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# Comparison of several hydrophilic extracts of *Polypodium leucotomos* reveals different antioxidant moieties and photoprotective effects *in vitro*

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**Oral photoprotection can be used to prevent photocarcinogenesis and photoaging. It is mainly based on the presence of antioxidant moieties in phytochemical extracts. The appearance of different botanical formulations endowed with apparently similar properties requires their side-by-side examination using an unbiased approach. The objectives of this study were to determine the antioxidant properties of several extracts of the fern *Polypodium leucotomos* and their ability to prevent ultraviolet (UV) damage *in vitro*. *In vitro* study with several extracts using cell-free and cellular assays was the design used. High performance liquid chromatography (HPLC), antioxidant assays, and cellular viability assays were the method used. Various extracts of the same plant species contain different amounts of antioxidant moieties. They bear different antioxidant and photoprotective capability at a cellular level, as determined by cellular viability assays and the appearance of DNA damage markers after UV exposure. Each botanical extract is endowed with different photoprotective properties. In these experiments, extracts from ferns' leaves are better photoprotectors than extracts from the rhizomes, but it is necessary to assess each formulation separately.**

**Key words:** *Polypodium*, antioxidant, ultraviolet (UV), photoprotection

## INTRODUCTION

Interest in photoprotection has grown recently. Photoprotection aims to prevent skin cancer in its various forms. Three main modes of photoprotection exist: (1) physical protection, which includes hats, sunglasses and clothes; (2) topical sunscreens; and (3) systemic photoprotectors. The three modes are not mutually exclusive; on the contrary, they all are necessary to prevent the deleterious effects of ultraviolet (UV) over-exposure. Of these, systemic photoprotectors are the most intriguing because, unlike the other two, they bear potential to revert some of the deleterious side-effects of previous unprotected exposure events, specifically photoaging and photo-induced carcinogenesis. This potential underlies the outburst of interest in this mode of photoprotection; however, it also provides ample opportunity for non-sanctioned, uncharacterized products

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under the promise of miraculous reversion of cancer, or aging.

Most systemic photoprotectors are phytochemicals endowed with potent antioxidant activities. Some of these can be obtained naturally from native sources, e.g. green Powerful antioxidants appear in phytochemicals obtained from non-edible, but non-toxic sources, e.g. ferns. For example, research on certain hydrophilic extracts obtained from the Central American fern *Polypodium leucotomos* (PLEs) has demonstrated their beneficial properties in terms of photoprotection (Berman et al., 2016).

Most *P. leucotomos* extracts studied to date are not toxic, even at high doses (Kuo and Yang, 2008; Nikitaki et al., 2015). *P. leucotomos* extracts inhibit UV-catalyzed formation of cyclobutane pyrimidine dimers (CPD) (Zattra et al., 2009) and prevent UV-mediated mitochondrial DNA damage (Villa et al., 2010). They also possess anti-inflammatory properties, decreasing sunburn and erythema (Gonzalez and Pathak, 1996). They also curb the expression of pro-inflammatory mediators such as tumor nuclear factor (TNF)-$\alpha$, inducible nitric oxide synthases (iNOS) and cyclooxygenase (COX)-2 (Janczyk et al., 2007; Zattra et al., 2009). An interesting property of most *P. leucotomos* extracts studied is that they prevent UV-induced immunosuppression (Middelkamp-Hup et al., 2004; Siscovick et al., 2008), which is one of the hallmarks of photo-induced carcinogenesis (Cavallo et al., 2011). This is due to the inhibition of the isomerization of *trans*-urocanic acid. *P. leucotomos* extracts prevent the appearance of *cis*-urocanic acid (UCA) (Capote et al., 2006), which is immunosuppressive (Walterscheid et al., 2006). *P. leucotomos* extracts also preserve the viability and function of skin myeloid cells (Middelkamp-Hup et al., 2004).

Different *P. leucotomos* extracts are currently commercialized by several companies, including Fernblock® (Cantabria Labs, Madrid, Spain). At face value, all these formulations are endowed with anti-aging, anti-oxidant and photoprotective properties. However, the proprietary extraction methods of each company (use of different solvents, different biochemical and biophysical methods and different parts of the plant) and the different geographical origin and growing conditions of the plants suggest that these extracts may have vastly different properties.

In this study, some of these extracts have been compared in an unbiased, objective manner, analyzing their known antioxidant moieties by high performance liquid chromatography (HPLC), as well as their antioxidant capability in cell-free assays. Emphasis was made on specific antioxidant moieties previously described in these extracts (Garcia et al., 2006). Finally, their effect on cell survival and the appearance of DNA damage markers upon UV exposure have been assessed. These experiments were carried out in dermal fibroblasts and a keratinocyte cell line (HaCat cell line), which are widely accepted models to dissect the effect of irradiation *in vitro* (Fernandez et al., 2014). The results indicate that the different origins, extraction methods and additives together with the use of different parts of the plant result in very different antioxidant profiles, which underlie most, but not all, of the beneficial effects of these extracts.

