scavenging ability in both DPPH (IC_{50}: 614.405 µg/mL) and ABTS (IC_{50}: 25.18 µg/mL) assays. Moreover, in the same study, *P. foetida* leaves were found to possess total antioxidant potential comparable to ascorbic acid. The antioxidant potential also correlated with the extract content in phenols, flavonoids, and saponins [30]. Hence, the relatively higher antioxidant potential of ethyl acetate extract obtained in the present study could be attributed to the comparatively higher phenolic content present. Indeed, phenolic compounds may exert their antioxidant activity in different ways [41].

Furthermore, high antiradical properties (EC_{50} of 1.37 ± 1.17 µg/mL) and moderate antioxidant reducing power (0.41 ± 0.03 mM FE), with 82.09 ± 13.82 mg gallic acid equivalent/g of TPC and 205.59 ± 6.57 mg quercetin equivalent/g of TFC values were displayed by *P. foetida* methanolic extract [29]. Similarly, reducing power and free radical scavenging potential was demonstrated by fruit and leaf extracts of *P. foetida*. However, the antioxidant activity of the leaf extract was higher than the fruit extract. Additionally, phytochemicals such as alkaloids, phenolics, flavonoids, saponins, and cardiac glycosides were found in the leaf extract [42].

Cholinesterase (ChE) inhibitors are a significant target in the treatment of Alzheimer's disease [43]. On the pharmacy shelf, some drugs such as tacrine and rivastigmine have been produced as cholinesterase inhibitors for alleviating Alzheimer's disease. As a result, a number of botanicals used in various traditional systems of medicines as memory enhancers have been evaluated for anti-cholinesterase activity [44]. In the present investigation, with the exception of ethyl acetate extract which was found to inhibit BChE selectively (2.45 ± 0.18 mg GALAE/g), *P. foetida* extracts inhibited AChE selectively. Particularly, the methanolic extract was observed to be the most potent AChE inhibitor (2.22 ± 0.04 mg GALAE/g), while water maceration, methanolic (80%) and infusion extracts showed moderate inhibition against AChE (0.50–0.92 mg GALAE/g) (Table 5). Previous studies have also reported the AChE inhibition induced by *P. foetida* extract [45]. An infusion was also reported as a moderate inhibitor in our previous paper [46].

Table 5. Enzyme inhibitory properties of the tested extracts.

Extracts	AChE	BChE	Tyrosinase	Amylase	Glucosidase
	(mg GALAE/g)		(mg KAE/g)	(mM ACAE/g)	
EA	na	2.45 ± 0.18	48.48 ± 3.68 ^{a}	0.59 ± 0.01 ^{a}	0.70 ± 0.01 ^{a}
MeOH	2.22 ± 0.04 ^{a}	na	29.33 ± 1.71 ^{b}	0.43 ± 0.03 ^{b}	0.37 ± 0.01 ^{b}
MeOH (80%)	0.92 ± 0.02 ^{b}	na	35.11 ± 5.77 ^{b}	0.35 ± 0.02 ^{c}	0.30 ± 0.01 ^{c}
Water maceration	0.77 ± 0.08 ^{c}	na	na	0.17 ± 0.01 ^{d}	0.11 ± 0.01 ^{d}
Infusion	0.50 ± 0.08 ^{d}	na	na	0.41 ± 0.01 ^{b}	0.28 ± 0.01 ^{c}

Values are reported as mean ± S.D. EA: Ethyl acetate; GALAE: Galantamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent. na: not active. Different letters (a, b, c, d) indicate significant differences in the extracts ($p < 0.05$).

Tyrosinase is a key enzyme in the synthesis of melanin, thus it can be regarded as the main target for controlling hyperpigmentation problems [47]. In the present study, only the ethyl acetate and the two methanolic extracts showed anti-tyrosinase inhibitory potentials, whereas water maceration and infusion extracts were both inactive against tyrosinase. Ethyl acetate extract exhibited the highest anti-tyrosinase activity (48.48 ± 3.68 mg KAE/g), followed by the methanolic extracts (29.33 ± 1.71 and 35.11 ± 5.77 mg KAE/g) (Table 5). Interestingly, in addition to inhibition of melanin formation in B16 melanoma cells and the exhibition of DPPH radical-scavenging activity, *P. foetida* was also found to inhibit tyrosinase enzyme [48]. It is noteworthy that antioxidants have been reported to exhibit potent inhibitory activities towards tyrosinase and melanin production and have consequently been used to inhibit melanogenesis [49].

In fact, a high correlation was obtained between mushroom tyrosinase inhibition and ABTS and DPPH radical scavenging activity in previous reports suggesting that free radical scavenging activity enhanced the inhibition of mushroom tyrosinase activity [50]. Hence, the relatively good anti-tyrosinase activity of the extracts reported in the present work also could be ascribed to their respective antioxidant potency.

Chronic hyperglycemia has been considered as one of the principal causes of several diabetic complications. Since the major source of glucose is dietary carbohydrates, the inhibition of key carbohydrates digesting enzymes, such as α-amylase and α-glucosidase, can be vital in preventing a postprandial rise in blood glucose, chronic hyperglycemia and hence diabetic complications. This is because inhibitors of these enzymes (α-amylase and α-glucosidase) delay the carbohydrate digestion

and reduce the rate of glucose absorption from the gut, lowering the postprandial rise in blood glucose level. Therefore, inhibition of α-amylase and α-glucosidase is key in the management and treatment of non-insulin-dependent diabetes mellitus or type 2 diabetes [51,52].

Interestingly, in the present study, all tested extracts demonstrated dual inhibition against α-amylase (0.17–0.59 mM/g) and α-glucosidase (0.11–0.70 mM/g). However, the ethyl acetate extract displayed the highest anti-amylase (0.59 ± 0.01 mM/g) and anti-glucosidase (0.70 ± 0.01 mM/g) effects. Comparatively, water maceration extract showed the least activity against these enzymes (0.17 ± 0.01 and 0.11 ± 0.01 mM/g respectively). Nonetheless, the other tested extracts displayed moderate inhibitory potentials α-amylase and α-glucosidase (Table 5).

Remarkably, the findings of the present study were in agreement with previous studies which also reported the antidiabetic potential of *P. foetida* by inhibiting the enzymes involved in postprandial hyperglycemia. Maximum α-amylase inhibitory activity was recorded in 100 µg/mL of aqueous and ethanolic extract of root with inhibition of 80.3% and 83.3%, respectively. In the α-glucosidase inhibitory activity, the aqueous extract of seed of *P. foetida* showed maximum inhibition of 72.3%, followed by the ethanolic extract of its root which demonstrated inhibition of 65.7% [53]. Likewise, several other *Passiflora* species have been reported to be potent inhibitors of α-amylase and α-glucosidase. This was largely attributed to their high phenolic/flavonoid content and antioxidant activity [54,55]. Recent studies have also established polyphenols rich plants to have the ability to chelate the enzymes α-amylase and α-glucosidase through alteration in a structure that is coupled with the loss of biological functions [54,56].

Based on the results of HPLC-MS fingerprint analysis, *in silico*, *in vitro*, and *ex vivo* studies were conducted with the aim to unravel putative pharmacokinetics, target proteins and toxicological effects underlying *P. foetida* extract rational use in phytotherapy. Formerly, the brine shrimp toxicological assay was conducted with the aim to define the biocompatibility limits of methanol and water extracts, in the range 1–20 mg/mL. The tests yielded LC_{50} values < 5 mg/mL for both *P. foetida* extracts. These data were also confirmed by *in silico* toxicological evaluation conducted through the "Toxicity Estimation Software Tool" (T.E.S.T.) developed by the United States Environmental Protection Agency (EPA). Specifically, the analyses were conducted on selected phytochemicals, namely apigenin, chrysoeriol, isovitexin, loliolide, luteolin, quercetin, and vitexin.

The *in silico* eco-toxicological tests were conducted on the *Daphnia magna* model that yielded LC_{50} values in the range 2–26 mg/mL. This is consistent, albeit partially, with the experimental LC_{50} values obtained with the brine shrimp test. The selected phytochemicals were also analyzed through the bioinformatics platforms SwissADME and SwissTargetPrediction, as well, with the aim to predict pharmacokinetics and putative targets, respectively. The results of the bioinformatics approach are fully reported in the Supplementary Materials (Bioinformatics Folder), whereas the synthetic components-targets analysis (Network Pharmacology) is included in Figure 1.

