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Phytochemical Screening, Antioxidant, and Enzyme Inhibitory Properties of Three *Prangos* Species (*P. heyniae*, *P. meliocarpoides* var. *meliocarpoides*, and *P. uechtritzi*) Depicted by Comprehensive LC-MS and Multivariate Data Analysis

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Abstract: The aim of the present study was to identify/quantify bioactive compounds and determine the antioxidant activity and enzyme inhibitory effects of various solvent extracts (n-hexane, ethyl acetate, methanol, and water) of *Prangos heyniae* H. Duman and M.F. Watson, *Prangos meliocarpoides* var. *meliocarpoides*, and *Prangos uechtritzi* Boiss. and Hausskn. This is the first time such a report has been designed to validate the phytochemical composition and bioactivity (especially enzyme inhibitory properties) of these plants. A combined approach of liquid chromatography (LC) with mass spectrometry (HR-MS and MSⁿ) allowed to identify that *P. heyniae* contains condensed tannins; *P. meliocarpoides* is rich in hydrolysable tannins; and *P. uechtritzi* possesses coumarins, flavonoids, and hydroxycinnamic acids. Different extracts were tested for antioxidant activities using a battery of assays, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), cupric reducing antioxidant capacity (CUPRAC), total antioxidant capacity (TAC) (phosphomolybdenum), and metal chelating. Enzyme inhibitory effects were investigated using acetylcholinesterase (AChE), butyrylcholinesterase (BChE), tyrosinase, α -amylase, and α -glucosidase as target enzymes. The obtained results depended on the extraction solvents used for each *Prangos* species. The methanol extract of *P. meliocarpoides* var. *meliocarpoides* exhibited significant radical scavenging activity (DPPH: 52.27 mg Trolox equivalent (TE)/g; ABTS: 92.84 mg TE/g), the most potent-reducing potential (CUPRAC: 154.04 mg TE/g; FRAP: 104.34 mg TE/g), and high TAC (2.52 mmol TE/g). Moreover, the strongest BChE (7.97 mg galantamine equivalent/g), α -amylase (0.46 mmol acarbose equivalent/g), and tyrosinase (81.15 mg kojic acid equivalent/g) inhibitory effects were observed for the hexane extract of *P. meliocarpoides* var. *meliocarpoides*. Correlation analysis showed a significant positive correlation between hydrolysable tannins and antioxidant activities. The same trend was also observed between the same class of compounds and the inhibitory effects on enzymatic activities. These results suggest a principal role of hydrolysable tannins in the observed bioactivities of *Prangos*. Our results suggested that the tested *Prangos* species could be valuable as sources of natural agents in the development of health-promoting applications.

Keywords: *Prangos*; antioxidants; enzyme inhibitors; phytochemicals; medicinal plants

1. Introduction

Plants are still significant sources of bioactive constituents, and many of the known secondary metabolites still need a lot of research to assess their potential usefulness as therapeutic agents. In the recent time, the development of new therapeutic agents has considered the so-called drug-repositioning and, in this regard, natural products show great potential because they have been used for various medical purposes for thousands of years [1,2]. Furthermore, many natural compounds have been investigated only for limited bioactivities, and they still can be used to assess new potential effects. Humans have used medicinal herbs empirically, with no rational knowledge of their pharmacological effects or active ingredients, but just learning from the experience. Plant-based rational drug discovery began in the early 19th century [3], trying to combine the chemical composition with bioactivity. Despite the importance of biotechnological drugs and monoclonal antibodies, medicinal plants still offer a unique opportunity to discover novel drugs thanks to their exceptional chemical diversity [4].

Plants are a rich source of chemicals, including flavonoids, anthocyanins, carotenoids, catechins, cinnamic acid derivatives, chalcones, stilbenes, and tocopherols, which can promote health through antioxidant action [5]. Phytochemicals can operate as multiple target molecules and can be valuable sources of health-promoting agents, particularly knowing that the pathophysiology of many illnesses is multifactorial and not caused by a single factor [6]. To ensure product safety, particular attention should be paid to pharmaceutical formulation, extraction and manufacture, and mode of action [7]. In this respect, medicinal plants must be investigated to identify possible antioxidants or enzyme inhibitors for use as nutraceuticals, functional foods, or medications.

The genus *Prangos* includes 45 species worldwide and the Irano-Turanian region is the gene center of the genus [8]. The genus is represented by 19 species in Turkey and most of them are distributed in the Central and East Anatolia Region [9]. In the literature, several studies have been performed on the members of the genus *Prangos*. For example, crude extracts or essential oil of *P. ferulacea* (L.) Lindl. are commonly investigated in terms of its volatile and non-volatile components, cytotoxic activity; antioxidant, antimicrobial, hypoglycemic, acetylcholinesterase, and analgesic activities [10–12]; wound healing property [13]; and antiviral activity [14]. Interestingly, a randomized controlled trial was even conducted to determine the effect of a vaginal cream containing *P. ferulacea* on accelerating the recovery of bacterial vaginosis. The results showed that the cream displayed a positive effect on patients with this type of inflammation [15]. A novel coumarin (yuganin A) identified in the roots of *P. pabularia* Lindl. exhibited potent effects on the proliferation of B16 melanoma cells [16]. *P. haussknechtii* Boiss containing coumarins 1 and 2, monoterpenoids, and amino acid derivatives inhibited lipid peroxidation with IC₅₀ values between 43 and 114 µM, and reduced MTT to formazan blue between 48 and 128 µM [17]. Tan, et al. [18] assessed the antibacterial activity of the pyrenylated coumarin 4'-senecioloxyosthol, identified in the roots of *P. hulusii* Senol, Yildirim, and Secmen. The results showed that the coumarin exhibited antimicrobial activity against *Bacillus subtilis* at a minimum inhibitory concentration of 5 µg/mL. The aim of this study is to assess the bioactive compounds and biological activities of three underexplored *Prangos* species, namely *P. heyniae* H.Duman and M.F.Watson, *P. meliocarpoides* var. *meliocarpoides*, and *P. uechtritzii* Boiss. and Hausskn. In the literature, the *Prangos* species have been studied for several biological abilities. For example, Ahmed et al. [19] investigated the total phenolic and DPPH radical scavenging abilities of the methanol and water extracts of the three *Prangos* species, and the water extract of *P. heyniae* was the richest in terms of total phenolic level, with 127.33 mg GAE/g. In addition, the extract was the most active one in DPPH radical scavenging ability with the lowest IC₅₀ values (20.96 µg/mL). In a recent study

performed by Albayrak et al. [20], two new coumarin glycosides (7-methoxy isoarnottinin 4'-O- β -D-glucopyranoside and 7-methoxy isoarnottinin 4'-O-rutinoside) were isolated from the roots of *P. heyniae* collected from Turkey. In addition to the studies on the extract of *P. heyniae*, several studies showed chemical characterization and some biological activities of the essential oil of the species [21,22]. In an earlier study conducted by Oke et al. [23], the radical scavenging and chemical composition of *P. meliocarpoides* fruit extracts were determined and the water extract was found to be the most active one. Moreover, chlorogenic and rutin were determined to be the main components in their study. The fruit extract had a higher radical scavenging ability as compared with the root and aerial parts in another study [19]. Among the targeted *Prangos* species, *P. uechtritzi* has been the most studied one and several studies reported its essential oil composition as well as biological activities [24–26]. A recent study by Sevin et al. [27] examined the erectile function of root extract of *P. uechtritzi* as well as *P. heyniae*, and the authors reported that oxypeucedanin was the most active coumarin. In our earlier paper [28], the chemical characterization and biological activities of the essential oils of three *Prangos* species were reported. Although many studies have examined the phytochemical composition and bioactivity of different *Prangos* species, the three species examined here still need to be evaluated and confirmed by scientists. To the best of our knowledge, this study is the first to report the phytochemical composition, antioxidant effects, and inhibitory activity against α -amylase, α -glucosidase, acetylcholinesterase, butyrylcholinesterase, and tyrosinase enzymes of secondary metabolites extracted from the same species. We believe that the results presented here could fill in the research gap and subsequently open new research avenues, particularly in the development of therapeutic bioproducts.

2. Materials and Methods

2.1. Plant Material and Extraction

The aerial parts of the *Prangos* species were collected during Summer 2020 from Central Anatolia Region of Turkey. The information of the collection area was reported in our earlier paper [28]. Voucher specimens were deposited at the herbarium of Selcuk University, Science Faculty (Voucher numbers: EY-3039, EY-2998, and EY-3023 for *P. heyniae*, *P. meliocarpoides* var. *meliocarpoides*, and *P. uechtritzi*, respectively). The plant samples were dried in shade conditions at room temperature for about one week. Then, the powdering procedure was performed using a mill, and the samples were stored in the dark.

Extracts were prepared using n-hexane, ethyl acetate, methanol, and water. Overnight, the plant material (10 g) was macerated at room temperature with 200 mL of solvents (hexane, ethyl acetate, and methanol, individually). Finally, solvents were evaporated from the mixtures. Plant materials (10 g) were extracted in 200 mL of boiling water for 15 min before being filtered. Water extracts were lyophilized. All extracts were stored at 4 °C until further analysis.

2.2. Total Phenolic and Flavonoid Contents

The total phenolic and flavonoid contents were determined using the Folin–Ciocalteu and AlCl_3 tests, respectively [29]. The results were presented as gallic acid equivalents (mg GAEs/g dry extract) and rutin equivalents (mg REs/g dry extract) for the assays. The experimental details are given in the supplemental section.

2.3. LC-DAD-MSⁿ

Extracts were dissolved in appropriate solvent: the more lipophilic extracts were dissolved in DMSO, methanol extracts in methanol, and water extracts were dissolved in water/DMSO mixtures. Samples were prepared weighting 10 mg (± 0.1 mg) of dried material and dissolved in 10 mL of solvent using an ultrasound bath (WWR, Ultrasonic bath 45 Hz, 60 W, WWR Milano, Italy) for 15 min [30]. Then, the volume was adjusted at 20 mL and solutions were centrifuged at 13,000 rpm prior to being transferred to glass vials for analyses [30].

