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Evaluation of Anti-Tyrosinase and Antioxidant Properties of Four Fern Species for Potential Cosmetic Applications

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Abstract: Ferns are poorly explored species from a pharmaceutical perspective compared to other terrestrial plants. In this work, the antioxidant and tyrosinase inhibitory activities of hydrophilic and lipophilic extracts, together with total polyphenol content, were evaluated in order to explore the potential cosmetic applications of four Spanish ferns collected in the Prades Mountains (*Polypodium vulgare* L., *Asplenium adiantum-nigrum* L., *Asplenium trichomanes* L., and *Ceterach officinarum* Willd). The antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, oxygen radical absorbance capacity (ORAC) and xanthine/xanthine oxidase (X/XO) assays. The potential to avoid skin hyperpigmentation was tested by inhibiting the tyrosinase enzyme, as this causes melanin synthesis in the epidermis. All ferns were confirmed as antioxidant and anti-tyrosinase agents, but interestingly hydrophilic extracts (obtained with methanol) were more potent and effective compared to lipophilic extracts (obtained with hexane). *Polypodium vulgare*, *Asplenium adiantum-nigrum*, and *Ceterach officinarum* methanolic extracts performed the best as antioxidants. *Polypodium vulgare* methanolic extract also showed the highest activity as a tyrosinase inhibitor.

Keywords: pteridophytes; ferns; antioxidant; tyrosinase inhibition; cosmetics; Polypodiopsida

1. Introduction

The incidence of cutaneous disorders and melanoma has increased worldwide [1]; in fact, non-melanoma skin cancer has become the principal skin cancer among fair-skinned people [2]. Sun radiation is known to accelerate photodamage of the skin, and ultraviolet radiation is one of the main factors that causes skin hyperpigmentation and skin aging [3].

Melanin, which is obtained by irreversible tyrosine catalyzed reactions, is an important epidermal agent that blocks ultraviolet radiation [4]. It has been noted that melanocyte cultures from black skin-types increase melanogenesis and melanosis more than in fair skin-types [5]. Consequently, lighter and thinner skin is 6–33 times more susceptible to developing minimal perceptible erythema than darker and thicker skin [6,7]. Ectopic dermal melanocytes, a result of successive erythemas, are shown to be directly dependent on increased melanin in the epidermis (hypermelanosis), which can trigger sun spots [8].

Primary photoprotection, also called non-systemic photoprotection, has traditionally been considered the main strategy against the harmful effects of sun radiation [9]. This method is based

on having healthy habits towards sun exposure and the use of physical photoprotective agents; however, some disadvantages have been described [10]. Antioxidant oral supplements from secondary metabolites of plants are an adjunctive to primary photoprotection [11]. This most recent strategy on photoprotection is known as secondary photoprotection, or systemic photoprotection [12,13], in which standardized aqueous extract from the fronds of *Polypodium leucotomos* L., which is marketed under the trade name Fernblock® (Cantabria Labs, Santander, Spain), has been one of the most popular systemic and topical photoprotective oral agents in cosmetic science [14,15]. The effectiveness and safety of the use of this fern is a consequence of its multiple pathways of action described by Palomino et al. [16].

Ferns (Polypodiopsida), formerly considered pteridophytes, have been reported as one of the least understood classes of tracheophyte plants from a phylogenetic perspective [17,18]. Recent reviews of the *Polypodium* genus have been published since the commercialization of Fernblock®, as shown in Berman et al. [19]. Other authors have recently published updated reviews on the phytochemistry and ethnopharmacology of ferns, highlighting the presence of polyphenols (particularly flavonoids), terpenoids, steroids, and alkaloids [20,21]. Most of these bioactive compounds are described as natural enzyme inhibitors in biomedical research drug discovery due to anticancer, antidiabetic, and antiaging properties [22–24]. The selected ferns in this study (*Polypodium vulgare* L., *Asplenium adiantum-nigrum* L., *Asplenium trichomanes* L., and *Ceterach officinarum* Willd) are some of the most common leptosporangiate ferns reported on the Prades Mountains (Spain, 41°18′43″ N 1°05′09″ E) [25].

Considering Fernblock® as a reference in skin photoprotection [26,27], the potential anti-aging and skin-whitening properties of four Spanish ferns collected in the Prades Mountains have been studied. In vitro antioxidant activities against different free radicals (2,2-diphenyl-1-picrylhydrazyl—DPPH, oxygen radical absorbance capacity—ORAC, and xanthine/xanthine oxidase—X/XO methods) and in vitro inhibition of the tyrosinase enzyme were evaluated.