All these compounds were predicted to be absorbed through the gastrointestinal tract, as well, whereas the sole loliolide was predicted to cross the blood brain barrier (BBB). Among all proteins identified by the bioinformatics analysis, we focused our attention on selected enzymes, namely AChE, cyclo-oxygenase (COX)-2, tyrosinase, and monoamine oxidase (MAO)-A. The selected enzymes were docked with apigenin, chrysoeriol, isovitexin, loliolide, luteolin, quercetin, and vitexin, in order to confirm the bioinformatics predictions and calculate putative affinity constants (Ki) that were in the submicromolar-micromolar range (0.2–10.5 µM) (Figure 2A–O).

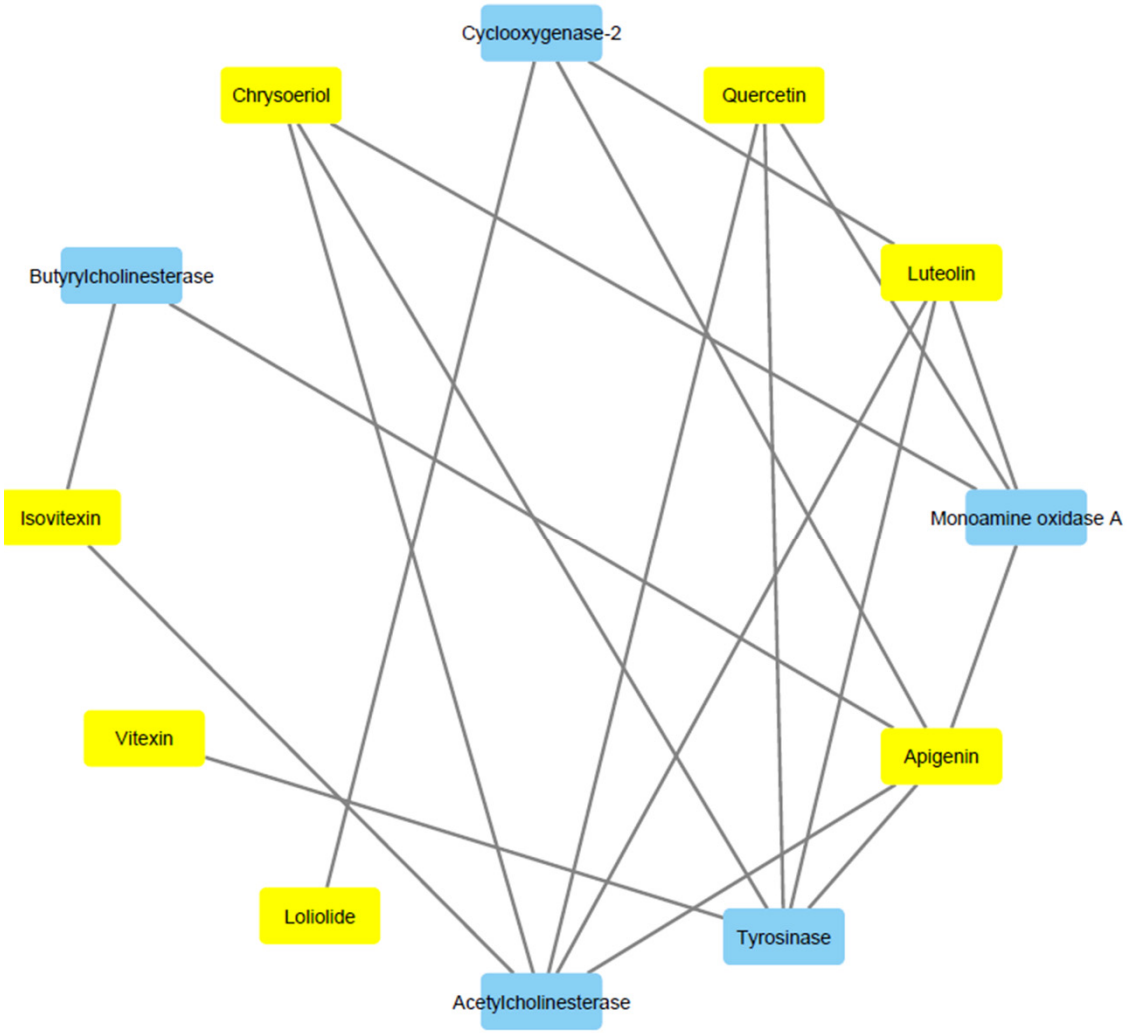


Figure 1. Components-Targets analysis.