LC-DAD-MSⁿ analyses were performed using an Agilent 1260 chromatograph equipped with autosampler, column oven, and diode array detector (DAD), all from the 1260 series. At the end of the chromatographic column, a “T” connector was fixed splitting the flow into two identical volumes: one was linked to DAD, while the other was connected to the electro spray (ESI) ion source of a Varian 500 Ion Trap mass spectrometer (MS). The spectrometer operated in both negative and positive ion modes. Data were acquired in the 100–2000 *m/z* range using the turbo data depending scanning (tdds) function, which allows the recording of the fragmentation of the most abundant ionic species. MS parameters were as follows: needle voltage: 4800 V, capillary voltage: 80 V, drying gas pressure: 25 psi, nebulizer pressure: 40 psi, drying gas temperature: 280 °C, and nebulizer temperature: 270 °C. For the identification of compounds, MSⁿ spectra were acquired and compared with the literature and available online databases (Human Metabolome Database (HMDB) and Food Metabolome Database (FOODB)). Furthermore, MS data were compared with those of reference compounds available in the laboratory or acquired by commercial sources. Quantification of compounds was obtained using selected reference standards for each class of identified constituents. The calibration curves were as follows: imperatorin (detected in MS, positive ion mode, and DAD at 320 nm), reference solutions from 90 to 1.5 µg/mL, $y = 35x - 0.320$; rutin (detected in MS, negative ion mode, and in DAD at 350 nm), reference solutions from 100 to 1.0 µg/mL, $y = 72x - 1.20$; ellagic acid (detected in MS, negative ion mode, and in DAD at 254 nm), reference solutions from 100 to 1.0 µg/mL, $y = 37.5x - 0.88$; and epigallocatechin gallate (detected in MS, negative ion mode, and in DAD at 280 nm), reference solutions from 120 to 1.0 µg/mL, $y = 83x - 0.992$.

Accurate *m/z* values of identified metabolites were obtained by UPLC-QToF analysis of the same *Prangos* extracts. The system used for the analysis was an Acquity UPLC (Waters) coupled with a QToF MS model Xevo G2 (Waters). Agilent Zorbax Eclipse Plus C18 (2.1 × 50 mm, 1.8 µm) was used as the stationary phase, which was kept at a constant temperature of 40 °C. As the mobile phase, a mixture of water with 1% formic acid (A) and acetonitrile (B) was used. The elution gradient started from 0 to 1 min, 98% A; then at 11 min, 15% A; at 16 min, 0% A; and kept isocratic until 20 min. Then, at 21 min, 98% A and 24 min, 98% A. The flow rate was 300 µL/min, and the injection volume was 2 µL. MS data were acquired in both ESI(+) and ESI(−) in the mass range of 50–2000 Da. The sampling cone voltage was adjusted at 40 V and the source offset was 80 V. The capillary voltage was set to 3.5 KV. Nitrogen was used as nebulizer gas at a flow rate of 800 L/h. The desolvation temperature was 450 °C. Mass accuracy and reproducibility were maintained by infusing lock mass (leucine–enkephalin, $[M + H]^+ = 556.2771$ *m/z*, and $[M - H]^- = 554.2620$ *m/z*) through Lockspray at a flow rate of 20 µL/min. The *m/z* value of all acquired spectra was automatically corrected during acquisition based on lock mass. MS^e experiment was simultaneously performed to collect structural information, setting the collision energy to 30 V.

2.4. Antioxidant Assays

Antioxidant assays were performed using methods that have been previously reported [31,32]. The antioxidant potential was calculated as follows: mg Trolox equivalents (TE)/g extract in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging tests; cupric-reducing antioxidant capacity (CUPRAC) and ferric-reducing antioxidant power (FRAP); and mmol TE/g extract in the (MCA). The experimental details are given in the supplemental section.

2.5. Enzyme Inhibitory Assays

The enzyme inhibition experiments were performed based on previously described procedures [31,32]. Amylase and glucosidase inhibition was expressed as mmol acarbose equivalents (ACAEs)/g extract, while acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition was expressed as mg galanthamine equivalents (GALAE

s)/g extract. Tyrosinase inhibition was expressed as mg kojic acid equivalents (KAEs)/g extract. The experimental details are given in the supplemental section.

2.6. Statistical Analysis

This study used ANOVA (Tukey's test) in order to determine whether there were any differences in the extract levels between the three samples. xStat was used to carry out the statistical analysis. p -values < 0.05 were considered as statistically significant. Multivariate statistical analyses were performed on chemical and biological data to uncover correlations between chemical constituents of *Prangos* extracts and their bioactivities, i.e., to describe possible activity biomarkers. Predictive OPLS models were developed using SIMCA (Umetrics, Sweden), and they were validated performing a permutation test ($n = 200$ permutations). Pearson's rank correlation test was performed using the open-source MetaboAnalyst platform (<https://www.metaboanalyst.ca/>, accessed on 14 July 2022). Prior to being analyzed, data were pre-processed as follows: variables with more than 80% missing values were removed, and the remaining missing values were imputed using the KNN algorithm; finally, they were normalized by log-transformation and Pareto scaling.

3. Results and Discussion

3.1. Characterization of Bioactive Secondary Metabolites

The choice of the most suitable extraction solvent is an important step in order to define the quality and yield of extraction of these compounds [33]. Solvents used to extract bioactive chemicals from plants are chosen based on the polarity of the solute of interest, because a solute with comparable polarity to the solvent will be adequately dissolved according to the rule of similarity and intermiscibility (like dissolves like) [34,35]. In this study, the polyphenolic compounds such as phenolics (TPCs) and flavonoids (TFCs) of *P. heyniae*, *P. meliocarpoides* var. *meliocarpoides*, and *P. uechtritzi* extracted with four solvents of different polarities (water > methanol > ethyl acetate > hexane) were quantified. The results are presented in Table 1.

Table 1. Total bioactive compounds and total antioxidant capacity (by phosphomolybdenum assay) of the tested extracts ($n = 3$).

| Species | Solvents | TPC (mg GAE/g) | TFC (mg RE/g) | PBD (mmol TE/g) |
|---|----------|---------------------------|---------------------------|----------------------------|
| <i>P. heyniae</i> | Hexane | 17.55 ± 0.16 ^g | 3.43 ± 0.14 ^l | 1.93 ± 0.02 ^b |
| | EA | 21.85 ± 0.36 ^f | 12.93 ± 0.35 ^f | 2.30 ± 0.10 ^a |
| | MeOH | 32.13 ± 0.76 ^c | 28.75 ± 0.36 ^b | 1.51 ± 0.11 ^{de} |
| | Water | 38.77 ± 0.01 ^b | 16.19 ± 0.24 ^d | 1.39 ± 0.03 ^{ef} |
| <i>P. meliocarpoides</i> var. <i>meliocarpoides</i> | Hexane | 22.63 ± 0.16 ^f | 8.19 ± 0.23 ^h | 2.52 ± 0.06 ^a |
| | EA | 26.15 ± 1.40 ^e | 19.40 ± 0.60 ^c | 2.49 ± 0.05 ^a |
| | MeOH | 40.03 ± 0.68 ^b | 44.66 ± 0.68 ^a | 1.82 ± 0.11 ^{bc} |
| | Water | 44.28 ± 0.27 ^a | 11.00 ± 0.18 ^g | 1.46 ± 0.02 ^{def} |
| <i>P. uechtritzi</i> | Hexane | 18.70 ± 0.08 ^g | 1.72 ± 0.07 ^k | 1.79 ± 0.04 ^{bc} |
| | EA | 25.45 ± 0.12 ^e | 5.72 ± 0.05 ⁱ | 2.35 ± 0.17 ^a |
| | MeOH | 31.20 ± 0.16 ^c | 28.22 ± 0.54 ^b | 1.67 ± 0.13 ^{cd} |
| | Water | 29.62 ± 0.07 ^d | 14.75 ± 0.30 ^e | 1.21 ± 0.03 ^f |

Values are reported as mean ± SD of three parallel experiments. EA: ethyl acetate; MeOH: methanol; TPC: total phenolic content; TFC: total flavonoid content; PBD: phosphomolybdenum; GAE: gallic acid equivalent; RE: rutin equivalent; TE: Trolox equivalent. Different letters indicate significant differences between the tested extracts ($p < 0.05$).

Overall, we observed a decline in TPC and TFC in the different plant species studied in the following order: *P. meliocarpoides* var. *meliocarpoides* (TPC: 44.28 mg GAE/g, and TFC: 44.66 mg RE/g), *P. heyniae* (TPC: 38.77 mg GAE/g, and 28.75 mg RE/g), and *P. uechtritzi* (TPC: 31.20 mg GAE/g, and TFC: 28.22 mg RE/g). The most polar solvents, namely water and methanol, were successful in extracting phenolics and flavonoids. As such, it can be

said that the compounds extracted were mostly polar. On the other hand, hexane extracted the least number of compounds. A number of publications have reported methanol and water as the most efficient solvents in obtaining a high yield of extracts, as well as in extracting polar bioactive compounds such as phenolics and flavonoids [36,37].