2. Material and Methods

2.1. Chemicals and Reagents

All reagents used were of analytical grade. Methanol, hexane, tyrosinase, and L-dihydroxyphenylalanine (L-DOPA) were acquired through Vidrafoc® (Barcelona, Spain). Dimethyl sulfoxide (DMSO) was obtained from Fisher Scientific® (Madrid, Spain). The provider of 5-hydroxy-2-(hydroxymethyl) pyran-4-one (kojic acid) was Alfa Aesar® (Karlsruhe, Germany). The reagents used to determine the antioxidant activity such as 2,2-diphenyl-1-picrylhydrazyl (DPPH radical), 2,6-dihydroxypurine (xanthine), xanthine oxidase, and (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) were supplied by Sigma-Aldrich® (Madrid, Spain). Sodium carbonate anhydrous (Na_2CO_3) and nitrotetrazolium blue chloride (NBT) were purchased from Laboaragon® (Cartuja Baja, Spain) and Sumalsa® (Zaragoza, Spain), respectively. All aqueous solutions were prepared with ultra-pure water.

2.2. Plant Material

It was checked that the subject species had been described in The Plant List [28] and by Banco de Datos de Biodiversidad de Cataluña [29].

The whole fresh fronds of selected fern species were identified and collected from the Prades Mountains, in the province of Tarragona (Spain), in November 2016 by Adrià Farràs and Josep Mª Farràs using botanical keys [30]. The samples were dried in the shade at room temperature.

A dried voucher specimen has been deposited at the Herbarium of Universidad San Jorge, Zaragoza, Spain (*Polypodium vulgare*: voucher no. 003-2016; *Asplenium adiantum-nigrum*: voucher no. 004-2016; *Asplenium trichomanes*: voucher no. 005-2016; *Ceterach officinarum* voucher no. 006-2016). These examples were authenticated by Dr. J.A. Vicente Orellana from Universidad CEU San Pablo (Madrid, Spain).

2.3. Extracts Preparation

Hydrophilic (= polar) and lipophilic (= non polar) extracts were prepared using methanol or hexane, respectively. Dried fronds of the four species were powdered mechanically until obtaining 40 mg of each. The powdered fronds of each species were split equally into two erlenmeyers of 500 mL. Each 20 mg of fern powder was macerated with 250 mL of solvent (hexane or methanol) at room laboratory temperature for 24 h. The extract was filtered using Whatman N°4 filter paper, and the solvent was evaporated using rotatory evaporator with a thermostatic bath at 30 °C. This process was completed two more times until exhaustion of plant material; extracts were stored at −20 °C until further experiments. Yields were calculated in percentages from the dry weight of fronds used and the quantity of dry mass obtained by extraction.

2.4. Phytochemical Screening by Thin Layer Chromatography (TLC) and Total Phenolic Content (TPC)

Silica gel TLC plates coated with fluorescent indicator F254 were used in order to detect phenolic compounds (flavonoids and phenolic acids) in the samples. 10 µL of hexane and methanolic extracts of the samples at concentrations of 10 mg/mL were run on the plates with EtOAc/MeOH/H_2O (65:15:5, *v*:*v*:*v*) as mobile phase. After eluting the samples, plates were dried, sprayed with the Natural Products polyethylene glycol (PEG) reagent, observed at 365 nm and retention factors (Rf) calculated [31].

TPC was quantified by the Folin Method as previously described using gallic acid for the standard calibration curve [32].

2.5. Determination of Antioxidant Activities

Antioxidant capacity was assessed by three complementary methods that were DPPH, ORAC, and superoxide radicals generated by X/XO.

2.5.1. DPPH Radical Scavenging Activity

The neutralization of DPPH radicals as antioxidant method was reported the first time by Blois et al. [33]. In this case, the assay was carried out according to the modifications described by Casedas et al. [34]. In 96-well microplates, each well contained 150 µL of extract and 150 µL of DPPH (0.04 mg/mL methanol solution). Antioxidant activity was determined measuring absorbance (Abs) at 515 nm after 30 min of dark incubation. Blank and control wells were also considered. The highest concentration of extracts tested was 1 mg/mL. Trolox, a water soluble derivate of vitamin E, was used as positive standard. Background interferences from solvents and samples were deducted from the activities prior to calculating radical scavenging capacity (RSC) as follows: RSC (%) = $[(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$.

2.5.2. ORAC Assay

ORAC assay was carried out to measure the capacity of extracts to scavenge peroxyl radicals. Samples and trolox were dissolved in PBS and methanol (50:50, *v:v*). Samples were incubated with fluorescein (0.07 µM) in 96-well plates for 10 min at 37 °C. Afterwards, AAPH (0.012 M) was supplemented and fluorescence was measured for 98 min at 485 nm of excitation and 520 nm of emission, in a FLUOstar Optima fluorimeter (BMG Labtech, Ortenberg, Germany) [35]. Results were expressed as µmol trolox equivalents (TE)/mg sample.