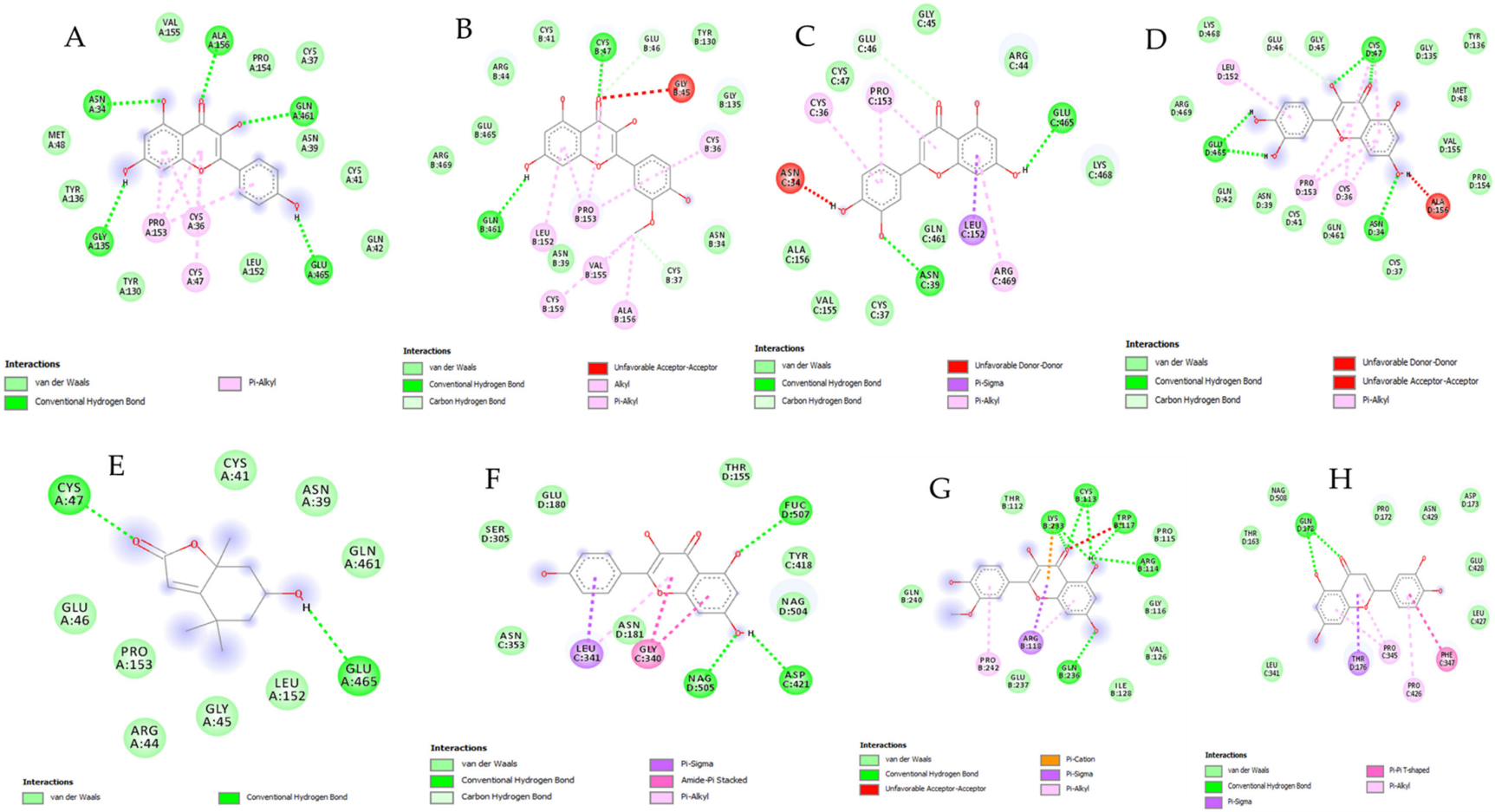


Figure 2. *Cont.*

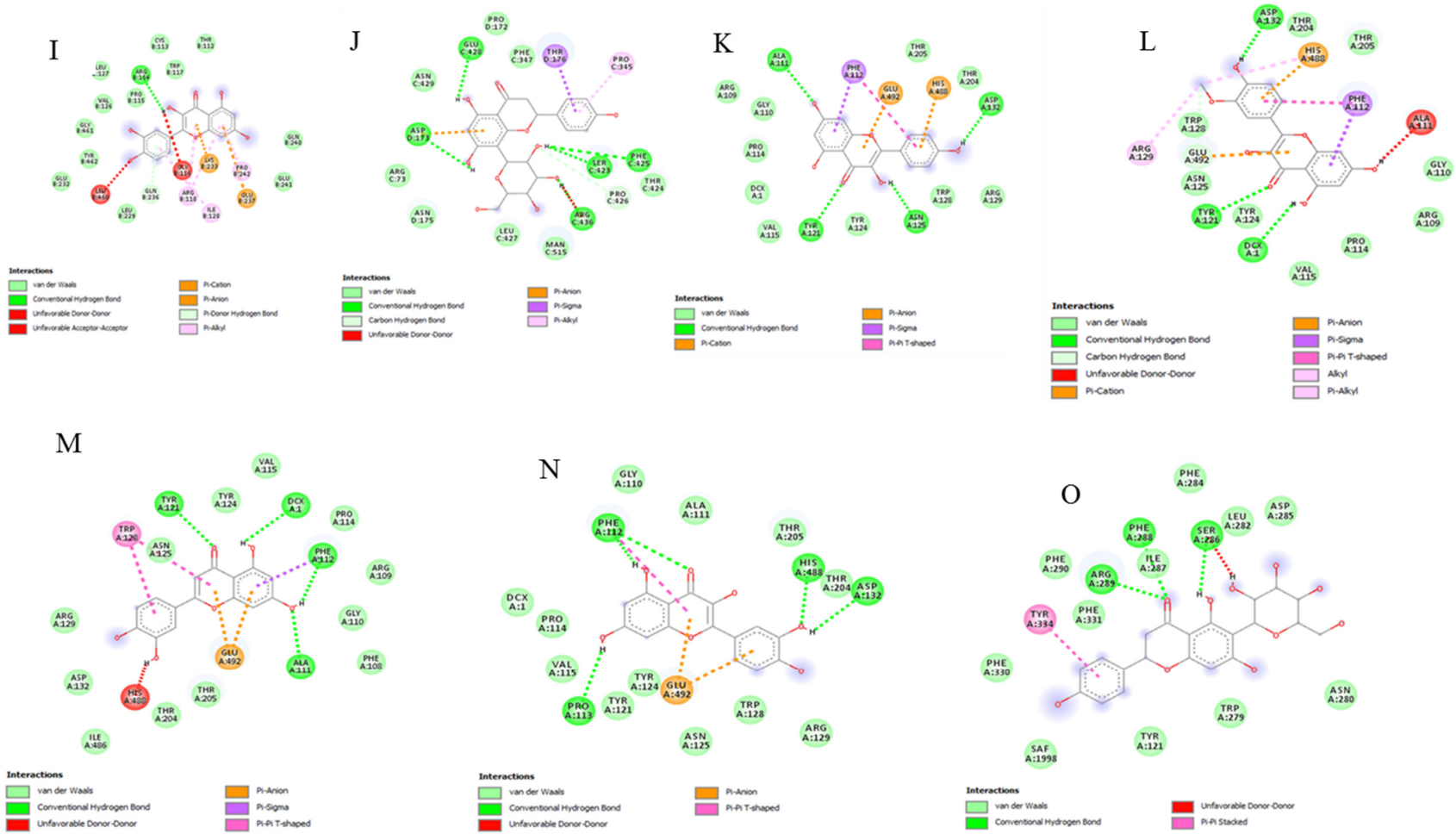


Figure 2. (**A**): Putative interaction between apigenin and COX-2 (PDB: 1CX2). Free energy of binding (ΔG) and affinity (Ki) are −8.8 kcal/mol and 0.4 μM, respectively; (**B**): Putative interaction between chrysoeriol and COX-2 (PDB: 1CX2). Free energy of binding (ΔG) and affinity (Ki) are −9.3 kcal/mol and 0.2 μM, respectively; (**C**): Putative interaction between luteolin and COX-2 (PDB: 1CX2). Free energy of binding (ΔG) and affinity (Ki) are −9.5 kcal/mol and 0.1 μM, respectively; (**D**): Putative interaction between quercetin and COX-2 (PDB: 1CX2). Free energy of binding (ΔG) and affinity (Ki) are −9.3 kcal/mol and 0.2 μM, respectively; (**E**): Putative interaction between loliolide and COX-2 (PDB: 1CX2). Free energy of binding (ΔG) and affinity (Ki) are −6.8 kcal/mol and 10.5 μM, respectively;

(**F**): Putative interaction between apigenin and tyrosinase (PDB: 5M8P). Free energy of binding (ΔG) and affinity (Ki) are −8.1 kcal/mol and 1.2 μM, respectively;
(**G**): Putative interaction between chrysoeriol and tyrosinase (PDB: 5M8P). Free energy of binding (ΔG) and affinity (Ki) are −7.8 kcal/mol and 1.9 μM, respectively;
(**H**): Putative interaction between luteolin and tyrosinase (PDB: 5M8P). Free energy of binding (ΔG) and affinity (Ki) are −8.2 kcal/mol and 0.9 μM, respectively;
(**I**): Putative interaction between quercetin and tyrosinase (PDB: 5M8P). Free energy of binding (ΔG) and affinity (Ki) are −8.3 kcal/mol and 0.8 μM, respectively;
(**J**): Putative interaction between vitexin and tyrosinase (PDB: 5M8P). Free energy of binding (ΔG) and affinity (Ki) are −8.6 kcal/mol and 0.5 μM, respectively;
(**K**): Putative interaction between apigenin and MAO-A (PDB: 2Z5X). Free energy of binding (ΔG) and affinity (Ki) are −8.2 kcal/mol and 0.9 μM, respectively;
(**L**): Putative interaction between chrysoeriol and MAO-A (PDB: 2Z5X). Free energy of binding (ΔG) and affinity (Ki) are −8.3 kcal/mol and 0.8 μM, respectively;
(**M**): Putative interaction between luteolin and MAO-A (PDB: 2Z5X). Free energy of binding (ΔG) and affinity (Ki) are −8.4 kcal/mol and 0.7 μM, respectively;
(**N**): Putative interaction between quercetin and MAO-A (PDB: 2Z5X). Free energy of binding (ΔG) and affinity (Ki) are −8.3 kcal/mol and 0.8 μM, respectively;
(**O**): Putative interaction between quercetin and AchE (PDB: 1GQR). Free energy of binding (ΔG) and affinity (Ki) are −8.7 kcal/mol and 0.4 μM, respectively [57].

The putative interactions between extracts' phytochemicals and selected enzymes were also confirmed, albeit partially, by the aforementioned colorimetric tests and the pharmacological paradigms discussed below. In this regard, methanol and water extracts were studied through *in vitro* and *ex vivo* models in order to confirm their efficacy in experimental paradigms mimicking the burden of oxidative stress and inflammation at both central and peripheral levels. Specifically, hypothalamic HypoE22 cells were treated with scalar extract concentrations (10–500 µg/mL), with the highest concentration being at least 10-fold lower than the LC_{50} calculated via brine shrimp test [22]. As evidenced by the MTT viability test, the methanol and water extracts were tolerated by HypoE22 cells, in the aforementioned concentration range, in both basal and hydrogen peroxide-induced oxidative stress conditions (Figure 3). Additionally, the lowest tested concentration of the water extract induced a mild stimulation of the cell viability, as well.