The bioactive compounds in *Prangos* species were investigated by combining positive and negative ion mode LC-MS (Table 2). The analysis revealed that the extraction of *Prangos* species yielded extracts containing different classes of secondary metabolites, which we grouped as condensed tannins, hydrolysable tannins, coumarins, flavonoids hydroxycinnamic acid derivatives, and “other constituents”. Schematic representations of the relative abundance of the main constituents are reported in the form of pie-charts in Supplementary Materials Figures S1–S3. The structures of the identified compounds were deduced owing to the HR-MS data as well as by MSn. Epigallocatechin gallate derivatives are characterized by the loss of one or two gallic acid moieties (m/z 152), leading in this latter case to ion m/z 305, corresponding to the epigallocatechin moiety [38,39] (Table 1). Other condensed tannins in *P. heyniae* were prodelphinidine gallate derivatives, compounds in fact present the molecular formula of $C_{37}H_{30}O_{18}$, which generate fragment because of -152 Da ascribable to gallic acid moiety. Further fragmentations of the species generated at m/z 611 are ascribable to prodelphinidin dimer gallate. Four different isomeric derivatives are present in *P. heyniae*. Two further peaks presenting similar fragmentation and showing the loss of two gallic acid moieties have also been detected and annotated as di-galloyl prodelphinidin dimers [40–42]. Epigallocatechin gallate and methyl ellagic acid were also detected and their presence was confirmed with an injection of authentic standards. An unusual derivative presenting molecular formula of $C_{14}H_{12}O_{11}$ was tentatively assigned to Chebulic acid due to the loss of water and CO_2 , leading to the fragment at m/z 293 that further presented loss of further CO_2 moieties leading to the species at m/z 249, 205, and 163; the mass spectra are reported in Supplementary Figure S4. A second peak presenting the same molecular ion and fragmentation pathway was observed and indicated as a chebulic acid isomer (Supplementary Figure S5). These compounds have been identified in the fruits of *Terminalia chebula* and LC methods have been proposed for their identification [43]. A series of condensed tannins formed by units of epigallo/gallo catechin with or without gallic acid esterification have been also identified [39,44,45]. Furthermore, the peak presenting molecular formula of $C_{44}H_{36}O_{22}$ was tentatively assigned to the Assamicain chalcanean flavan dimer previously identified in *Camelia sinensis* [46]. Several hydrolysable tannins have been identified specifically in *P. meliocarpoides* var. *meliocarpoides* and most of the compounds were ester of gallic acid with glucose, quinic acid; moreover, different derivatives formed by mixed esters of gallic, synaptic, and shikimic acid have been detected, and identification was based on the obtained MS measurements and predicted MS fragmentation from database and software, as well as by comparison with the literature [44,47–50]. Some coumarin derivatives were identified, especially in the *P. meliocarpoides* extracts obtained with methanol or water. Coumarins have been reported in *Prangos* species [8,51], where the identity of compounds was confirmed with the injection of reference purified substances. A series of glycosidic flavonoids, mainly quercetin, myricetin, and isorhamnetin derivatives, were also identified [52,53] and quantified, and they mostly were present in *P. uechritzii*.

Table 2. Cont.

| M-H | Molecular Formula | Fragments | Name and References | <i>P. heyniae</i> -Hexane | <i>P. heyniae</i> -EA | <i>P. heyniae</i> -MeOH | <i>P. heyniae</i> -Water | <i>P. meliocar-poides</i> -Hexane | <i>P. meliocar-poides</i> -EA | <i>P. meliocar-poides</i> -MeOH | <i>P. meliocar-poides</i> -Water | <i>P. uechtritzi</i> -Hexane | <i>P. uechtritzi</i> -EA | <i>P. uechtritzi</i> -MeOH | <i>P. uechtritzi</i> -Water |
|----------|---|-------------|---|---------------------------|-----------------------|-------------------------|--------------------------|-----------------------------------|-------------------------------|---------------------------------|----------------------------------|------------------------------|--------------------------|----------------------------|-----------------------------|
| 761,136 | C ₃₇ H ₃₀ O ₁₈ | 593 423 | (epi)gallo catechin-gallo catechin-gallate dimer [39,45] | nd | 0.35 ± 0.01 | 3.00 ± 0.05 | 1.63 ± 0.05 | nd | nd | nd | nd | nd | nd | nd | nd |
| 761,136 | C ₃₇ H ₃₀ O ₁₈ | 593 423 | (epi)gallo catechin-gallo catechin-gallate dimer [39,45] | nd | 3.12 ± 0.06 | 23.54 ± 0.25 | 12.4 ± 0.11 | nd | nd | nd | nd | nd | nd | nd | nd |
| 761,136 | C ₃₇ H ₃₀ O ₁₈ | 593 423 | (epi)gallo catechin-gallo catechin-gallate dimer [39,45] | nd | 2.22 ± 0.05 | 13.64 ± 0.11 | 7.52 ± 0.11 | nd | nd | nd | nd | nd | nd | nd | nd |
| 745,141 | C ₃₇ H ₃₀ O ₁₇ | | Epicatechin-(4beta->8)-epigallo catechin 3'-gallate [39,45] | nd | 0.59 ± 0.06 | 7.31 ± 0.07 | 4.48 ± 0.10 | nd | nd | nd | nd | nd | nd | nd | nd |
| 913,1468 | C ₄₄ H ₃₃ O ₂₂ | 761 423 | (epi)gallo catechin-gallate dimer [39,45] | nd | 3.46 ± 0.11 | 18.02 ± 0.11 | 12.39 ± 0.22 | nd | nd | nd | nd | nd | nd | nd | nd |
| 913,1468 | C ₄₄ H ₃₃ O ₂₂ | 761 423 | (epi)gallo catechin-gallate dimer [39,45] | nd | 1.85 ± 0.09 | 9.71 ± 0.11 | 8.46 ± 0.10 | nd | nd | nd | nd | nd | nd | nd | nd |
| 745,141 | C ₃₇ H ₃₀ O ₁₈ | | Epicatechin-(4beta->8)-epigallo catechin 3'-gallate [39,45] | nd | 0.40 ± 0.05 | 5.79 ± 0.08 | 6.57 ± 0.05 | nd | nd | nd | nd | nd | nd | nd | nd |
| 745,141 | C ₃₇ H ₃₀ O ₁₉ | | Epicatechin-(4beta->8)-epigallo catechin 3'-gallate [39,45] | nd | 0.86 ± 0.05 | 5.89 ± 0.09 | 1.96 ± 0.02 | nd | nd | nd | nd | nd | nd | nd | nd |
| 915,162 | C ₄₄ H ₃₆ O ₂₂ | 457 | Assamicain [46] | nd | 0.17 ± 0.05 | 3.52 ± 0.22 | 4.58 ± 0.04 | nd | nd | nd | nd | nd | nd | nd | nd |
| 457,0771 | C ₂₂ H ₁₇ O ₁₁ | 331 305 169 | gallo catechin gallate* [46] | 0.01 ± 0.01 | 1.61 ± 0.05 | 7.23 ± 0.09 | 7.32 ± 0.05 | nd | nd | nd | nd | nd | nd | nd | nd |
| 457,0771 | C ₂₂ H ₁₇ O ₁₁ | 331 305 169 | epi-gallo catechin gallate* | nd | 1.77 ± 0.05 | 8.61 ± 0.11 | 6.61 ± 0.09 | nd | nd | nd | nd | nd | nd | nd | nd |
| 915,162 | C ₄₄ H ₃₆ O ₂₂ | 457 | Assamicain B [46] | nd | 0.05 ± 0.01 | 7.24 ± 0.09 | 5.73 ± 0.07 | nd | nd | nd | nd | nd | nd | nd | nd |
| | | | TOTAL | 0.24 | 17.12 | 141.95 | 103.77 | 1.29 | 2.15 | 2.97 | 4.73 | nd | 5.09 | 53.74 | 31.49 |
| | M-H | | Hydroly-sable tannins | | | | | | | | | | | | |
| 801,1135 | C ₃₅ H ₂₉ O ₂₃ | | tetra galloylquinic acid [39,47–49] | nd | nd | 0.99 ± 0.05 | 1.51 ± 0.09 | 0.26 ± 0.03 | 0.21 ± 0.01 | 1.33 ± 0.06 | 3.02 ± 0.06 | nd | nd | nd | nd |

Table 2. Cont.