2.5.3. Superoxide Radicals Generated by Xanthine/Xanthine Oxidase (X/XO)

Xanthine oxidase and xanthine as substrate are responsible for the production of superoxide radicals [36]. The effects of fern extracts on superoxide radicals generated by X/XO were evaluated by measuring the formation of the NBT (nitrotetrazolium blue chloride)-radical superoxide complex [37] using a described procedure [34]. The reaction mixture was prepared every day as a consequence of reduced stability. This mixture was composed of 90 µM xanthine, 16 mM Na_2CO_3, and 22.8 µM NBT

in phosphate buffer (pH 7.0). 240 µL of the reaction mixture in each well with 30 µL of extract solution and XO was incubated in the dark for 2 min at 37 °C and absorbance read at 560 nm. Blank and control wells were also considered, and background interferences from solvents and samples were also deducted from the activities previous to calculating the RSC (%). The reference substance (trolox) was the same used in DPPH and ORAC assays.

2.6. Inhibition of Tyrosinase Activity

The inhibition of tyrosinase was performed following a previous method [38]. Samples were mixed with 40 µL L-DOPA and 80 µL potassium phosphate buffer (pH 6.8). Finally, 40 µL of tyrosinase (200 U/mL) was added in the wells. L-DOPA and tyrosinase were solved in buffer. The inhibition of tyrosinase was determined at 475 nm. Methanolic extracts were dissolved in methanol, and hexane extracts were dissolved in DMSO. Kojic acid was the reference inhibitor substance. Background interferences from solvents and samples were previously deducted from the activities to calculate the percentage of enzymatic inhibition (compared to control activity). Control wells had the same mix except the sample/inhibitor, which was replaced by the solvents of these.

2.7. Statistical Analysis

All samples were analyzed in triplicates (n = 3), at least, on different days. Statistical significance was analyzed by using GraphPad Prism version 6, San Diego, CA, USA. Data are presented as mean +/- standard error. The half maximal inhibitory concentration (IC_{50}) values were obtained by non-linear regression. Activities have been compared using a one-way analysis of variance (ANOVA). Statistical differences were considered as follows: $p \leq 0.05$ (*), $p \leq 0.01$ (**), and $p \leq 0.001$ (***). Correlations were performed between TPC and ORAC values, and TPC and tyrosinase IC_{50} values; Pearson values were also obtained using GraphPad Prism version 6.

3. Results

3.1. Plant Material and Yields

Table 1 shows the scientific names and Spanish common names of the collected samples as well as yields of extraction in each case. The hydrophilic extracts obtained with methanol have higher yields (ranging from 16.27% to 29.55%) than lipophilic extracts obtained with hexane (ranging from 1.49% to 2.34%).

3.2. Polyphenol Content by Thin Layer Chromatography (TLC) and Folin Method

TLC plates sprayed with Natural Products-PEG reagent revealed the presence of flavonoids in methanolic samples obtained from *Asplenium trichomanes* (ATM, Rf = 0.97, 0.92, 0.86) and *Ceterach officinarum* (COM, Rf = 0.66, 0.5). Spots corresponding to phenolic acids were also detected with similar retention factor (Rf) values in the methanolic samples from *Polypodium vulgare* (PVM, Rf = 0.81), *Asplenium adiantum-nigrum* (AAM, Rf = 0.81), *Ceterach officinarum* (COM, Rf = 0.81), and hexane extract of *Ceterach officinarum* (COH, Rf = 0.81). An image of the TLC plate sprayed with Natural Products-PEG reagent can be downloaded from Supplementary Materials (Figure S1). Total Phenolic Content (TPC) was quantified using the Folin–Ciocalteu reagent; as observed in Table 1, methanolic extracts contained higher amounts of polyphenols, with PVM and COM showing the highest values. As predicted, methanol seems to be a better solvent to extract polyphenols.

Table 1. Fern samples, botanical names, yields of extraction, and total polyphenol content (TPC).

Species	Spanish Common Name	Methanol Extract			Hexane Extract		
		Code	Yield (%) [a]	TPC (μGAE/mg)	Code	Yield (%) [a]	TPC (μGAE/mg)
Polypodium vulgare L. (*Polypodiaceae*)	"Polipodio"	PVM	23.53	172.8 ± 3.8	PVH	1.49	74.7 ± 5.8
Asplenium adiantum-nigrum L. (*Aspleniaceae*)	"Culantrillo negro"	AAM	16.27	113.5 ± 5.8	AAH	1.57	96.0 ± 3.8
Asplenium trichomanes L. (*Aspleniaceae*)	"Culantrillo rojo"	ATM	29.55	100.4 ± 0.7	ATH	2.01	70.3 ± 6.2
Ceterach officinarum Willd (*Aspleniaceae*)	"Doradilla"	COM	28.04	193.2 ± 3.8	COH	2.34	70.3 ± 7.6

[a] The yield is a relation between the weight of the dried extract and the weight of dried plant material expressed as percentage (%). GAE: gallic acid equivalents; PVM: *P. vulgare* methanol extract; AAM: *A. adiantum-nigum* methanol extract; ATM: *A. trichomanes* methanol extract; COM: *C. officinarum* methanol extract; PVH: *P. vulgare* hexane extract; AAH: *A. adiantum-nigum* hexane extract; ATH: *A. trichomanes* hexane extract; and COH: *C. officinarum* hexane extract.