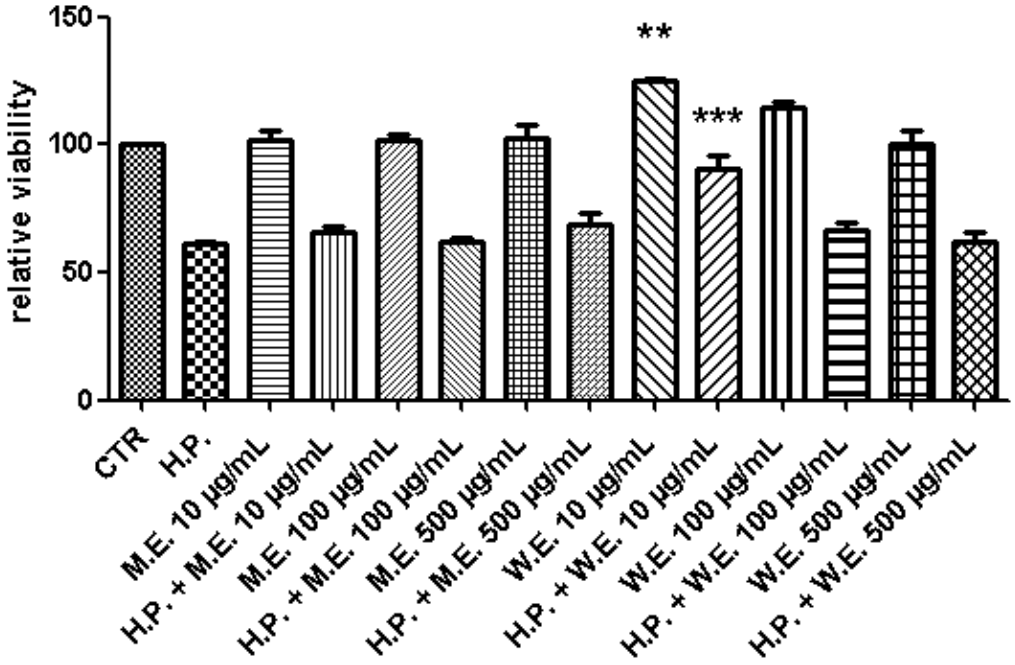
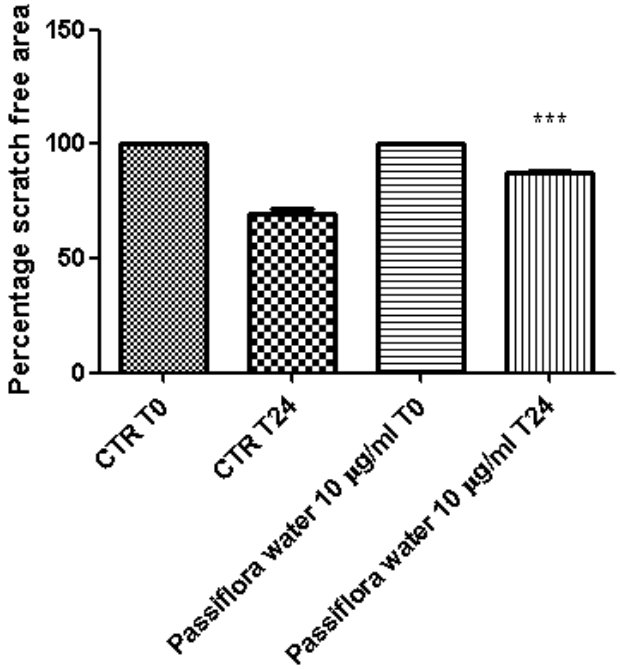


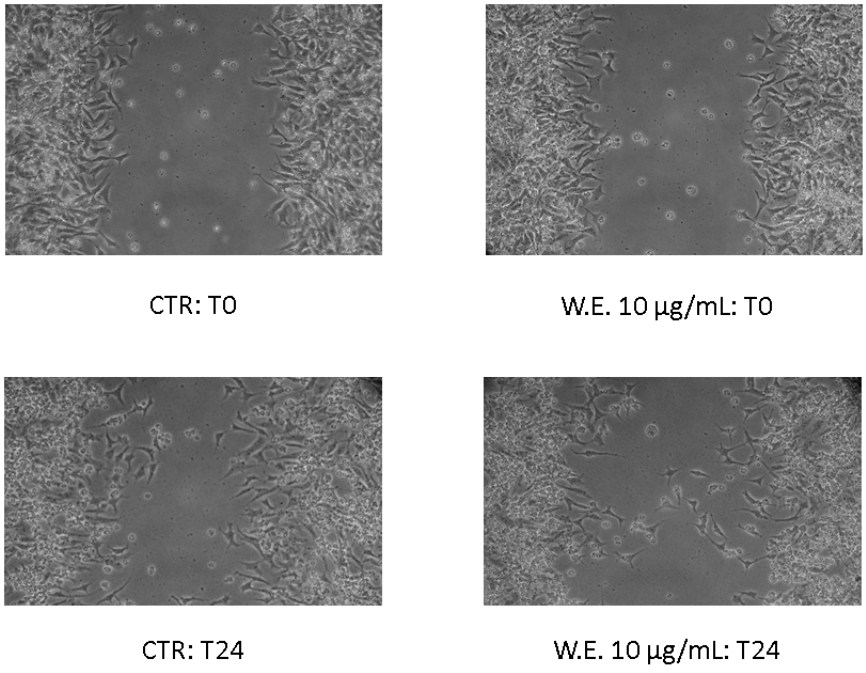
Figure 3. Effects of *P. foetida* methanol (M.E.) and water (W.E.) extracts on basal and hydrogen peroxide 300 µM (H.P.)-induced hypothalamic HypoE22 cell viability (MTT test). Cell viability was relatively calculated towards the untreated control (CTR) group. Data were analyzed through analysis of variance (ANOVA), followed by post hoc Newman-Keuls test. ANOVA, $p < 0.001$; ** $p < 0.01$ vs. H.P; *** $p < 0.001$.

By contrast, the wound healing test showed the water extract-induced decrease in the spontaneous migration (expressed as percentage of scratch-free area) of HypoE22 cells, in the 24 h following the experimental lesion of cell monolayer (Figure 4 A,B). This suggests a minor role exerted by extract as a neuroprotective agent.

Additionally, none of the two extracts were able to prevent the hydrogen peroxide-induced decrease of extracellular 5-HT level (Figure 5).



(**A**)



(**B**)

Figure 4. Effects of subtoxic concentration (10 µg/mL) of *P. foetida* water extract on spontaneous hypothalamic HypoE22 cell migration. Quantification of free cell area (**A**) and representative images (**B**) of wound healing test recorded at different time (T) points: 0 and 24 h following stimulation. ANOVA, $p < 0.001$; *** $p < 0.001$ vs. CTR T24.

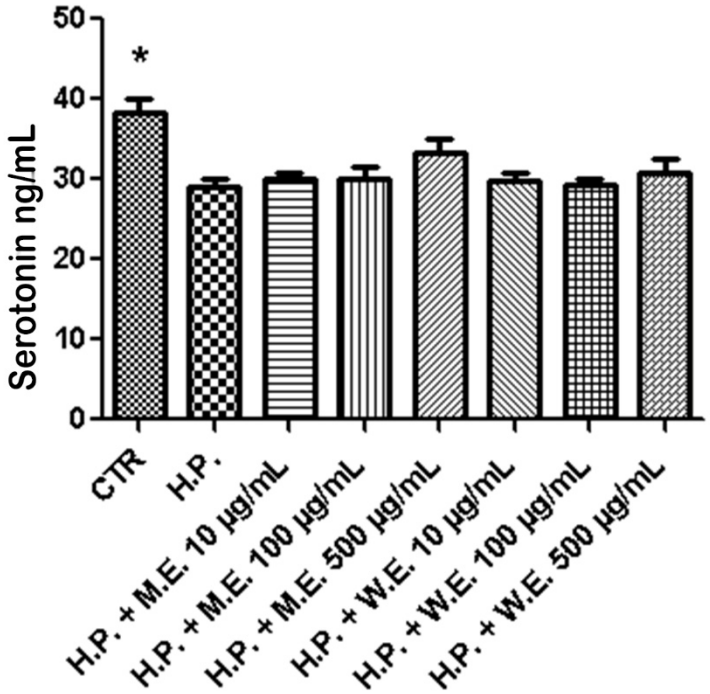


Figure 5. Effects of *P. foetida* methanol (M.E.) and water (W.E.) extracts on hydrogen peroxide 300 µM (H.P.)-induced decrease of serotonin (5-HT) release from hypothalamic HypoE22 cell. 5-HT release was quantified through HPLC coupled to coulometric detection and expressed as ng/mL. Data were analyzed through analysis of variance (ANOVA), followed by post hoc Newman-Keuls test. ANOVA, $p < 0.05$; * $p < 0.05$ vs. H.P.