| M-H | Molecular Formula | Fragments | Name and References | <i>P. heyniae</i> -Hexane | <i>P. heyniae</i> -EA | <i>P. heyniae</i> -MeOH | <i>P. heyniae</i> -Water | <i>P. meliocar-poides</i> -Hexane | <i>P. meliocar-poides</i> -EA | <i>P. meliocar-poides</i> -MeOH | <i>P. meliocar-poides</i> -Water | <i>P. uechtritzi</i> -Hexane | <i>P. uechtritzi</i> -EA | <i>P. uechtritzi</i> -MeOH | <i>P. uechtritzi</i> -Water |
|-----------|--|-------------|---|---------------------------|-----------------------|-------------------------|--------------------------|-----------------------------------|-------------------------------|---------------------------------|----------------------------------|------------------------------|--------------------------|----------------------------|-----------------------------|
| 618,0935 | C ₂₇ H ₂₃ O ₁₇ | | 3-O-Galloyl-4,6-(S)-HHDP-d-glucose [39,47–49] | nd | nd | nd | nd | 0.19 ± 0.02 | 0.16 ± 0.03 | 13.35 ± 0.36 | 6.11 ± 0.06 | nd | nd | nd | nd |
| 787,0994 | C ₃₄ H ₂₇ O ₂₂ | 617 321 | 1,3-Digalloyl-4,6-HHDP-glucose/Heterophyllin A [39,47–49] | nd | nd | nd | nd | 1.28 ± 0.04 | 0.01 ± 0.01 | 26.06 ± 0.35 | 12.48 ± 0.12 | nd | nd | nd | nd |
| 787,1152 | C ₃₅ H ₃₀ O ₂₁ | | 2'',3'',6''-Tris-O-(3,4,5-trihydroxybenzoyl)-3'-Glucosyl-2',4',6'-trihydroxyacetophenone [39,47–49] | nd | nd | 1.71 ± 0.06 | 1.16 ± 0.06 | 0.22 ± 0.03 | 0.15 ± 0.03 | 37.97 ± 0.99 | 18.84 ± 0.17 | nd | nd | nd | nd |
| 787,0994 | C ₃₄ H ₂₇ O ₂₂ | 617 321 | Heterophyllin A isomer | nd | nd | 1.86 ± 0.05 | 1.16 ± 0.09 | 0.47 ± 0.05 | nd | 36.29 ± 0.85 | 22.62 ± 0.23 | nd | nd | nd | nd |
| 801,155 | C ₃₅ H ₂₉ O ₂₂ | | tetragalloylquinic acid [39,47–49] | nd | nd | nd | nd | 8.58 ± 0.06 | 0.36 ± 0.03 | 18.63 ± 0.88 | 12.13 ± 0.21 | nd | nd | nd | nd |
| 1237,71 | C ₃₅ H ₃₄ O ₃₄ | | Prostratin A or Tirucallin A | nd | nd | nd | nd | 0.11 ± 0.02 | 0.04 ± 0.02 | 0.32 ± 0.01 | 2.11 ± 0.06 | nd | nd | nd | nd |
| 1083.0581 | C ₄₈ H ₂₇ O ₃₀ | | alfa-Punicalagin * [48] | nd | nd | nd | nd | 4.26 ± 0.14 | 8.82 ± 0.11 | 0.11 ± 0.01 | 0.65 ± 0.05 | nd | nd | nd | nd |
| 1083.0581 | C ₄₈ H ₂₇ O ₃₀ | | beta-punicalagin * [48] | nd | nd | nd | nd | 9.12 ± 0.16 | 14.64 ± 0.23 | 0.13 ± 0.01 | 1.13 ± 0.02 | nd | nd | nd | nd |
| 1083.0581 | C ₄₈ H ₂₇ O ₃₀₀ | 601 | Punicalagin derivative [48] | nd | nd | nd | nd | 4.72 ± 0.22 | 8.40 ± 0.22 | 0.32 ± 0.01 | 4.93 ± 0.09 | nd | nd | nd | nd |
| 1083.0581 | C ₄₈ H ₂₈ O ₃₀ | 601 | Terchebuline [39,45,48] | nd | nd | nd | nd | 8.71 ± 0.03 | 17.53 ± 0.85 | nd | nd | nd | nd | nd | nd |
| 637.1052 | C ₂₇ H ₂₄ O ₁₈ | 483 465 313 | trigalloyl glucose [39,47–49] | nd | nd | nd | nd | 0.11 ± 0.03 | 0.23 ± 0.09 | 1.32 ± 0.02 | 3.67 ± 0.04 | nd | nd | nd | nd |
| 637.1048 | C ₂₇ H ₂₄ O ₁₈ | 483 465 313 | trigalloyl glucose [39,47–49] | nd | nd | nd | nd | 0.04 ± 0.01 | 0.07 ± 0.03 | 1.43 ± 0.04 | 4.48 ± 0.04 | nd | nd | nd | nd |
| 637.1048 | C ₂₇ H ₂₄ O ₁₈ | 483 465 313 | trigalloyl glucose [39,47–49] | nd | nd | nd | nd | 0.08 ± 0.01 | 0.13 ± 0.02 | 0.37 ± 0.02 | 27.63 ± 0.34 | nd | nd | nd | nd |
| 971.1733 | C ₄₃ H ₃₈ O ₂₆ | 817 635 465 | tetragalloyl-syrigylglucose [39,47–49] | nd | nd | nd | nd | 0.01 ± 0.01 | 0.07 ± 0.03 | 3.43 ± 0.08 | 4.37 ± 0.06 | nd | nd | nd | nd |
| 637.1045 | C ₂₇ H ₂₄ O ₁₈ | | trigalloyl glucose [39,47–49] | nd | nd | nd | nd | 0.46 ± 0.04 | 1.35 ± 0.05 | 7.63 ± 0.09 | 28.29 ± 0.33 | nd | nd | nd | nd |

Table 2. Cont.

| M-H | Molecular Formula | Fragments | Name and References | <i>P. heyniae</i> -Hexane | <i>P. heyniae</i> -EA | <i>P. heyniae</i> -MeOH | <i>P. heyniae</i> -Water | <i>P. meliocarpoides</i> -Hexane | <i>P. meliocarpoides</i> -EA | <i>P. meliocarpoides</i> -MeOH | <i>P. meliocarpoides</i> -Water | <i>P. uechtritzi</i> -Hexane | <i>P. uechtritzi</i> -EA | <i>P. uechtritzi</i> -MeOH | <i>P. uechtritzi</i> -Water |
|----------|---|----------------------------------|--|---------------------------|-----------------------|-------------------------|--------------------------|----------------------------------|------------------------------|--------------------------------|---------------------------------|------------------------------|--------------------------|----------------------------|-----------------------------|
| 247,097 | C ₁₄ H ₁₄ O ₄ | 188 146 | 8-(2',3'-dihydroxyisopentyl)-7-hydroxycoumarin /marmesin* [8,51] | nd | nd | 0.74 ± 0.05 | 3.25 ± 0.09 | 0.52 ± 0.03 | nd | 2.41 ± 0.04 | 7.06 ± 0.04 | nd | nd | 4.71 ± 0.06 | 5.87 ± 0.11 |
| 271,091 | C ₁₆ H ₁₃ O ₄ | 203 175 | Imperatorin* [8,51] | nd | nd | nd | nd | 0.25 ± 0.03 | 0.21 ± 0.03 | 8.28 ± 0.11 | 14.72 ± 0.33 | nd | nd | nd | nd |
| 327,166 | C ₂₀ H ₂₃ O ₄ | | Dentatin * [8,51] | nd | nd | nd | nd | nd | nd | nd | nd | 4.05 ± 0.03 | 1.54 ± 0.03 | 2.61 ± 0.05 | 3.68 ± 0.11 |
| | | | TOTAL | 0.07 | 0.04 | 4.18 | 9.11 | 0.94 | 0.43 | 16.51 | 22.82 | 4.12 | 1.64 | 25.59 | 28.05 |
| M-H | | | Flavonoid derivatives | | | | | | | | | | | | |
| 785,162 | C ₃₆ H ₃₂ O ₂₀ | | Degalloyltheaflavonin | nd | nd | nd | nd | 2.04 ± 0.03 | 2.65 ± 0.11 | 2.17 ± 0.09 | 3.16 ± 0.22 | nd | nd | nd | nd |
| 771,2372 | C ₃₄ H ₄₂ O ₂₀ | 625,1824 479,0928 317,0729 | Typhaneoside | nd | nd | nd | nd | 1.37 ± 0.05 | 1.05 ± 0.08 | 30.82 ± 0.55 | 15.9 ± 0.78 | nd | nd | nd | nd |
| 433,1171 | C ₂₁ H ₂₁ O ₁₀ | | Apigenin-C-hexoside | nd | nd | nd | nd | 1.26 ± 0.09 | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.28 ± 0.02 | nd | nd | nd | nd |
| 601,1193 | C ₂₈ H ₂₅ O ₁₅ | 431 329 | 2'-O-Galloylquercitrin | nd | nd | nd | nd | 1.25 ± 0.02 | 1 | 29.98 ± 0.66 | 14.72 ± 0.22 | nd | nd | nd | nd |
| 585,1269 | C ₂₈ H ₂₅ O ₁₄ | 431 415 285 | Kaempferol-deoxyhexoside-gallate [39,45,49] | nd | nd | nd | nd | 0.12 ± 0.01 | 0.32 ± 0.03 | 19.61 ± 0.44 | 9.87 ± 0.08 | nd | nd | nd | nd |
| 769.2205 | C ₃₄ H ₄₂ O ₂₀ | 605 314 | Isorhamnetin-7-O-hexoside-3-O-hexosidedeoxyhexoside [39,45,49] | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.24 ± 0.02 | 0.11 ± 0.01 |
| 625.1198 | C ₃₀ H ₂₆ O ₁₅ | 448 301 | Quercetin-7-O-hexoside-caffeoyl [39,45,49] | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 3.08 ± 0.09 | 1.4 ± 0.02 |
| 625.1196 | C ₃₀ H ₂₆ O ₁₅ | 449 301 | Quercetin-7-O-hexoside-caffeoyl isomer [39,45,49] | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.94 ± 0.06 | 0.25 ± 0.01 |
| 479.0815 | C ₂₁ H ₂₀ O ₁₃ | 316 271 179 | Myricetin-3-O-glucopyranoside* | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 4.52 ± 0.09 | 0.71 ± 0.02 |
| 769.2204 | C ₃₄ H ₄₂ O ₂₀ | 605 314 | Isorhamnetin-7-O-hexoside-3-O-hexosidedeoxyhexoside [39,45,49] | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 1.54 ± 0.11 | 0.71 ± 0.03 |

Table 2. Cont.