3.3. Antioxidant Activity

3.3.1. DPPH Radical Scavenging Activity

As seen in Figure 1, methanolic extracts had a more powerful capacity for DPPH reduction than hexane extract for each fern. Methanolic extracts had a very similar profile of antiradical activity reaching 100% of radical inhibition at concentrations between 0.01 and 0.1 mg/mL. PVM and COM had the lowest IC_{50} values and can therefore be considered as the best antioxidants. Extracts obtained with hexane were also antioxidants, but the concentrations needed to scavenge 100% of DPPH radicals were superior compared to samples extracted with methanol as solvent.

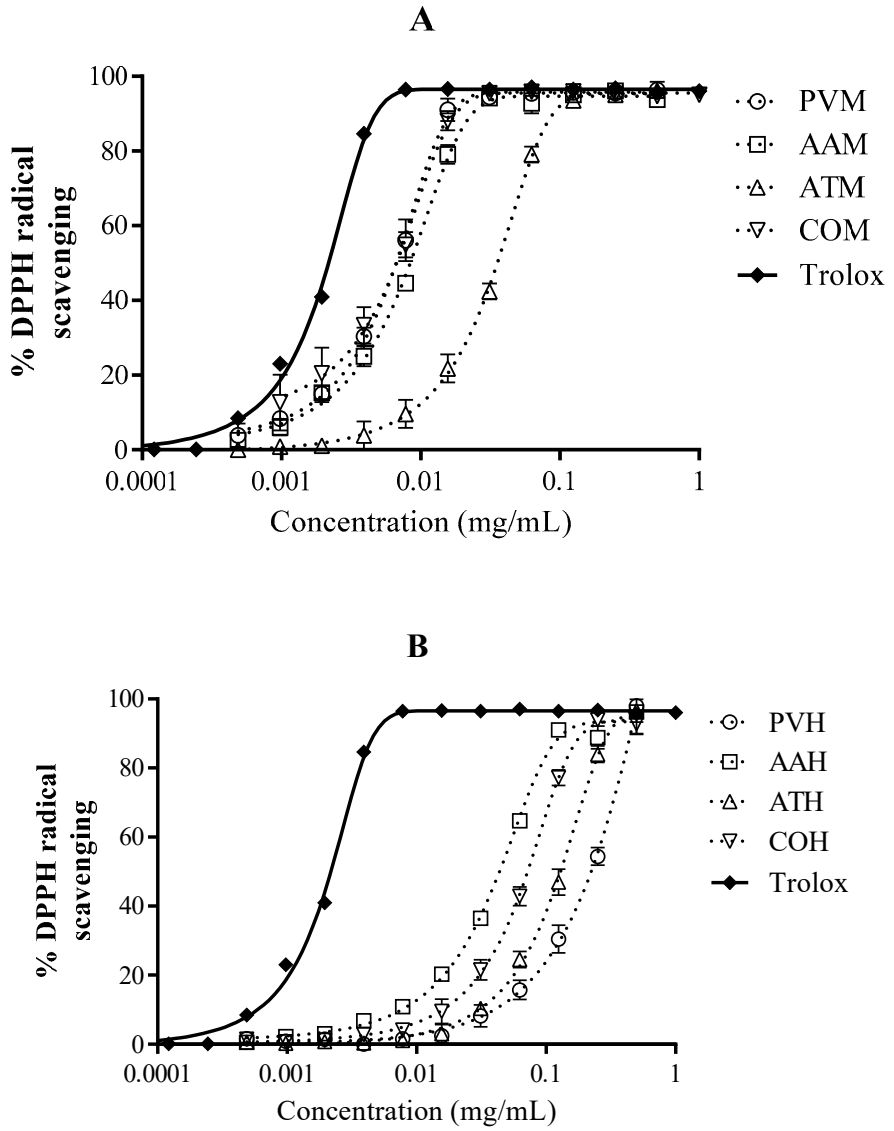

Figure 1. Antioxidant activity against DPPH radicals of methanol extracts (**A**) and hexane extracts (**B**) using trolox as a reference.

3.3.2. ORAC Assay

The ORAC assay is an internationally recognized method to measure antioxidant capacity. In Table 2, the ORAC values were also higher for methanolic extracts, particularly for PVM and COM, which is in accordance with data obtained in the DPPH assay. In Figure 2A, there is a positive correlation between ORAC values and TPC, which seems to indicate that the antioxidant activity may be mediated by polyphenols.