As a consequence, the putative MAO-A inhibition, at least in part predicted by docking calculations, is questionable. MAO-A is deeply involved in 5-HT catabolism, and increased oxidative stress and inflammatory conditions in the brain are able to upregulate MAO-A activity [58,59]. Although most of the selected phytochemicals showed good affinities towards MAO-A, the two extracts were ineffective in preventing hydrogen-peroxide depletion of hypothalamic 5-HT. Additionally, none of these phytochemicals was predicted to cross BBB, with the exception of the sole loliolide that was found to interact with the COX-2. In this context, we can hypothesize that the traditional uses of *P. foetida* in treating neurological and psychiatric disorders [9] could derive, at least partially, from the potential efficacy of plant-deriving phytochemicals in counteracting the inflammatory components underlying these chronic diseases [60].

P. foetida was also reported to exert protective effects in skin inflammatory models [8]. Based on the aforementioned anti-tyrosinase effects induced by the extracts, and further confirmed by docking calculations on apigenin, chrysoeriol, luteolin, quercetin, and vitexin, we conducted an *ex vivo* study in order to verify the efficacy of *P. foetida* water and methanol extracts on isolated mouse skin specimens challenged with hydrogen peroxide (1 mM), that, besides functioning as a classical oxidative stress stimulus, is also able to increase tyrosinase activity [61]. The increased tyrosinase activity was monitored through the evaluation of the skin L-dopa level [62], compared to the untreated control (CTR) group (Figure 6). By contrast, the extract efficacy was evaluated as the prevention of hydrogen peroxide-induced L-dopa turnover. Both extracts were able to prevent L-dopa turnover, thus further confirming the anti-tyrosinase activity suggested by both colorimetric and docking analyses.

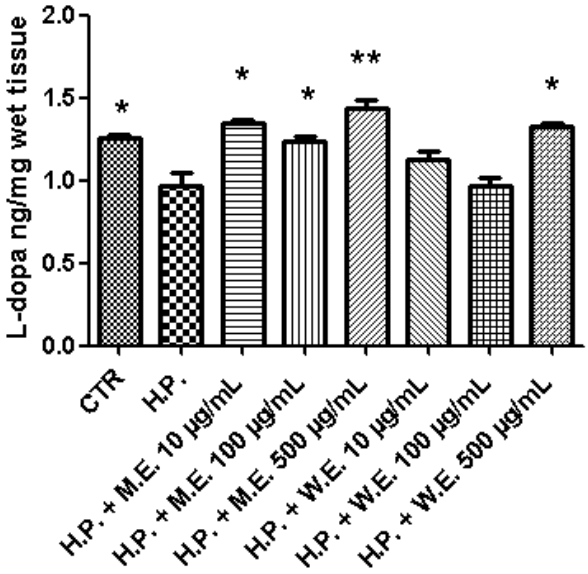


Figure 6. Effects of *P. foetida* methanol (M.E.) and water (W.E.) extracts on hydrogen peroxide 1 mM (H.P.)-induced decrease of l-dopa release from isolated mouse skin. l-dopa release was quantified through HPLC coupled to coulometric detection and expressed as ng/mg wet tissue. Data were analyzed through analysis of variance (ANOVA), followed by post hoc Newman-Keuls test. ANOVA, $p < 0.001$; * $p < 0.05$, ** $p < 0.01$ vs. H.P.

Additionally, the extracts were also effective in blunting hydrogen peroxide-induced level of PGE_2 (Figure 7). This also agrees with the calculated affinity of apigenin, chrysoeriol, luteolin, quercetin, and loliolide towards COX-2.

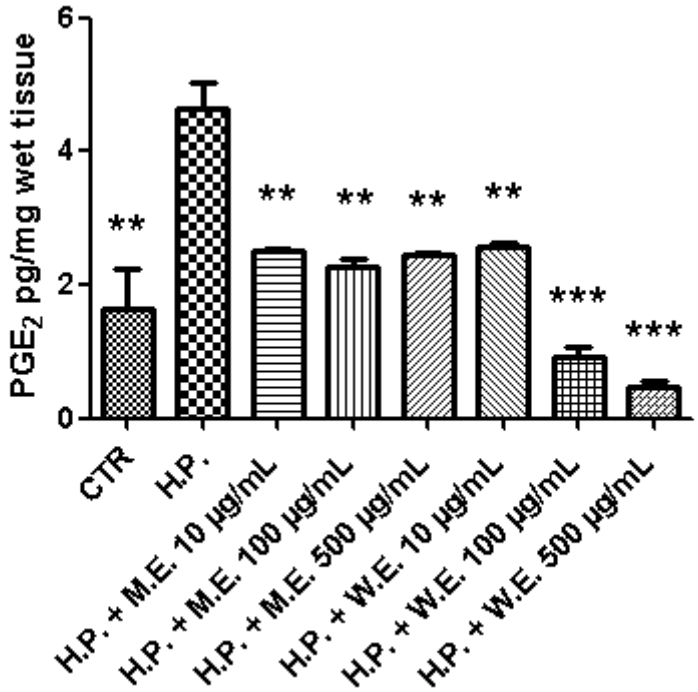


Figure 7. Effects of *P. foetida* methanol (M.E.) and water (W.E.) extracts on hydrogen peroxide 1 mM (H.P.)-induced decrease of PGE_2 release from isolated mouse skin. ANOVA, $p < 0.001$; ** $p < 0.01$, *** $p < 0.001$ vs. H.P.

Finally, considering the potential antimicrobial applications of *P. foetida* [1], methanol and water extracts were also tested against multiple dermatophytes strains, namely *Tricophyton* and *Aerthrodrma* species (Table 6), that are involved in skin disorders, including hyperpigmentation [63–65]. Both extracts were able to inhibit fungal growth and these results add to the aforementioned inhibitory activity against tyrosinase, which is crucial in the pharmacotherapy of hyperpigmentation. The present antimycotic activity could be related, at least partially, to the extract phenol and flavonoid content of *P. foetida* [66] and further supports the phytotherapy use of *P. foetida* as a skin protective agent.

Table 6. Minimal inhibitory concentrations (MICs) of *P foetida* methanol and water extracts, and griseofulvin towards selected dermatophytes.

Dermatophytes (ID strain) A3:D15	Minimum Inhibitory Concentration (MIC)		
	Methaol Extract (μg mL^{-1}) *	Water Extract (μg mL^{-1}) *	Griseofulvin (μg mL^{-1}) *
Arthroderma crocatum (CCF 5300)	12.4 (7.81–15.625)	39.37(31.25–62.5)	>8
Arthroderma curreyi (CCF 5207)	6.19 (3.9–7.81)	12.4(7.81–15.625)	>8
Arthroderma gypseum (CCF 6261)	157.49 (125–250)	396(250–500)	1.587(1–2)
Arthroderma insingulare (CCF 5417)	12.4 (7.81–15.625)	19.68(15.625–31.25)	>8
Arthroderma quadrifidum (CCF 5792)	78.74 (62.5–125)	314.98(250–500)	>8
Trichophyton mentagrophytes (CCF 4823)	49.6 (31.25–62.5)	99.21(62.5–125)	2.52(2–4)
Trichophyton mentagrophytes (CCF 5930)	157.49 (125–250)	396(250–500)	3.174(2–4)
Trichophyton rubrum (CCF 4933)	39.37 (31.25–62.5)	99.21(62.5–125)	1.26(1–2)
Trichophyton rubrum (CCF 4879)	99.21 (62.5–125)	198,42(125–250)	3.175(2–4)
Trichophyton tonsurans (CCF 4834)	9.84 (7.81–15.625)	9.84(7.81–15.625)	0.198(0.125–0.25)

* MIC values are reported as geometric means of three independent replicates (n = 3); MIC ranges are reported within brackets. MIC values are reported as < [lowest concentration tested].