| M-H | Molecular Formula | Fragments | Name and References | <i>P. heyniae</i> -Hexane | <i>P. heyniae</i> -EA | <i>P. heyniae</i> -MeOH | <i>P. heyniae</i> -Water | <i>P. meliocarpoides</i> -Hexane | <i>P. meliocarpoides</i> -EA | <i>P. meliocarpoides</i> -MeOH | <i>P. meliocarpoides</i> -Water | <i>P. uechtritzi</i> -Hexane | <i>P. uechtritzi</i> -EA | <i>P. uechtritzi</i> -MeOH | <i>P. uechtritzi</i> -Water |
|----------|---|---------------------|--|---------------------------|-----------------------|-------------------------|--------------------------|----------------------------------|------------------------------|--------------------------------|---------------------------------|------------------------------|--------------------------|----------------------------|-----------------------------|
| 769.2200 | C ₃₄ H ₄₂ O ₂₀ | 605 314 | Isorhamnetin-7-O-hexoside-3-O-hexosidedeoxyhexoside [39,45,49] | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.78 ± 0.08 | 0.43 ± 0.01 |
| 609.1464 | C ₂₇ H ₃₀ O ₁₆ | 301 271 179 | Rutin * | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 8.55 ± 0.11 | 2.75 ± 0.11 |
| 463.0884 | C ₂₁ H ₂₀ O ₁₂ | 316 271 | Myricetin-3-O-rhamnopyranoside * | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 11.7 ± 0.22 | 3.07 ± 0.11 |
| 463.0887 | C ₂₁ H ₂₀ O ₁₂ | 316 271 | Myricetin-7-O-rhamnopyranoside * | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 7.37 ± 0.13 | 1.11 ± 0.22 |
| 623.1627 | C ₂₈ H ₃₂ O ₁₆ | 461 314 315 299 | Isorhamnetin-7-O-rhamnopyranosyl-3-O-glucopyranoside * | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 5.51 ± 0.22 | 1.86 ± 0.22 |
| 463.0969 | C ₂₁ H ₂₀ O ₁₂ | 301 | Quercetin-3-O-galactopyranoside * | nd | nd | nd | nd | 0.06 ± 0.03 | 0.16 ± 0.03 | 1.05 ± 0.02 | 3.69 ± 0.05 | nd | nd | nd | nd |
| 477.0684 | C ₂₁ H ₁₈ O ₁₃ | 301 271 | Quercetin-3-O-glucuronide * | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 16.33 ± 0.99 | 10.17 ± 0.22 |
| 623.1638 | C ₂₈ H ₃₂ O ₁₆ | 315 300 | Isorhamnetin-3-O-hexosyl-deoxyhexoside [39,45,49] | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 2.18 ± 0.11 | 0.73 ± 0.05 |
| 623.1622 | C ₂₈ H ₃₂ O ₁₆ | 315 300 | Isorhamnetin-3-O-rutinoside * | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 5.05 ± 0.09 | 1.61 ± 0.07 |
| 477.1054 | C ₂₂ H ₂₂ O ₁₂ | 314 300 271 255 | Isorhamnetin-3-O-glucopyranoside * | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 2.02 ± 0.05 | 1.4 ± 0.07 |
| 463.0854 | C ₂₁ H ₂₀ O ₁₂ | 301 271 255 179 151 | Quercetin-3-O-glucopyranoside * | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 34.3 ± 0.51 | 10.57 ± 0.33 |
| 477.1043 | C ₂₂ H ₂₂ O ₁₂ | 314 300 271 255 | Isorhamnetin-3-O-galactopyranoside * | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 10.03 ± 0.11 | 3.11 ± 0.05 |

Table 2. Cont.

| M-H | Molecular Formula | Fragments | Name and References | <i>P. heyniae</i> -Hexane | <i>P. heyniae</i> -EA | <i>P. heyniae</i> -MeOH | <i>P. heyniae</i> -Water | <i>P. meliocarpoides</i> -Hexane | <i>P. meliocarpoides</i> -EA | <i>P. meliocarpoides</i> -MeOH | <i>P. meliocarpoides</i> -Water | <i>P. uechtritzi</i> -Hexane | <i>P. uechtritzi</i> -EA | <i>P. uechtritzi</i> -MeOH | <i>P. uechtritzi</i> -Water |
|----------|---|-------------------|--|---------------------------|-----------------------|-------------------------|--------------------------|----------------------------------|------------------------------|--------------------------------|---------------------------------|------------------------------|--------------------------|----------------------------|-----------------------------|
| 491.0840 | C ₂₂ H ₂₀ O ₁₃ | 315 300 271 255 | Isorhamnetin-3-O-glucuronide * | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 11.93 ± 0.22 | 6.18 ± 0.07 |
| 431.0978 | C ₂₁ H ₂₀ O ₁₀ | 285 | Kaempferol-3-O-rhamnoside * | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 8.18 ± 0.08 | 1.71 ± 0.06 |
| 461.1083 | C ₂₂ H ₂₁ O ₁₁ | 314 300 | Isorhamnetin-7-O-rhamnopyranoside * | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 4.12 ± 0.11 | 1.06 ± 0.05 |
| | | | TOTAL | nd | nd | nd | nd | 6.10 ± 0.05 | 5.19 ± 0.09 | 83.65 ± 0.99 | 47.62 ± 0.08 | nd | 2.68 ± 0.03 | 138.03 ± 1.25 | 48.92 ± 0.66 |
| M + H | | | Hydroxycinnamic derivatives | | | | | | | | | nd | nd | | |
| 339.11 | C ₁₆ H ₁₉ O ₈ | 191 | 1-coumaroyl quinic acid [39,45,49] | 1.83 ± 0.01 | 5.48 ± 0.07 | 8.52 ± 0.05 | 6.32 ± 0.07 | 0.13 ± 0.01 | 1.44 ± 0.07 | 3.06 ± 0.04 | 0.75 ± 0.05 | nd | nd | 4.11 ± 0.04 | 3.91 ± 0.04 |
| M-H | | | | | | | | | | | | nd | nd | | |
| 341.0873 | C ₁₅ H ₁₇ O ₉ | 179 | Caffeoyl hexose [39,45,49] | nd | 0.16 ± 0.05 | 0.76 ± 0.06 | 1.31 ± 0.09 | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.02 ± 0.01 | nd | nd | 0.08 ± 0.01 | 0.23 ± 0.02 |
| 501.1619 | C ₂₂ H ₂₉ O ₁₃ | 417 399 285 152 | Clemomandshuricoside B | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 2.09 ± 0.06 | 0.55 ± 0.02 |
| | | | TOTAL | 1.83 | 5.64 | 9.26 ± 0.10 | 7.61 ± 0.09 | 0.14 ± 0.05 | 1.46 ± 0.06 | 3.07 ± 0.05 | 0.77 ± 0.03 | nd | nd | 6.27 ± 0.07 | 4.69 ± 0.02 |
| M + H | | | Other compounds | | | | | | | | | nd | nd | | |
| 225.1399 | C ₁₃ H ₂₀ O ₃ | 147 119 103 79 88 | 6-hydroxy-3-oxo-alpha-ionol | 3.72 ± 0.01 | 3.12 ± 0.05 | 10.2 ± 0.20 | 10.8 ± 0.11 | nd | 0.89 ± 0.03 | 5.05 ± 0.11 | 0.08 ± 0.01 | nd | nd | 6.52 ± 0.05 | 6.57 ± 0.02 |
| 381.1658 | C ₁₆ H ₂₉ O ₁₀ | 364 219 200 | Prenyl arabinosyl-(1->6)-glucoside | 0.11 ± 0.01 | 0.27 ± 0.05 | 7.36 ± 0.11 | 7.92 ± 0.10 | nd | nd | nd | nd | nd | nd | 3.25 ± 0.05 | 3.61 ± 0.04 |
| 787.1152 | C ₃₅ H ₃₁ O ₃₁ | | 2'',3'',6''-Tris-O-(3,4,5-trihydroxybenzoyl)-3'-Glucosyl-2',4',6'-trihydroxyacetophenone | nd | nd | 1.71 ± 0.06 | 1.16 ± 0.05 | 0.21 ± 0.05 | 0.15 ± 0.03 | 37.97 ± 0.54 | 18.84 ± 0.22 | nd | nd | nd | nd |
| 285.0974 | C ₁₃ H ₁₇ O ₇ | | p-hydroxy-benzoic acid rhamnosyl ester | 4.92 ± 0.02 | 3.83 ± 0.06 | 3.52 ± 0.09 | 3.65 ± 0.09 | nd | nd | nd | nd | 0.57 ± 0.03 | 2.54 ± 0.05 | 5.46 ± 0.11 | 2.91 ± 0.03 |
| 315.1088 | C ₁₄ H ₁₈ O ₈ | 167 | glucovanillin | nd | nd | 2.98 ± 0.09 | 2.27 ± 0.11 | nd | nd | nd | nd | 2.21 ± 0.09 | 6.78 ± 0.09 | 10.27 ± 0.22 | 3.67 ± 0.05 |
| | | | TOTAL | 8.73 | 7.22 | 25.77 | 25.8 | 0.2 | 1.04 | 43.02 | 18.92 | 2.78 | 9.32 | 25.5 | 16.75 |

nd: not detected; * identity confirmed by injection of reference compound.

As clearly shown in Supplementary Figures S1–S3, composition is different considering the various species and extraction solvents. Chromatograms are also given in Supplementary Figures S5 and S6. *P. heyniae* extracts are characterized by large abundance of condensed tannins (except for the hexane extract, which is rich in 6-hydroxy-3-oxo-alpha-ionol), while *P. meliocalpoides* mostly contains hydrolysable tannins. More complex is the situation of *P. uechtrizii*, which presents coumarins in the hexane extract, other constituents in the ethyl acetate extract, and flavonoids in methanol and water extracts.

Thus, the three *Prangos* species differ in composition and the solvent used for the extraction can influence the composition of the obtained products. Positive and negative ion mode LC-MS revealed that the most abundant compounds in the most lipophilic extracts (hexane and ethyl acetate) of *P. heyniae* are 6-hydroxy-3-oxo-alpha-ionol and the p-hydroxybenzoic acid rhamnosyl ester. The extraction is influenced by the nature of the solvent and, using methanol and water, other derivatives have been extracted from *P. heyniae* in higher yields such as epigallocatechin digallate, and galloyl prodelphinidine resulted in more abundant compounds of the methanol and water extracts. The *P. heyniae* samples also contained the coumarins heraclenin, 8-methoxy coumarin, and marmesin. Considering *P. meliocalpoides*, all of the different extracts present as the most abundant constituents' hydrolysable tannins. Nevertheless, chebulic acid (a tricarboxylic acid forming specific ellagitannin derivatives), as well as chebulinic acid (an ellagitannin), were specifically detected only in this species and, to the best of our knowledge, this is the first report of these derivatives in *Prangos*, while previous identification of such compounds occurred in *Terminalia* spp and in *Phyllanthus emblica* [54,55]. *P. uechtrizii* is characterized by the presence of flavonoid glycosides and coumarins, while it contains a negligible amount of condensed or hydrolysable tannin.

Some compounds can be considered characteristic for the different species. For example, condensed tannins, mostly derivatives of epigallocatechin gallate and galloprodelphinidine, are found mainly in *P. heyniae*. On the other hand, chebulic acid, i.e., a tetracarboxylic acid, was found only in *P. meliocalpoides*, which is also characterized by the large presence of hydrolysable tannins, which were not detected in the other two species. Considering the coumarins, heraclenin characterized *P. heyniae* while imperatorin and dentatin were specifically found only in *P. meliocalpoides* and *P. uechtrizii*, respectively.