Table 2. ORAC values of methanolic and hexane extracts of fern species.

Ferns	ORAC (μmol Trolox Equivalents/mg Sample)	
	Methanolic Extract	**Hexane Extract**
Polypodium vulgare (PV)	2.34 ± 0.04	0.38 ± 0.02
Asplenium adiantum-nigrum (AA)	2.25 ± 0.03	0.34 ± 0.11
Asplenium trichomanes (AT)	2.25 ± 0.14	0.44 ± 0.01
Ceterach officinarum (CO)	2.93 ± 0.23 *	0.84 ± 0.06 #

* $p < 0.05$ versus PV, AA, and AT methanol extracts. # $p < 0.05$ versus PV, AA, and AT hexane extracts. Data analyzed using a one-way ANOVA and Tukey post-hoc test.

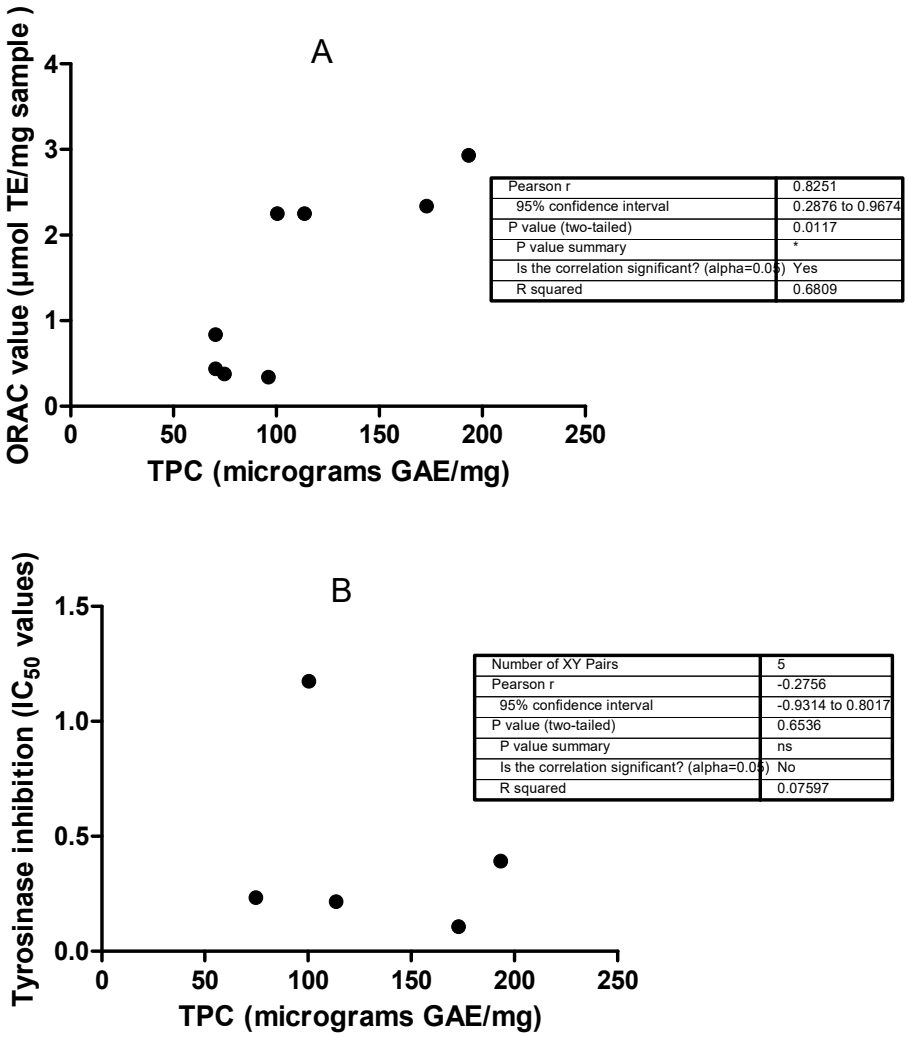

Figure 2. Correlation studies between polyphenol content and antioxidant activity (**A**) and between polyphenol content and tyrosinase inhibition (**B**). Pearson r values confirm that there is a correlation between polyphenol content and antioxidant activity measured by the ORAC method, whereas no correlation exists between polyphenol content and the inhibition of the tyrosinase enzyme.

3.3.3. Superoxide Radicals Generated by Xanthine/Xanthine Oxidase (X/XO)

In order to determine if the extracts were able to scavenge physiological radicals like superoxide anion (O_2^-) generated by X/XO, the extracts were tested using this methodology at various concentrations [15]. There were significant differences between methanolic and hexane extracts (Figure 3), but surprisingly, the activity of certain methanolic extracts was superior to the reference compound trolox (Figure 3A). Table 3 reveals that PVM, AAM, and COM showed lower IC_{50} values than trolox, which confirms their potential as antiradical agents.