4. Conclusions

Passiflora species have been greatly appraised for their vast range of bioactive compounds and pharmacological properties. In this study, *P. foetida* extracts prepared using different solvents were found to yield a varying amount of total phenolic and flavonoid contents. Particularly, ethyl acetate and methanolic (80%) extracts were found to contain the highest phenolic and flavonoid contents respectively. Nonetheless, the infusion and methanolic extracts were both found to be rich in flavonoids with the presence of flavones, flavone glycosides, amongst others. Additionally, *P. foetida* extracts displayed notable enzyme inhibitory effects by acting as inhibitors of α-amylase, α-glucosidase, tyrosinase, acetyl- and butyryl-cholinesterase, although the extracts differed in their inhibition capacities. All *P. foetida* extracts also showed good overall antioxidant potentials by acting as radical scavenging, reducing, and metal chelating mechanisms. The enzyme inhibition and antioxidant effects were also confirmed by both docking and pharmacological evaluations. Finally, the extracts were also effective against multiple dermatophytes strains involved in skin inflammation. Hence, the results obtained from the current investigation evidently support the therapeutic properties of *P. foetida* as antioxidant, antidiabetic, and anti-hyperpigmentation agents and thus could help to stimulate further consideration to understand their potential influence on human health as potential pharmaceuticals and nutraceuticals.

Supplementary Materials: The following are available online at http://www.mdpi.com/2227-9717/8/9/1034/s1, Supplementary Folder.

Author Contributions: Conceptualization, C.F., P.A., G.O., G.Z., L.M.; methodology, C.F, P.A., G.O., G.Z., L.M.; software, C.F., G.Z.; validation, C.F., P.A., G.O., G.Z., L.M.; formal analysis, C.F., P.A., G.O., G.Z., L.M.; investigation, A.C., S.C.D.S., M.C.C., K.I.S., G.A.F., L.R., S.L., R.V., O.K.E., G.A., S.J., J.J., Z.C., C.F., P.A., G.O., G.Z., L.M.; data curation, C.F., P.A., G.O., G.Z., L.M.; writing—original draft preparation, M.F.M.; writing—review and editing, C.F., P.A., G.O., G.Z., L.M., M.F.M.; visualization, L.B.; supervision, L.B.; project administration, C.F., G.O., L.M.; funding acquisition, C.F., G.O., L.M. All authors have read and agreed to the published version of the manuscript.

Funding: The study was supported by funds from Cristalfarma S.r.l. (Milan, Italy) within joint projects coordinated by Giustino Orlando, Luigi Menghini and Claudio Ferrante.

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

References

1. Simão, M.J.; Barboza, T.J.; Vianna, M.G.; Garcia, R.D.O.; Mansur, E.; Ignacio, A.C.P.; Pacheco, G. A comparative study of phytoconstituents and antibacterial activity of in vitro derived materials of four Passiflora species. *Anais da Academia Brasileira de Ciências* **2018**, *90*, 2805–2813. [CrossRef] [PubMed]
2. Casierra-Posada, F.; Jarma-Orozco, A. Nutritional Composition of Passiflora Species. In *Nutritional Composition of Fruit Cultivars*; Elsevier BV: Amsterdam, The Netherlands, 2016; pp. 517–534.
3. Gadioli, I.L.; Cunha, M.D.S.B.D.; De Carvalho, M.V.O.; Costa, A.M.; Pineli, L.D.L.D.O. A systematic review on phenolic compounds inPassifloraplants: Exploring biodiversity for food, nutrition, and popular medicine. *Crit. Rev. Food Sci. Nutr.* **2017**, *58*, 785–807. [CrossRef] [PubMed]
4. Dhawan, K.; Dhawan, S.; Sharma, A. Passiflora: A review update. *J. Ethnopharmacol.* **2004**, *94*, 1–23. [CrossRef] [PubMed]
5. Chen, Y.-Z.; Wei, X.; Li, M.; Duan, X.; Sun, Y.-M.; Yang, R.-L.; Su, X.-D.; Huang, R.; Wang, H. Nutritional Composition and Antioxidant Properties of the Fruits of a Chinese Wild *Passiflora foetida*. *Molecules* **2018**, *23*, 459. [CrossRef]
6. Ramaiya, S.D.; Bujang, J.S.; Zakaria, M.H.; King, W.S.; Sahrir, M.A.S. Sugars, ascorbic acid, total phenolic content and total antioxidant activity in passion fruit (Passiflora) cultivars. *J. Sci. Food Agric.* **2012**, *93*, 1198–1205. [CrossRef]
7. Revathy, S.; Sunilkumar, T.C. Phytochemical and nutritional studies on the fruit pulp extract of *Passiflora foetida* Linn. *J. Pharmacogn. Phytochem.* **2019**, *8*, 732–734.
8. Mohanasundari, C.; Natarajan, D.; Srinivasan, K.; Umamaheswari, S.; Ramachandran, A. Antibacterial properties of *Passiflora foetida* L. a common exotic medicinal plant. *Afr. J. Biotechnol.* **2007**, *6*, 2650–2653. [CrossRef]
9. Patil, A.S.; Paikrao, H.M.; Patil, S.R. *Passiflora foetida* Linn: A complete morphological and phytopharmacological review. *Int J Pharma. Bio. Sci.* **2013**, *4*, 285–296.
10. Sasikala, V.; Saravanan, S.; Parimelazhagan, T. Analgesic and anti–inflammatory activities of *Passiflora foetida* L. *Asian Pac. J. Trop. Med.* **2011**, *4*, 600–603. [CrossRef]
11. Sutar, V.; Bhosale, U.P. Screening of *Passiflora foetida* extracts as anticancer agents on MCF 7 cell line. *BIOINFOLET-A* **2013**, *10*, 808–810.
12. Anandan, R.; Jayakar, B.; Jeganathan, S.; Manavalan, R.; Kumar, S.R. Effect of ethanol extract of fruits of *Passiflora foetida* Linn. on CCl. *J. Pharm. Res.* **2009**, *2*, 413–415.
13. Rahman, M.A.; Hossain, M.A.; Hasan, M.S.; Hossain, M.G. Antinociceptive, antidiarrhoeal and cytotoxic activities of *Passiflora foetida* linn. *Pharmacologyonline* **2011**, *1*, 228–236.
14. Santosh, P.; Venugopl, R.; Nilakash, S.; Kunjbihari, S.; Mangala, L. Antidepressant activity of methanolic extract of *Passiflora foetida* leaves in mice. *Int. J. Pharm. Pharm. Sci.* **2011**, *3*, 112–115.
15. Asadujjaman, M.; Mishuk, A.U.; Hossain, A.M.; Karmakar, U.K. Medicinal potential of *Passiflora foetida* L. plant extracts: Biological and pharmacological activities. *J. Integr. Med.* **2014**, *12*, 121–126. [CrossRef]
16. Sathish, R.; Sahu, A.; Natarajan, K. Antiulcer and antioxidant activity of ethanolic extract of *Passiflora foetida* L. *Indian J. Pharmacol.* **2011**, *43*, 336–339. [CrossRef] [PubMed]
17. Ranganatha, N.; Kuppast, D.I.J.; Veerashekar, T. Study of Anti-Hypertension Activity of Aerial Parts of *Passiflora foetida* Linn. *Int. Res. J. Pharm. Plant Sci.* **2013**, *1*, 1–12.
18. Nguyen, T.Y.; To, D.C.; Tran, M.H.; Lee, J.S.; Lee, J.-H.; Kim, J.A.; Woo, M.H.; Min, B.S. Anti-inflammatory Flavonoids Isolated from *Passiflora foetida*. *Nat. Prod. Commun.* **2015**, *10*, 929–931. [CrossRef]
19. Dall'Acqua, S.; Aktumsek, A. Investigation of Antioxidant Potentials of Solvent Extracts From Different Anatomical Parts of Asphodeline Anatolica E. Tuzlaci: An Endemic Plant to Turkey. *Afr. J. Tradit. Complement. Altern. Med.* **2014**, *11*, 481–488. [CrossRef]
20. Dall'Acqua, S.; Uysal, A.; Diuzheva, A.; Gunes, E.; Jekő, J.; Cziáky, Z.; Picot-Allain, C.M.N.; Mahomoodally, F.M. Characterization of phytochemical components of Ferula halophila extracts using HPLC-MS/MS and their pharmacological potentials: A multi-functional insight. *J. Pharm. Biomed. Anal.* **2018**, *160*, 374–382. [CrossRef]