The results revealed that, considering the whole phytochemical profile of extracts obtained from this plant material, methanol and ethyl acetate are the most suitable solvents, despite water and hexane. It is possible that the condensed tannins and epigallocatechin derivatives are not soluble enough, neither in the too nonpolar hexane nor in water. What is notable compared with other *Prangos* species reported in the literature is the limited amount of detected coumarins, as only low amounts of imperatorin were detected.

A similar behavior was observed for *P. meliocalpoides*. In fact, the LC-MS analysis revealed that more compounds were detected in the ethyl acetate and methanol extracts. The main detected compounds in this second case were tetragalloyl quinic acid and digalloyltheaflavin. Marmesin and imperatorin were also detected, showing the presence of different coumarins compared with the previous plant species. Furthermore, condensed tannins as punicalagins; the ellagitannin terchebulin; as well as esters of gallic, shikimic, and synapoyl acid with glucose represented the most notable constituents. *P. uechtrizii* extracts were characterized by the presence of a large amount of the coumarins 8-methoxy coumarin and marmesin, as well as a significant amount of epigallocatechin-gallate. Furthermore, this species was the only one showing a pattern of glycosidic flavonoids, myricetin, and quercetin.

3.2. Antioxidant Effects

The antioxidant activities of *P. heyniae*, *P. meliocalpoides* var. *meliocalpoides*, and *P. uechtrizii* extracts were evaluated using different assays. In our model, DPPH and ABTS assays were used to assess radical quenching ability, while FRAP and CUPRAC assays results indicated reducing power. The capacity of extracts to chelate ferrous ions was

measured by metal chelating assay, while the phosphomolybdenum test was useful to establish the total antioxidant capacity. Each assay presents strengths and limitations, as detailed elsewhere [56]. The results are presented in Tables 1 and 3.

Table 3. Antioxidant properties of the tested extracts ($n = 3$).

| Species | Solvents | DPPH (mg TE/g) | ABTS (mg TE/g) | CUPRAC (mg TE/g) | FRAP (mg TE/g) | MCA (mg EDTAE/g) |
|---|----------|----------------------------|---------------------------|----------------------------|----------------------------|----------------------------|
| <i>P. heyniae</i> | Hexane | 5.42 ± 0.37 ^g | na | 35.97 ± 1.80 ^h | 20.27 ± 0.55 ⁱ | 27.34 ± 0.34 ^a |
| | EA | 10.15 ± 0.47 ^e | 10.44 ± 0.91 ^g | 59.74 ± 0.63 ^{ef} | 31.60 ± 0.62 ^{gh} | 26.18 ± 0.16 ^{ab} |
| | MeOH | 46.65 ± 0.34 ^c | 60.98 ± 0.04 ^d | 108.43 ± 1.52 ^c | 70.21 ± 3.22 ^d | 15.53 ± 0.90 ^e |
| | Water | 50.66 ± 0.42 ^b | 90.74 ± 1.35 ^a | 129.89 ± 3.24 ^b | 93.14 ± 1.75 ^b | 27.79 ± 0.32 ^a |
| <i>P. meliocarpoides</i> var. <i>meliocarpoides</i> | Hexane | 4.13 ± 0.33 ^g | 8.90 ± 0.95 ^g | 55.98 ± 0.84 ^f | 28.61 ± 1.00 ^h | 26.38 ± 0.15 ^{ab} |
| | EA | 9.70 ± 0.09 ^e | 17.72 ± 0.08 ^f | 62.26 ± 1.02 ^e | 34.22 ± 0.55 ^g | 19.91 ± 0.22 ^{cd} |
| | MeOH | 52.27 ± 0.28 ^a | 77.88 ± 1.07 ^b | 133.19 ± 1.09 ^b | 80.79 ± 1.03 ^c | 18.55 ± 0.61 ^d |
| | Water | 52.01 ± 0.52 ^{ab} | 92.84 ± 0.44 ^a | 154.04 ± 2.10 ^a | 104.34 ± 1.07 ^a | 21.17 ± 0.54 ^c |
| <i>P. uechtritzi</i> | Hexane | 2.18 ± 0.62 ^h | 17.00 ± 0.74 ^f | 35.91 ± 1.32 ^h | 21.79 ± 0.85 ⁱ | 18.50 ± 1.92 ^d |
| | EA | 8.11 ± 0.35 ^f | 26.29 ± 0.44 ^e | 45.62 ± 2.18 ^g | 27.99 ± 1.69 ^h | 20.75 ± 0.25 ^c |
| | MeOH | 34.55 ± 0.63 ^d | 75.03 ± 0.43 ^c | 96.69 ± 2.56 ^d | 56.31 ± 0.36 ^f | 17.88 ± 0.83 ^d |
| | Water | 34.52 ± 0.96 ^d | 74.74 ± 0.58 ^c | 95.43 ± 0.80 ^d | 65.86 ± 0.82 ^e | 24.57 ± 0.40 ^b |

Values are reported as mean ± SD of three parallel experiments. EA: ethyl acetate; MeOH: methanol; TE: Trolox equivalent; EDTAE: EDTA equivalents. na: not active. Different letters in the same column indicate significant differences between the tested extracts ($p < 0.05$).

Overall, the findings showed that a correlation exist between DPPH, ABTS, FRAP, and CUPRAC assays, except with the metal chelating and phosphomolybdenum assay. Such a correlation was observed in several other studies [56–59]. The methanolic and aqueous extracts exhibited the highest antioxidant activities in most of the assays, suggesting an important role of the phenolics and flavonoids of the extracts. The polarity-dependent increase in total antioxidant activity and reducing properties indicates that the extraction of strong antioxidant compounds is more favorable in polar solvents [60]. Furthermore, in a previous paper that highly polar solvents, such as methanol, can have a high effectiveness in the extraction of antioxidants [35], supporting our findings.

Among the tested *Prangos* species, *P. meliocarpoides* var. *meliocarpoides* exhibited the strongest antioxidant activity in DPPH (52.27 mg TE/g), ABTS (92.84 mg TE/g), CUPRAC (154.04 mg TE/g), FRAP (104.34 mg TE/g), and phosphomolybdenum (2.52 mmol TE/g) assays. However, *P. heyniae* displayed the highest metal chelation effects (27.79 mg EDTAE/g) considering metal-chelating properties. Previous studies have also reported a weak correlation between metal-chelating assays and DPPH, ABTS, CUPRAC, and FRAP methods [61–64]. An explanation of this discrepancy is related to the different chemical reactions involved in the assays. The DPPH, ABTS, CUPRAC, FRAP, and phosphomolybdenum assays are single-electron transfer or hydrogen atom transfer reaction-based assays. On the other hand, the metal-chelating assay involves sequestration of transition metals [56]. This can justify the difference in the results obtained with DPPH, ABTS, CUPRAC, and FRAP methods and metal-chelating tests.

3.3. Enzyme Inhibitory Activities

In the present study, the ability of *P. heyniae*, *P. meliocarpoides* var. *meliocarpoides*, and *P. uechtritzi* extracts to modulate the activity of enzymes related to Alzheimer's disease (acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)), diabetes type 2 (α -amylase and α -glucosidase), and skin hyperpigmentation (tyrosinase) was investigated, and the results are presented in Table 4.

Table 4. Enzyme inhibitory effects of the tested extracts ($n = 3$).

| Species | Solvents | AChE (mg GALAE/g) | BChE (mg GALAE/g) | Tyrosinase (mg KAE/g) | Amylase (mmol ACAE/g) | Glucosidase (mmol ACAE/g) |
|---|----------|---------------------------|---------------------------|----------------------------|-----------------------------|---------------------------------|
| <i>P. heyniae</i> | Hexane | 2.39 ± 0.06 ^a | 7.83 ± 0.18 ^a | 56.07 ± 1.46 ^{ef} | 0.36 ± 0.01 ^c | 0.67 ± 0.04 ^{bc} |
| | EA | 1.58 ± 0.38 ^{cd} | 7.64 ± 0.15 ^{ab} | 54.21 ± 1.32 ^f | 0.41 ± 0.01 ^b | 0.62 ± 0.04 ^{cd} |
| | MeOH | 2.36 ± 0.18 ^a | 4.28 ± 0.16 ^c | 65.20 ± 0.89 ^c | 0.17 ± 0.01 ^e | 0.46 ± 0.08 ^e |
| | Water | 0.35 ± 0.08 ^e | na | 17.34 ± 0.38 ⁱ | 0.06 ± 0.01 ^g | na |
| <i>P. meliocarpoides</i> var. <i>meliocarpoides</i> | Hexane | 1.16 ± 0.27 ^d | 7.97 ± 0.06 ^a | 81.15 ± 0.19 ^a | 0.46 ± 0.01 ^a | 0.61 ± 0.02 ^{cd} |
| | EA | na | 7.32 ± 0.80 ^{ab} | 59.92 ± 0.96 ^d | 0.40 ± 0.02 ^b | 0.56 ± 0.01 ^d |
| | MeOH | na | 3.34 ± 0.46 ^d | 70.57 ± 0.59 ^b | 0.21 ± 0.01 ^d | 0.74 ± 0.01 ^{ab} |
| | Water | 0.19 ± 0.01 ^e | na | 21.23 ± 1.33 ^h | 0.05 ± 0.01 ^g | na |
| <i>P. uechtritzi</i> | Hexane | 2.34 ± 0.12 ^{ab} | 7.63 ± 0.39 ^{ab} | 58.77 ± 1.76 ^{de} | 0.39 ± 0.01 ^b | 0.64 ± 0.01 ^{cd} |
| | EA | 2.13 ± 0.18 ^{ab} | 6.91 ± 0.17 ^b | 61.03 ± 1.10 ^d | 0.40 ± 0.01 ^b | 0.59 ± 0.01 ^{cd} |
| | MeOH | 1.76 ± 0.14 ^{bc} | 1.58 ± 0.12 ^e | 68.03 ± 0.39 ^{bc} | 0.20 ± 0.01 ^d | 0.78 ± 0.01 ^a |
| | Water | na | 0.34 ± 0.04 ^f | 27.54 ± 1.03 ^g | 0.09 ± 0.01 ^f | na |

Values are reported as mean ± SD of three parallel experiments. EA: Ethyl acetate; MeOH: Methanol; GALAE: Galantamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent; na: not active. Different letters in the same column indicate significant differences between the tested extracts ($p < 0.05$).