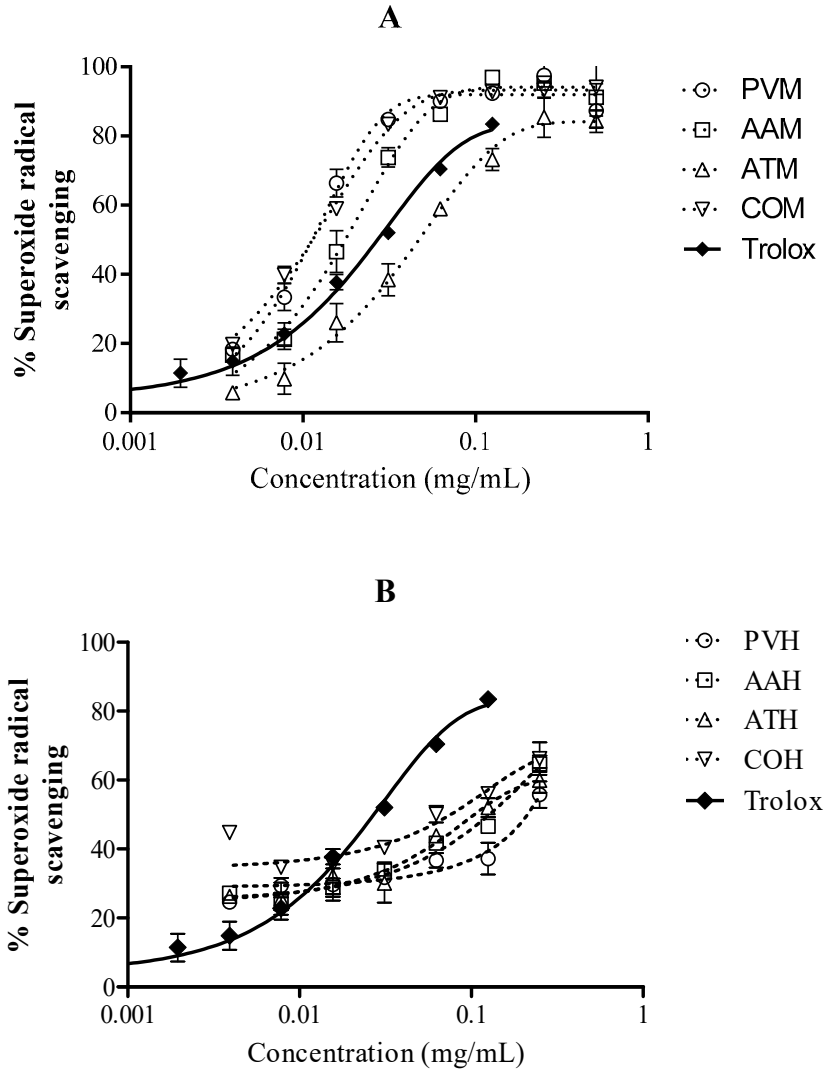

Figure 3. Antioxidant activity of fern methanolic (**A**) and hexane (**B**) extracts against superoxide radicals generated by xanthine/xanthine oxidase using trolox as a reference. *** $p < 0.001$ versus the hexane extract in the same species.

Table 3. Summary of IC_{50} values of methanolic and hexane extracts in the DPPH, xanthine/xanthine oxidase, and tyrosinase assays.

Species	IC_{50} (mg/mL) [a]					
	DPPH Radical		O_2^- Radical		Tyrosinase Inhibition	
	Methanol Extract	Hexane Extract	Methanol Extract	Hexane Extract	Methanol Extract	Hexane Extract
Polypodium vulgare (PV)	0.007	0.233	0.011	0.201	0.107	0.233
Asplenium adiantum-nigrum (AA)	0.008	0.044	0.011	0.128	0.216	ND
Asplenium trichomanes (AT)	0.036	0.129	0.047	0.090	1.175	ND
Ceterach officinarum (CO)	0.007	0.072	0.012	0.073	0.392	ND
Kojic acid	-		-		0.063	
Trolox	0.002		0.026		-	

[a] Each value is expressed as the mean of at least three independent measurements. ND: Not determined at assayed concentration (consequence of low activity). IC_{50} value is defined as the effective concentration of extract at which 50% DPPH radicals, 50% of superoxide radicals generated by xanthine/xanthine oxidase, or 50% of the tyrosinase enzyme are inactivated. IC_{50} value was obtained by interpolation from non-linear regression analysis using GraphPad Prism version 6.