21. Grochowski, D.; Uysal, S.; Aktumsek, A.; Granica, S.; Dall'Acqua, S.; Ceylan, R.; Locatelli, M.; Tomczyk, M. In vitro enzyme inhibitory properties, antioxidant activities, and phytochemical profile of Potentilla thuringiaca. *Phytochem. Lett.* **2017**, *20*, 365–372. [CrossRef]
22. Ferrante, C.; Recinella, L.; Ronci, M.; Menghini, L.; Brunetti, L.; Chiavaroli, A.; Leone, S.; Di Iorio, L.; Carradori, S.; Tirillini, B.; et al. Multiple pharmacognostic characterization on hemp commercial cultivars: Focus on inflorescence water extract activity. *Food Chem. Toxicol.* **2019**, *125*, 452–461. [CrossRef] [PubMed]
23. Menghini, L.; Ferrante, C.; Leporini, L.; Recinella, L.; Chiavaroli, A.; Leone, S.; Pintore, G.; Vacca, M.; Orlando, G.; Brunetti, L. An Hydroalcoholic Chamomile Extract Modulates Inflammatory and Immune Response in HT29 Cells and Isolated Rat Colon. *Phytotherapy Res.* **2016**, *30*, 1513–1518. [CrossRef] [PubMed]
24. Iodice, P.; Ferrante, C.; Brunetti, L.; Cabib, S.; Protasi, F.; Walton, M.E.; Pezzulo, G. Fatigue modulates dopamine availability and promotes flexible choice reversals during decision making. *Sci. Rep.* **2017**, *7*, 535. [CrossRef] [PubMed]
25. Gu, L.; Lu, J.; Li, Q.; Wu, N.; Zhang, L.; Li, H.; Xing, W.; Zhang, X. A network-based analysis of key pharmacological pathways of Andrographis paniculata acting on Alzheimer's disease and experimental validation. *J. Ethnopharmacol.* **2020**, *251*, 112488. [CrossRef]
26. Chiavaroli, A.; Sinan, K.I.; Zengin, G.; Mahomoodally, F.M.; Sadeer, N.B.; Etienne, O.K.; Cziáky, Z.; Jekő, J.; Glamočlija, J.; Sokovic, M.; et al. Identification of Chemical Profiles and Biological Properties of Rhizophora racemosa G. Mey. Extracts Obtained by Different Methods and Solvents. *Antioxidants* **2020**, *9*, 533. [CrossRef]
27. Van Ngo, T.; Scarlett, C.J.; Bowyer, M.C.; Ngo, P.D.; Van Vuong, Q. Impact of Different Extraction Solvents on Bioactive Compounds and Antioxidant Capacity from the Root of *Salacia chinensis* L. *J. Food Qual.* **2017**, *2017*, 1–8. [CrossRef]
28. Asir, P.J.; Priyanga, S.; Hemmalakshmi, S.; Devaki, K. In Vitro Free Radical Scavenging Activity and Secondary Metabolites in *Passiflora foetida* L. *Asian. J. Pharmaceut. Res. Health Care.* **2014**, *6*, 3–11.
29. Sisin, N.N.T.; Abdullah, H.; Sul'ain, M.D. Antiproliferative, antioxidative and compounds identification from methanolic extract of *Passiflora foetida* and its fractions. *J. Anal. Pharm. Res.* **2017**, *6*, 00166. [CrossRef]
30. Ajane, G.; Patil, A.S. Evaluation of Antioxidant Potential of *Passiflora foetida* Extract and Quantitative Evaluation of its Phytochemical Content-A Possible Natural Antioxidant. *Pharm. Chem. J.* **2019**, *6*, 14–24.
31. Ulrih, N.P.; Capanoglu, E.; Jassbi, A.R.; Miron, A. Advance on the Flavonoid C-glycosides and Health Benefits. *Crit. Rev. Food Sci. Nutr.* **2015**, *56*, S29–S45. [CrossRef]
32. Zucolotto, S.M.; Fagundes, C.; Reginatto, F.H.; Ramos, F.A.; Castellanos, L.; Duque, C.; Schenkel, E.P. Analysis of C -glycosyl Flavonoids from South American Passiflora Species by HPLC-DAD and HPLC-MS. *Phytochem. Anal.* **2011**, *23*, 232–239. [CrossRef] [PubMed]
33. Simirgiotis, M.; Schmeda-Hirschmann, G.; Borquez, J.; Kennelly, E.J. The Passiflora tripartita (Banana Passion) Fruit: A Source of Bioactive Flavonoid C-Glycosides Isolated by HSCCC and Characterized by HPLC–DAD–ESI/MS/MS. *Molecules* **2013**, *18*, 1672–1692. [CrossRef] [PubMed]
34. Dall'Acqua, S.; Mahomoodally, F.M.; Paksoy, M.Y.; Picot-Allain, C.; Glamocilja, J.; Sokovic, M.; Diuzheva, A.; Jekő, J.; Cziáky, Z.; Rodrigues, M.J.; et al. Phytochemical characterization and bioactivities of five Apiaceae species: Natural sources for novel ingredients. *Ind. Crop. Prod.* **2019**, *135*, 107–121. [CrossRef]
35. Echeverri, F.; Cardona, G.; Torres, F.; Peláez, C.; Quiñones, W.; Renteria, E. Ermanin: An insect deterrent flavonoid from *Passiflora foetida* resin. *Phytochem.* **1991**, *30*, 153–155. [CrossRef]
36. Shukla, R.; Pandey, V.; Vadnere, G.P.; Lodhi, S. Role of Flavonoids in Management of Inflammatory Disorders. In *Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases*, 2nd ed.; Watson, R.R., Preedy, V.R., Eds.; Elsevier: London, UK, 2019; pp. 293–322.
37. López-Lázaro, M. Distribution and biological activities of the flavonoid luteolin. *Mini-Rev. Med. Chem.* **2009**, *9*, 31–59. [CrossRef]
38. Salehi, B.; Fokou, P.V.T.; Sharifi-Rad, M.; Zucca, P.; Pezzani, R.; Martins, N.; Sharifi-Rad, J. The Therapeutic Potential of Naringenin: A Review of Clinical Trials. *Pharmaceuticals* **2019**, *12*, 11. [CrossRef]
39. Davis, J.M.; Murphy, E.A.; Carmichael, M.D. Effects of the Dietary Flavonoid Quercetin Upon Performance and Health. *Curr. Sports Med. Rep.* **2009**, *8*, 206–213. [CrossRef]
40. Bendini, A.; Cerretani, L.; Pizzolante, L.; Toschi, T.G.; Guzzo, F.; Ceoldo, S.; Marconi, A.M.; Andreetta, F.; Levi, M. Phenol content related to antioxidant and antimicrobial activities of Passiflora spp. extracts. *Eur. Food Res. Technol.* **2005**, *223*, 102–109. [CrossRef]