## MATERIALS AND METHODS

### *P. leucotomos* extracts (PLEs)

Six different *P. leucotomos* extracts were included in the study: 1 to 3 are from aerial parts of the plant (leaves) and 4 to 6 are rhizome extracts. Specifically, Sample #1 consists of a buffered aqueous extract of *P. leucotomos* leaves at pH=7; Sample #2 Fernblock® is an aqueous extract of *P. leucotomos* leaves extracted at basic pH; Sample #3 is a hydro alcoholic, acidic extract of *P. leucotomos* leaves; (4) Sample #4, a granular hydro alcoholic extract of the rhizome of *P. leucotomos*; (5) Sample #5, a hydro alcoholic extract of the rhizome of *P. leucotomos*; (6) Sample #6, consisting of a hydro glycolic extract from the rhizome of *P. leucotomos*.

### HPLC

Samples were prepared at 10 mg/mL by mixing 0.5 g of each dry extract with 50 mL double distilled water for 30 min at room temperature under constant stirring. Samples were filtered through a 0.45 µm-pore polyvinylidene difluoride (PVDF) membrane and 0.1 mL was loaded into a liquid HPLC device from Waters Alliance equipped with a Luna C18(2) column (5 µm, 100 A, 250 × 4.6 mm) from Phenomenex. Flux remained constant at 1 mL/min. Three mobile phases were used; 100% milliQ water (A); 10:90 (v/v) mixture of glacial acetic acid: milliQ water (B); 50:50 (v/v) mixture of acetonitrile: methanol. The elution gradient was carried out at 30°C.

Samples were monitored continuously at 260 and 290 nm, and represented at 260 nm from 0 to 65 min and 290 nm from 65 min to the end of the experiment (140 min). The reason for this switch is that 4-hydroxybenzoic and 4-hydroxy-3-methoxybenzoic acid are better detected at 260 nm, whereas the rest of the species analyzed are better detected at 290 nm.

### *HPLC standards*

3,4-Dihydroxybenzoic (protocatechuic), 4-hydroxybenzoic, 4-hydroxy-3-methoxybenzoic acid (vanillic), 3,4-dihydroxycinnamic (caffeic), 4-hydroxycinnamic, and 4-Hydroxy-3-methoxycinnamic (ferulic) acid were from Sigma-Aldrich. Standards were diluted at 10 µg/mL in double distilled water and 0.1 mL was loaded into the column as described earlier.

### ABTS antioxidant assay

ABTS antioxidant assay is a gold standard to study the antioxidant capability of a given substance, as reviewed previously (Dong et al., 2015). 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulphate (PPS), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox C), and 3,3’,4’,5,7-pentahydroxyflavone were from Sigma-Aldrich and prepared at 7 mM in $H_2O$ (ABTS), 2.5 mM in $H_2O$ (PPS) and 0.2 mM in ethanol (Trolox C). ABTS+* (reactive) was prepared by mixing equal volumes of ABTS and PPS solutions, incubating the

mixture in the dark for 16 h at room temperature and adding PBS until 734 nm absorbance = 0.7-1.0. Extracts were prepared at 1 mg/mL in $H_2O$. The experiment was performed by mixing 0.3 mL of vehicle ($H_2O$), 0.3 mL of a 0.2 mM Trolox C solution or 0.3 mL of each extract with 2.7 mL of ABTS+*. The mixtures were incubated for 15 min in the dark and absorbance at 734 nm was measured immediately in a Biorad Smartspec 3000 spectrophotometer. Data were collected in triplicate and represented as the relative percentage of ABTS reduction compared to Trolox C, which is considered 100%.

#### FRAP antioxidant assay

FRAP is another classic method to assess the antioxidant capability of a substance (Amorati and Valgimigli, 2015). 2,4,6-Tripyridyl-s-triazine (TPTZ) and ferric chloride ($FeCl_3$) were from Sigma-Aldrich, and prepared at 10 mM in 0.04 M HCl (TPTZ) and 20 mM in 0.3 M acetate buffer pH 3.6 ($FeCl_3$). The protocol was as follows: the reactive solution (FRAP) was prepared by mixing TPTZ: $FeCl_3$: acetate buffer in a 1:1:10 proportion. Extracts were prepared as for the ABTS assay. The experiment was carried out by mixing 2.85 mL of FRAP solution with 150 µL of vehicle, each extract or Trolox C. Mixtures were incubated for 15 min in the dark and absorbance at 593 nm measured immediately. Data were collected in triplicate and represented as the relative percentage of FRAP reduction as compared to Trolox C, which is considered 100%.