3.4. Tyrosinase Inhibition

Figure 4 shows that methanolic extracts were also better than hexane extracts as anti-tyrosinase agents. All methanolic samples presented IC_{50} values between 0.107 mg/mL and 1.175 mg/mL, whereas in the case of hexane samples, only PVH was able to reach the IC_{50} value (Table 3). According to the lowest IC_{50} values, PVM was the best sample as tyrosinase inhibitor, followed by AAM. In this case, tyrosinase inhibition was not correlated with total phenolic content (Figure 2B).

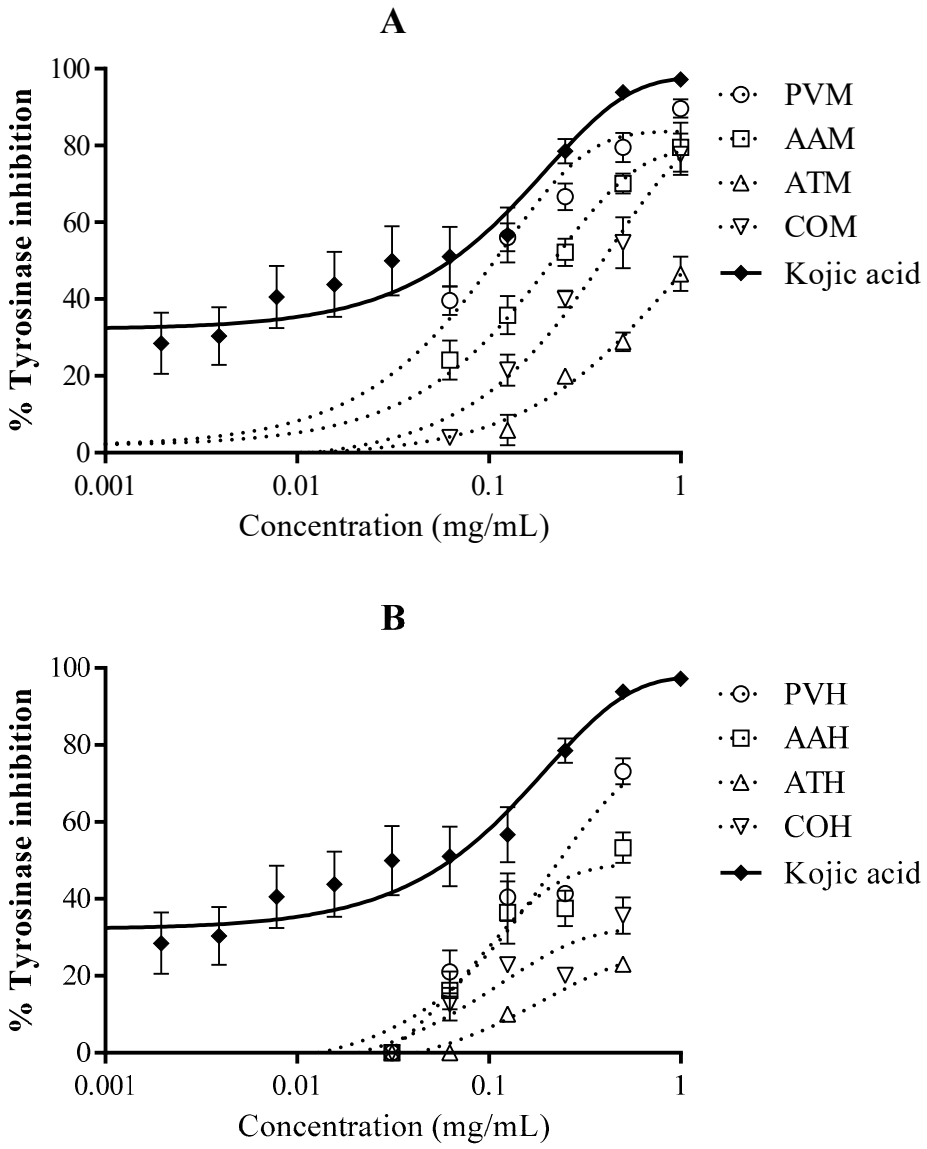

Figure 4. Inhibition of tyrosinase by methanolic (**A**) and hexane (**B**) extracts. Kojic acid was used as reference.

4. Discussion

Ferns have been used in traditional medicine in Central and South America. In fact, two of the selected species (*P. vulgare* and *A. adiantum-nigrum*) are known to possess anti-inflammatory and expectorant properties and are used in traditional medicine for colds [39]. In 2008, the European Medicines Agency (EMA) also approved the monograph of *Polypodium vulgare* rhizome for the treatment of cough and colds [40].

The antioxidant properties of ferns are not new; in fact, extracts of some ferns, such as *Pityrogramma calomelanos* and *Polypodium leucotomos*, were recently reported as antioxidants due to the presence of

polyphenols and flavonoids [21,41]. Different types of flavonoids have been described on selected Aspleniaceae ferns [42] and in ferns of the *Dryopteris* genus [43]. It has also been described that the antioxidant mechanisms of flavonoids can be based on hydrogen atom transfer (HAT), single electron transfer (SET), and transition metals chelation (TMC) [44]; however, the antioxidant mechanisms for phenolic acids are predominantly HAT rather than SET [45]. The differing nature of the antioxidant methods tested in this article allows us to determine and characterize the antiradical activity [46,47].