41. Walter, M.; Marchesan, E. Phenolic compounds and antioxidant activity of rice. *Braz. Arch. Boil. Technol.* **2011**, *54*, 371–377. [CrossRef]
42. Tandoro, Y.; Widyawati, P.S.; Budianta, T.D.W.; Sumargo, G. Phytochemical identification and antioxidant activity of passiflora foetida fruits and leaves extracts: A comparative study. *Int. J. Pharm. Pharm. Sci.* **2020**, *12*, 55–58. [CrossRef]
43. Gauthier, S.; Emre, M.; Farlow, M.R.; Bullock, R.; Grossberg, G.T.; Potkin, S.G. Strategies for continued successful treatment of Alzheimer's disease: Switching cholinesterase inhibitors. *Curr. Med Res. Opin.* **2003**, *19*, 707–714. [CrossRef] [PubMed]
44. Ahmed, F.; Ghalib, R.M.; Sasikala, P.; Ahmed, K.K.M. Cholinesterase inhibitors from botanicals. *Pharmacogn. Rev.* **2013**, *7*, 121. [CrossRef] [PubMed]
45. Melo Filho, A.A.; Kamezaki, A.K.; Estevam Ribeiro, P.R.; Goncalves Reis De Melo, A.C.; Montero Fernandez, I.; Carvalho Dos Santos, R.; Alves Chagas, E.; Cardoso Chagas, P. Chemical Composition, Antioxidant and Biological Activity of Leaves *Passiflora foetida*. *Chem. Eng. Trans.* **2018**, *64*, 241–246. [CrossRef]
46. Zhang, L.; Rocchetti, G.; Zengin, G.; Ak, G.; Yıldıztugay, E.; Mahomoodally, F.M.; Picot-Allain, M.C.N.; Lucini, L. Profiling of polyphenols and sesquiterpenoids using different extraction methods in Muscari turcicum, an endemic plant from Turkey. *Ind. Crop. Prod.* **2020**, *154*, 112626. [CrossRef]
47. Pillaiyar, T.; Manickam, M.; Namasivayam, V. Skin whitening agents: Medicinal chemistry perspective of tyrosinase inhibitors. *J. Enzym. Inhib. Med. Chem.* **2017**, *32*, 403–425. [CrossRef]
48. Arung, E.T.; Kusuma, I.W.; Christy, E.O.; Shimizu, K.; Kondo, R. Evaluation of medicinal plants from Central Kalimantan for antimelanogenesis. *J. Nat. Med.* **2009**, *63*, 473–480. [CrossRef]
49. Cui, H.-X.; Duan, F.-F.; Jia, S.-S.; Cheng, F.-R.; Yuan, K. Antioxidant and Tyrosinase Inhibitory Activities of Seed Oils from Torreya grandis Fort. ex Lindl. *BioMed Res. Int.* **2018**, *2018*, 1–10. [CrossRef]
50. Chatatikun, M.; Supjaroen, P.; Promlat, P.; Chantarangkul, C.; Waranuntakul, S.; Nawarat, J.; Tangpong, J.; Chiabchalard, A. Antioxidant and Tyrosinase Inhibitory Properties of an Aqueous Extract of Garcinia atroviridis Griff. ex. T. Anderson Fruit Pericarps. *Pharmacogn. J.* **2020**, *12*, 71–78. [CrossRef]
51. Hullatti, K.; Telagari, M. In-vitro α-amylase and α-glucosidase inhibitory activity of Adiantum caudatum Linn. and Celosia argentea Linn. extracts and fractions. *Indian J. Pharmacol.* **2015**, *47*, 425–429. [CrossRef]
52. Islam, S.; Chipiti, T.; Ibrahim, M.; Singh, M. In vitro α-amylase and α-glucosidase inhibitory and cytotoxic activities of extracts from Cissus cornifolia planch parts. *Pharmacogn. Mag.* **2017**, *13*, 329. [CrossRef]
53. Paulraj, J.A.; Subharamanian, H.; Suriyamoorthy, P.; Kanakasabapathi, D. Phytochemical screening, GC-MS analysis and enzyme inhibitory activity of *Passiflora foetida* L. *Indo Am. J. Pharm. Res.* **2014**, *4*, 3526–3534.
54. Saravanan, S.; Parimelazhagan, T. In vitro antioxidant, antimicrobial and anti-diabetic properties of polyphenols of Passiflora ligularis Juss. fruit pulp. *Food Sci. Hum. Wellness* **2014**, *3*, 56–64. [CrossRef]
55. Shanmugam, S.; Gomes, I.A.; Denadai, M.; Lima, B.D.S.; Araújo, A.A.D.S.; Narain, N.; Neta, M.T.S.L.; Serafini, M.R.; Quintans, J.S.; Thangaraj, P. UHPLC-QqQ-MS/MS identification, quantification of polyphenols from Passiflora subpeltata fruit pulp and determination of nutritional, antioxidant, α-amylase and α-glucosidase key enzymes inhibition properties. *Food Res. Int.* **2018**, *108*, 611–620. [CrossRef] [PubMed]
56. Nagappan, H.; Pee, P.P.; Kee, S.H.Y.; Ow, J.T.; Yan, S.W.; Chew, L.Y.; Kong, K.-W. Malaysian brown seaweeds Sargassum siliquosum and Sargassum polycystum: Low density lipoprotein (LDL) oxidation, angiotensin converting enzyme (ACE), α-amylase, and α-glucosidase inhibition activities. *Food Res. Int.* **2017**, *99*, 950–958. [CrossRef]
57. Artuğer, F.; Özkaynak, F. A Novel Method for Performance Improvement of Chaos-Based Substitution Boxes. *Symmetry* **2020**, *12*, 571. [CrossRef]
58. Lee, J.J.; Chang, C.K.; Liu, I.M.; Chi, T.C.; Yu, H.-J.; Cheng, J.T. Changes in endogenous monoamines in aged rats. *Clin. Exp. Pharmacol. Physiol.* **2001**, *28*, 285–289. [CrossRef]
59. Di Giacomo, V.; Ferrante, C.; Ronci, M.; Cataldi, A.; Di Valerio, V.; Rapino, M.; Recinella, L.; Chiavaroli, A.; Leone, S.; Vladimir-Knežević, S.; et al. Multiple pharmacological and toxicological investigations on Tanacetum parthenium and Salix alba extracts: Focus on potential application as anti-migraine agents. *Food Chem. Toxicol.* **2019**, *133*, 110783. [CrossRef]
60. Beheshti, F.; Hashemzehi, M.; Hosseini, M.; Marefati, N.; Memarpour, S. Inducible nitric oxide synthase plays a role in depression- and anxiety-like behaviors chronically induced by lipopolysaccharide in rats: Evidence from inflammation and oxidative stress. *Behav. Brain Res.* **2020**, *392*, 112720. [CrossRef]

61. Karg, E.; Odh, G.; Wittbjer, A.; Rosengren, E.; Rorsman, H. Hydrogen peroxide as an inducer of elevated tyrosinase level in melanoma cells. *J. Invest. Dermatol.* **1993**, *100*, 209–2013. [CrossRef]
62. Kim, Y.-J.; Kim, M.-J.; Kweon, D.-K.; Lim, S.-T.; Lee, S.-J. Quantification of Hypopigmentation Activity In Vitro. *J. Vis. Exp.* **2019**, *145*, e58185. [CrossRef]
63. Zhang, M.; Jiang, L.; Li, F.; Xu, Y.; Lv, S.; Wang, B. Simultaneous dermatophytosis and keratomycosis caused by Trichophyton interdigitale infection: A case report and literature review. *BMC Infect. Dis.* **2019**, *19*, 983–988. [CrossRef] [PubMed]
64. Isa-Isa, R.; Arenas, R.; Isa, M. Inflammatory tinea capitis: Kerion, dermatophytic granuloma, and mycetoma. *Clin. Dermatol.* **2010**, *28*, 133–136. [CrossRef] [PubMed]
65. El-Zawawy, N.A.; Ali, S.S. Pyocyanin as anti-tyrosinase and anti tinea corporis: A novel treatment study. *Microb. Pathog.* **2016**, *100*, 213–220. [CrossRef] [PubMed]
66. Orlando, G.; Recinella, L.; Chiavaroli, A.; Brunetti, L.; Leone, S.; Carradori, S.; Di Simone, S.; Ciferri, M.C.; Zengin, G.; Ak, G.; et al. Water Extract from Inflorescences of Industrial Hemp Futura 75 Variety as a Source of Anti-Inflammatory, Anti-Proliferative and Antimycotic Agents: Results from In Silico, In Vitro and Ex Vivo Studies. *Antioxidants* **2020**, *9*, 437. [CrossRef]