Although AChE inhibition is regarded as a very promising technique for symptomatic therapy of Alzheimer's disease, the involvement of BChE in late Alzheimer's disease has been established [65,66]. Among the prepared extracts, hexane extracts demonstrated the highest anti-AChE and anti-BChE activities for the three *Prangos* species, despite that these extracts yielded the lowest phenolic and flavonoid content. In terms of plants, *P. heyniae* demonstrated the highest inhibitory property against AChE (2.39 mg GALAE/g). However, the three plants exhibited relatively similar activity against BChE, with galantamine equivalent ranging from 7.63 to 7.97 mg GALAE/g. Taken together, the three members of the *Prangos* genus could be considered as valuable sources of AChE and BChE inhibitors. It is important to highlight that, despite that aqueous extracts possessed high levels of phenolic and flavonoid, the extracts were not good inhibitors of AChE and BChE. In an earlier study by Abbas-Mohammadi et al. [67], who tested twenty-five Iranian plants for AChE inhibition, found the n-hexane fraction of *P. ferulacea* to be the most active (75.6%) at 50 µg/mL concentration, and the results are consistent with our presented results, where n-hexane extracts were the most active on AChE. In addition, several furanocoumarins were isolated in their study, which showed more potent AChE inhibitory effects with low IC₅₀ values. However, we did not find a good correlation between coumarins and AChE inhibitory properties of the tested extracts. This fact could be explained by the interaction of phytochemicals (antagonistic and so on) in the present study. Thus, we strongly suggested to conduct further studies on the cholinesterase inhibitory properties of isolated compounds from the tested *Prangos* species. In another study by Bahadori [68], the AChE and BChE inhibitory effects of *P. gaubae* extracts (n-hexane, dichloromethane, and methanol) were detected, and the dichloromethane extract was the most active on both enzymes (AChE: 2.62 mg GALAE/g; BChE: 3.51 mg GALAE/g). The AChE inhibitory effects for the methanol extracts of *P. ferulacea* and *P. peucedanifolia* were also reported as 1.47 and 4.09 mg GALAE/g, respectively [69].

α-Amylase and α-glucosidase inhibitors delay the breakdown of carbohydrates in the small intestine and, as a consequence, decrease the post-prandial blood glucose level, which is considered as an important treatment strategy to manage blood glucose levels in type 2 diabetic patients [70]. As shown in Table 4, the three *Prangos* species exhibited similar inhibition against α-amylase enzyme (0.40–0.46 mmol ACAE/g). However, the highest anti-glucosidase activity was observed with *P. uechtritzi* (0.78 mmol ACAE/g), followed by *P. meliocarpoides* var. *meliocarpoides* (0.74 mmol ACAE/g) and *P. heyniae* (0.67 mmol ACAE/g). No activity against α-glucosidase was noted with aqueous extracts. Inhibition of α-amylase and α-glucosidase enzymes has been reported to be an intriguing target for the

management of type II diabetes because of less side effects when compared with standard therapies [71,72]. Few studies have been reported in the literature on the amylase and glucosidase inhibitory effects of members of the genus *Prangos*. Loizzo et al. [73] tested the amylase and glucosidase inhibitory activity of *P. asperula* extracts (n-hexane, chloroform, and methanol), and the n-hexane extract was the most active for the enzymes, consistent with our findings. However, the best amylase and glucosidase inhibitory activity was detected by dichloromethane extract (amylase: 0.93 mmol ACAE/g and glucosidase: 20.07 mmol ACAE/g) of *P. gaubae* [68]. The amylase and glucosidase inhibitory properties were also reported for *P. ferulacea* (amylase: 0.77 mmol ACAE/g and glucosidase: 4.45 mmol ACAE/g) and *P. peucedanifolia* (amylase: 0.83 mmol ACAE/g and glucosidase: 4.97 mmol ACAE/g) [69]. From Figure 1, the observed amylase and glucosidase inhibitory abilities of the tested extracts were also moderately correlated with punicalagins and the compounds were reported as antidiabetic agents in earlier studies [74,75].

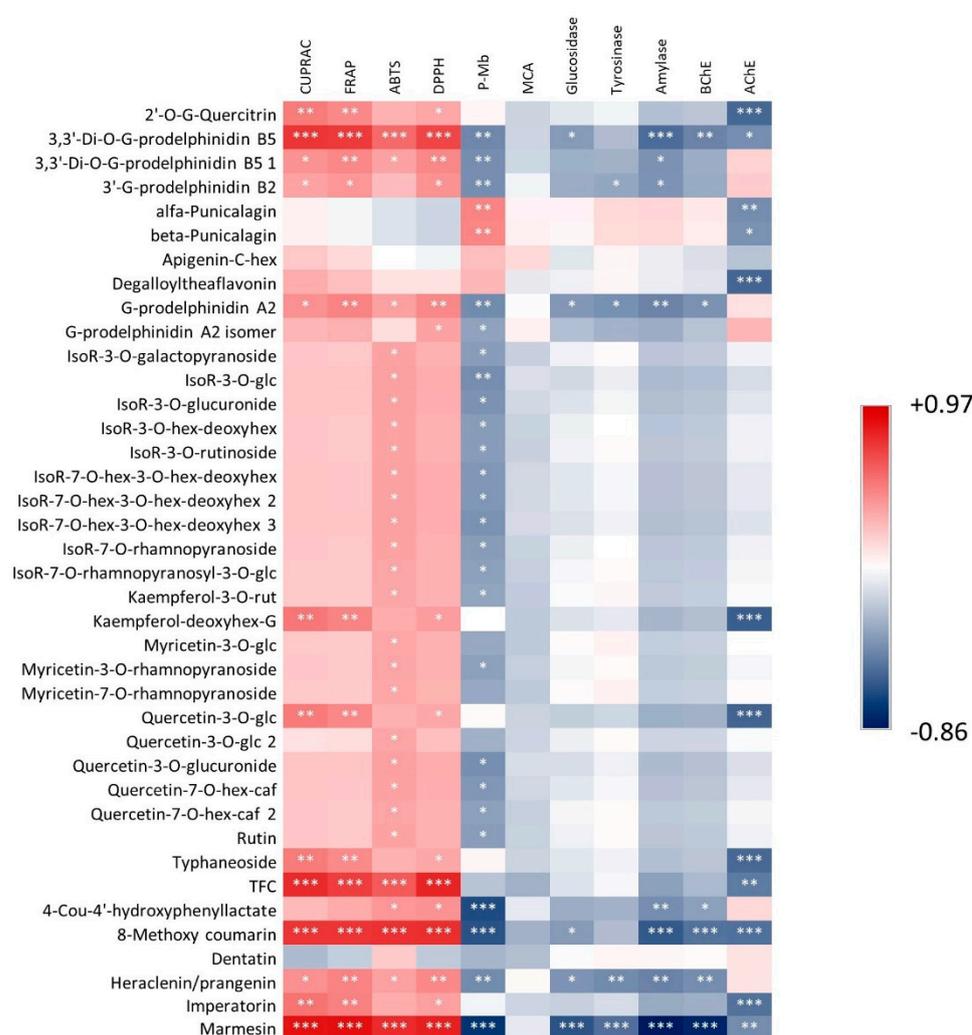


Figure 1. Correlation heatmap showing positive (red colour) and negative (blue colour) correlations between flavonoids and coumarins and measured bioactivities. Color scale is dependent on Pearson’s r value. Significance levels are indicated by asterisks in the plot. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Tyrosinase is a rate-limiting enzyme that is responsible for the manufacture of melanin, and it is regarded as a critical therapeutic approach for the treatment of skin hyperpigmentation problems [76]. The methanolic extract of *P. meliocarpoides* var. *meliocarpoides* displayed the best tyrosinase inhibitory effect (81.15 mg KAE/g), followed by *P. uechtritzi* (68.03 mg KAE/g) and *P. heyntiae* (65.20 mg KAE/g). Aqueous extracts exhibited the lowest

inhibition against tyrosinase (Table 4). In a previous study by Zengin et al. [69], the tyrosinase inhibitory properties of the methanol extract of *P. ferulacea* (131.94 mg KAE/g) and *P. peucedanifolia* (128.54 mg KAE/g) were higher than those of the presented study. The tyrosinase inhibitory properties of the extracts of *P. gaubae* varied from 16.85 mg KAE/g (in methanol) to 36.33 mg KAE/g (in n-hexane) [68].

Heatmaps were generated to establish possible correlations between the chemical composition of extracts and the observed bioactivities. One of these plots considered flavonoids and coumarins, while the second considered all of the other polyphenols (tannins and other constituents). These heatmaps are reported in Figures 1 and 2, respectively. Clearly, the results show a strong positive correlation between antioxidant activities and all of the different tannins, as well as between prodelphinidine and total flavonoid content. Considering the enzymatic activities, epigallocatechin derivatives were moderately correlated with AChE inhibition, suggesting a potential role of these compounds on this specific enzyme. As reported by Jabir et al. [77], different polyphenols have been considered as potential inhibitors of AChE, but their mode of action should be specifically studied, considering not only the possible different interactions with the active site of the enzyme, but also the potential modification to polyphenol structures due to metabolism of the host. Thus, our data suggest a possible role of gallicocatechin derivatives as potential inhibitors of AChE, and they can lead to further studies on these natural chemical compounds.

Furthermore, the OPLS model was generated using the data from the chemical analysis and combining the results of the bioassays. Figure 3 shows the score scatter plot, while the loading plot is shown in Figure 4.