All methanolic samples gave strong positive results in the three tested antioxidant methods. This fact suggests the presence of phenolic acids and flavonoids with hydroxyls groups in the B-ring in the samples [48]. However, the corresponding hexane extracts displayed higher IC_{50} values and weaker antioxidant properties in the DPPH, ORAC, and X/XO assays. The lower antioxidant activity of hexane extracts could be due to the lower polarity of the solvent system, indicating that the majority of phenol and flavonoid compounds are present in the methanolic extracts as determined by Folin and TLC analysis. Additionally, all methanolic extracts showed higher extraction yields compared to the corresponding hexane extract, indicating that the majority of phytoconstituents are hydrophilic molecules. ORAC is a method for antioxidant activity widely used in food sciences, but it is not the first time that certain ferns have also been evaluated using this methodology. The main advantage of this methodology is that the use of fluorescence in the ORAC assay avoids interference with the colored samples [49].

In the superoxide method, superoxide radicals are reduced by receiving one electron (SET mechanism). Flavonoids are known to possess antiradical activity by SET mechanisms; for this reason, the reported activity in this method may be due to flavonoids [50]. The successful results with IC_{50} values better than trolox demonstrate an exploitable antioxidant activity in line with previous results for *Polypodium leucotomos* [51].

The isolation of some phytoecdysteroids in certain ferns has been the focus for certain medicinal applications [52]. For example, phytoecdysteroids have already been isolated in *Polypodium vulgare* [53]. The antioxidative properties by singlet oxygen quenching (SET) and the promotion of differentiation of human keratinocytes of these components may be the reason for obtaining certain bioactivities in hexane extracts [54,55]. Contrary to expectation, all hexane extracts exerted antioxidant properties in the different tested methods. The content of terpenoids in ferns could be responsible for these results in relation to the antioxidant activity of the hydrofobic (hexane) extracts [43,56]. Additionally, carotenoids, which are tetraterpenoids, have been reported in a number of fern species [57].

According to tyrosinase inhibition, *Selaginella tamariscina* and *Stenoloma chusanum* are the only ferns that have been described as anti-tyrosinase agents, with flavonoids involved in this activity [42,58,59]. In our study, methanolic extracts also displayed the lowest IC_{50} values, displaying better anti-tyrosinase activity than hexane extracts. This might ascribe the activity to phenolic compounds; nevertheless, the Pearson values dismiss the positive correlation between IC_{50} values in the tyrosinase assay and TPC. Due to the fact that flavonoids have been described in certain species of the *Polypodiaceae* family [60,61], we might assume that anti-tyrosinase activity of methanolic and hexane extracts could be due to flavonoids; however, this is not completely in agreement with our results, and other authors have found that the inhibition of tyrosinase could be also due to cycloartanes derivatives isolated in PV [62–64].

5. Conclusions

Regarding the results obtained, particularly for the methanolic extracts, antioxidant and potential depigmenting activity has been reinforced for the species *Polypodium vulgare*, *Asplenium adiantum-nigrum*, *Asplenium trichomanes*, and *Ceterach officinarum*. Hydrophilic extracts of these species could be of interest to develop pharmaceutical or cosmetic products, but further studies are needed to better understand the properties and safety aspects of these species.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/10/2/179/s1, Figure S1: TLC analysis of methanol and hexane extracts of the four fern samples.

Author Contributions: Conceptualization, V.L. and A.F.; Methodology, V.L. and A.F.; Software, V.L. and F.L.; Validation, V.L. and F.L.; Formal Analysis, V.L.; Investigation, A.F., G.C. and F.L.; Resources, V.L. and E.M.T.; Data Curation, A.F., F.L. and V.L; Writing—Original Draft Preparation, A.F.; Writing—Review & Editing, V.L.; Visualization, V.L.; Supervision, V.L., E.M.T. and M.M.; Project Administration, V.L.; Funding Acquisition, V.L. and E.M.T.

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Conflicts of Interest: The authors declare that they do not have any conflict of interest.

Abbreviations

AA	*Asplenium adiantum-nigum*
AAPH	2,2′-azobis(2-amidinopropane) dihydrochloride
AT	*Asplenium trichomanes*
CO	*Ceterach officinarum*
DMSO	dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
GAE	gallic acid equivalents
NBT	nitrotetrazolium blue chloride
ORAC	oxygen radical absorbance capacity
PV	*Polypodium vulgare*
TE	trolox equivalents
TLC	thin layer chromatography
TPC	total phenolic content
X/XO	xanthine/xanthine oxidase

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