In the score scatter plot (Figure 3), a clear distinction can be observed considering the extracts obtained with the lipophilic solvents, namely hexane and ethyl acetate, and the extract obtained with the more hydrophilic methanol and water, with the first being all in the -x part of the plot and the other at the opposite side. As shown by the loading plot (Figure 4) and in the biplot (Figure 5), the more relevant results related to antioxidant activities are obtained with the methanol and water extracts, while the enzyme inhibitory activities appear to be related mostly to the more lipophilic extracts. This result suggests proceeding with the studies using the water and methanol extracts of *Prangos* species as sources of antioxidant constituents, while using the lipophilic solvents to extract compounds that can be evaluated as inhibitors of cholinesterase, tyrosinase, amylase, or glucosidase. Considering the other assays, we can observe that CUPRAC appears to be related to the hydrophylic extracts, while the metal-chelating assay results were ascribed to lipophilic extracts. Thus, the application of multivariate analysis can be used to select the proper extraction solvent to improve or to search for compounds or mixtures presenting efficient activity on target assays.

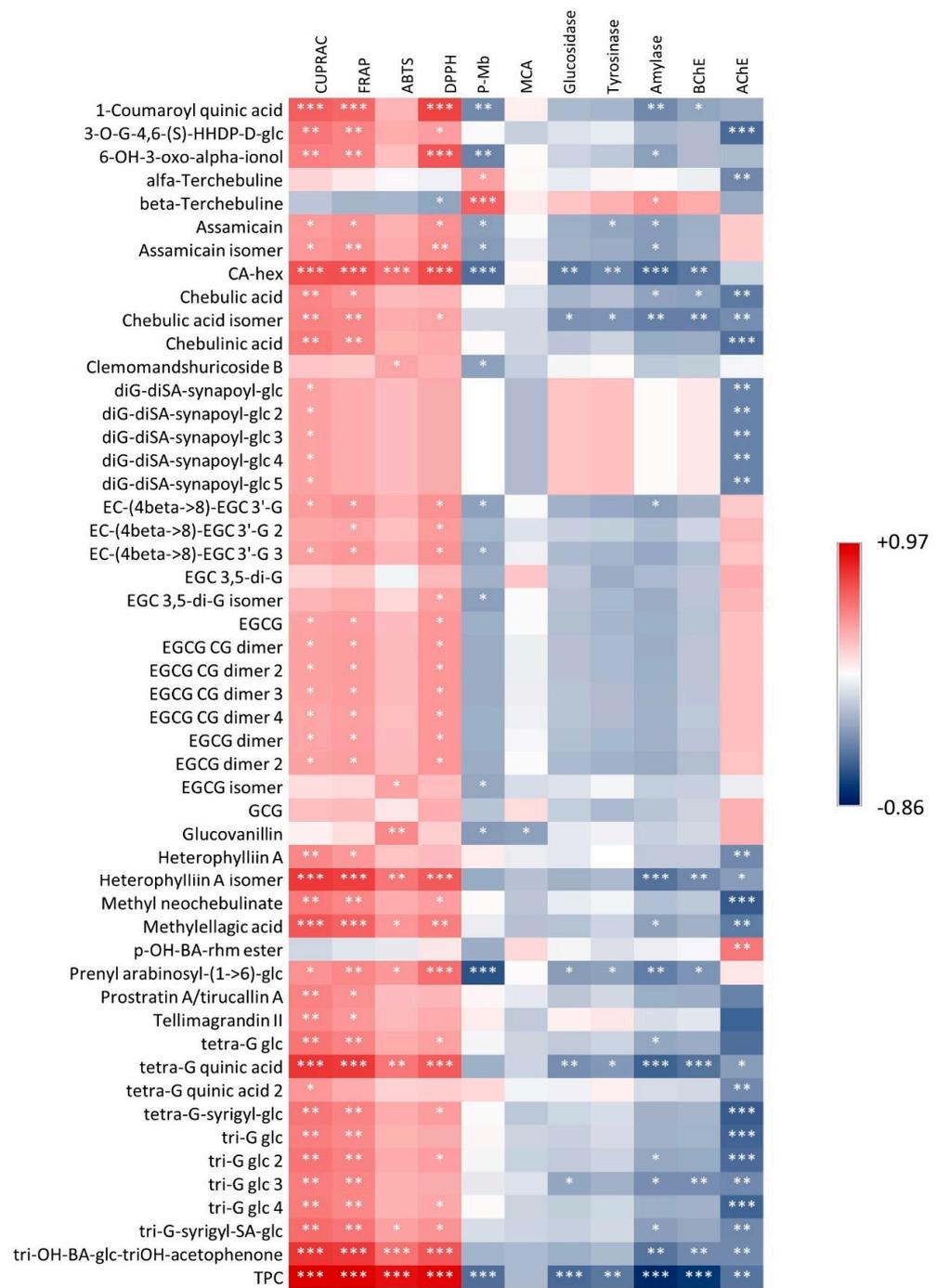


Figure 2. Correlation heatmap showing positive (red colour) and negative (blue colour) correlations between specific polyphenols and measured bioactivities. Color scale is dependent on Pearson’s r value. Significance levels are indicated by asterisks in the plot. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

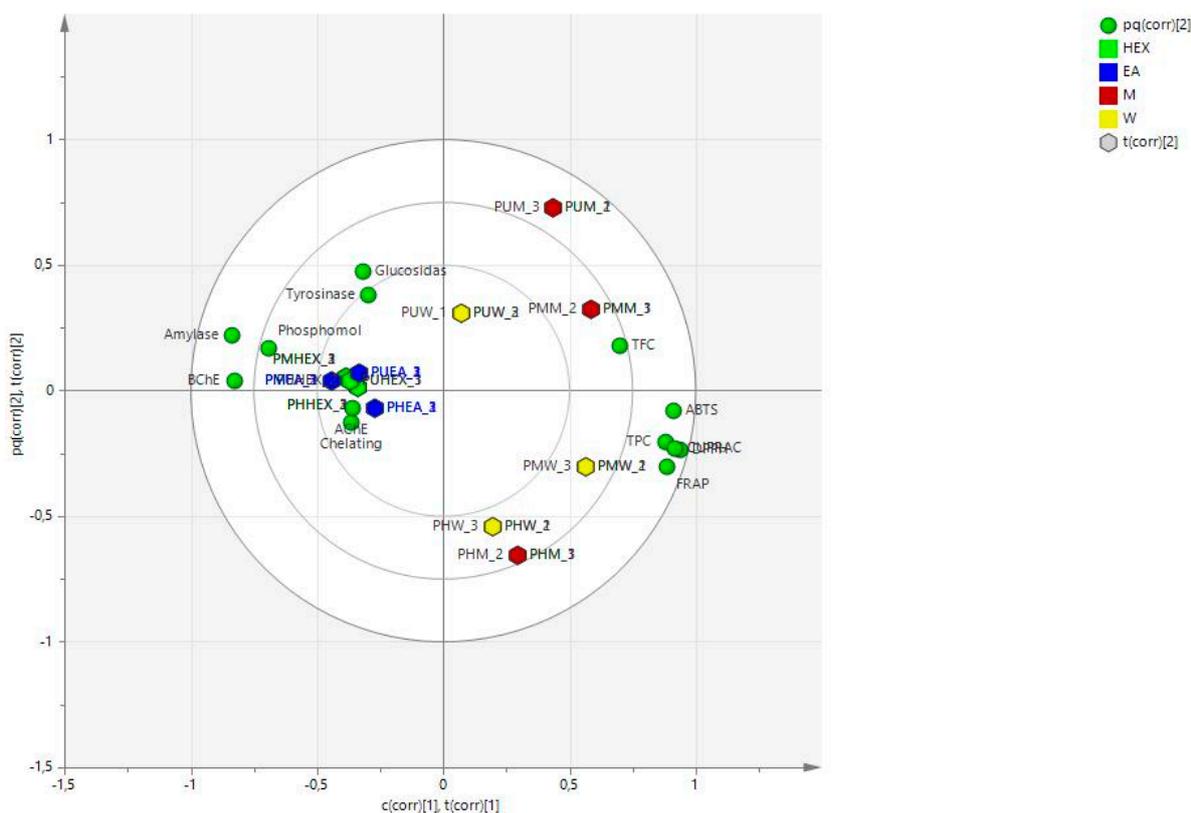


Figure 5. Biplot summarising the results of the assays and the different extracts.

4. Conclusions

In this study, the different phytochemical composition and biological activities (antioxidant and enzyme inhibitory effects) of three *Prangos* species, namely, *P. heyntiae*, *P. meliocarpoides* var. *meliocarpoides*, and *P. uechtritzi*, are described. The present study is the first report on the detailed chemical profiling and biological activities of the tested species. Thus, this work could provide valuable contributions to the scientific pool for the member of the genus *Prangos*. For each *Prangos* species, the chemical profile and biological activity depended on the extraction solvents used. Among these species, *P. meliocarpoides* var. *meliocarpoides* yielded the highest phenolic and flavonoid contents in the obtained extracts. Additionally, in terms of biological assays, *P. meliocarpoides* var. *meliocarpoides* displayed the most potent antioxidant activity as well as a significant inhibitory property on BChE, α -amylase, and tyrosinase, and these effects appear to be correlated to the presence of hydrolysable tannins. This research work presents valuable preliminary data on the three members of *Prangos* genus, displaying *P. meliocarpoides* var. *meliocarpoides* as the most promising one. However, further investigations such as in vivo bioavailability and toxicity studies need to be performed in the future, before projecting the plant for possible nutraceutical/functional food and/or pharmaceutical applications.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/antiox11091712/s1>, Figure S1: Pie charts summarising the relative percentage of phytochemicals identified and quantified in *P. heyntiae* extracts; Figure S2: Pie charts summarising the relative percentage of phytochemicals identified and quantified in *P. meliocarpoides* extracts; Figure S3: Pie charts summarising the relative percentage of phytochemicals identified and quantified in *P. uechtritzi* extracts; Figure S4: MSn spectra showing the loss of water and proposed fragmentation for the peak assigned to chebulic acid; Figure S5: MSn spectra showing the loss of water and proposed fragmentation for the peak assigned to chebulic acid.

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