#### Cellular assessment of the photoprotective activity of the *P. leucotomos* extracts

All the experiments involving primary cells were done in agreement with the Helsinki declaration, and the specific protocols were approved by the Research Ethics committee of the Universidad Autónoma de Madrid, Spain. Human retroauricular fibroblasts were obtained from healthy donors, who received thorough information regarding the use of their cells and signed an informed consent. HaCaT immortalized keratinocytes have been described previously (Boukamp et al., 1988). All the reagents were from Sigma-Aldrich unless otherwise specified. Both cell types were cultured in DMEM medium supplemented with 50 U/mL penicillin, 50 U/mL streptomycin, 2 mM L-glutamine and 10% fetal bovine serum and routinely passaged using trypsin-EDTA 0.25%. Fibroblasts were used between passages 12 and 15. To assess the photoprotective activity of the extracts, cells were cultured in 100 µL of DMEM in flat bottomed, 96-well dishes at $10^5$ cells/well. After reaching confluence, medium was replaced with 100 µL of non-supplemented DMEM without phenol red containing the appropriate dilution of each extract (data shown at 1 mg/mL). Cells were immediately irradiated with an UV lamp (Micro-UV Irradiator UV-1047Xe from Frontier Laboratories) in the 280 to 400 nm range. Cells were irradiated at 5 cm for 7 min for a total energy of 2.2 J/cm$^2$. After irradiation, medium was replaced with supplemented DMEM and dishes were incubated for 2.5 h at 37°C, after which cells were fixed, stained with crystal violet, solubilized with 0.1% sodium dodecyl sulfate (SDS) and measured in a spectrophotometer at 543 nm. Data was relativized to the survival in the Fernblock condition, which was reported previously (Alonso-Lebrero et al., 2003). Each experiment was performed three times in triplicate.

#### Assessment of the appearance of DNA damage markers

HaCaT cells were cultured as described, treated and UV-irradiated as described earlier. Cells were allowed to recover for 4 h, fixed with 4% paraformaldehyde in PBS, permeabilized with 0.5% Triton X-100 and stained with a p139Ser-γH2AX antibody (Novus Bio) followed by AlexaFluor568-conjugated goat anti-rabbit antibody (Invitrogen). Alternatively, cells were stained with the CPD staining kit from Cell Biolabs Inc., according to the manufacturer's instructions. Flow cytometry was carried out in a FACS Canto machine (Becton-Dickinson) and immunofluorescence images were obtained with an epifluorescence microscope fitted with an Olympus DP50 digital camera and using the following excitation filters: UVA (360-370 nm, exciting filter UG-1) for DAPI or blue (450-490 nm, exciting filter BP 490) filers. Images were processed using the Adobe PhotoShop 7.0 software (Adobe Systems).

#### Statistical analysis

Data are given as mean ± standard deviation (SD) from three independent experiments. Statistical significance was evaluated using the Student's t-test, and differences were considered to be significant at a value of $p < 0.05$. Where indicated, *p<0.05; **p<0.01; n.s: not significant.

## RESULTS

### Antioxidant moieties present in different *P. leucotomos* extracts measured by HPLC

First, the different *P. leucotomos* extracts were analyzed by HPLC to determine their relative antioxidant content. Based on previous evidence gathered using some of these extracts (Garcia et al., 2006), these experiments focused on several specific antioxidant aromatic acids, including 3,4-dihydroxybenzoic (protocatechuic), 4-hydroxybenzoic, 4-hydroxy-3-methoxybenzoic acid (vanillic), 3,4-dihydroxycinnamic (caffeic), 4-hydroxycinnamic, and 4-Hydroxy-3-methoxycinnamic (ferulic). The first two were analyzed by absorbance at 260 nm and the rest at 290 nm to improve resolution. Samples were prepared as indicated in Material and Methods. Figure 1A shows the HPLC standards used at 10 µg/mL (0.1 mL). Samples #1 and #2 (Figure 1B to C) contained detectable amounts of these species. Sample #3 (Figure 1D) also contained most of these antioxidant moieties. Conversely, sample #4 contained very small amounts of 4-hydroxybenzoic and caffeic acids; whereas vanillic and cinnamic acids were almost undetectable (Figure 1E). Sample #5 contained smaller amounts of all these antioxidants, particularly ferulic acid, which was almost undetectable (Figure 1F). Sample #6 contained almost none of these antioxidant moieties, although it displayed a very prominent peak similar to ferulic acid in terms of retention time (98.56 vs. 97.386, Figure 1G and 1A, respectively).

### Antioxidant activity of the different *P. leucotomos* extracts

To measure the actual antioxidant capability of the different *P. leucotomos* extracts, two gold-standard

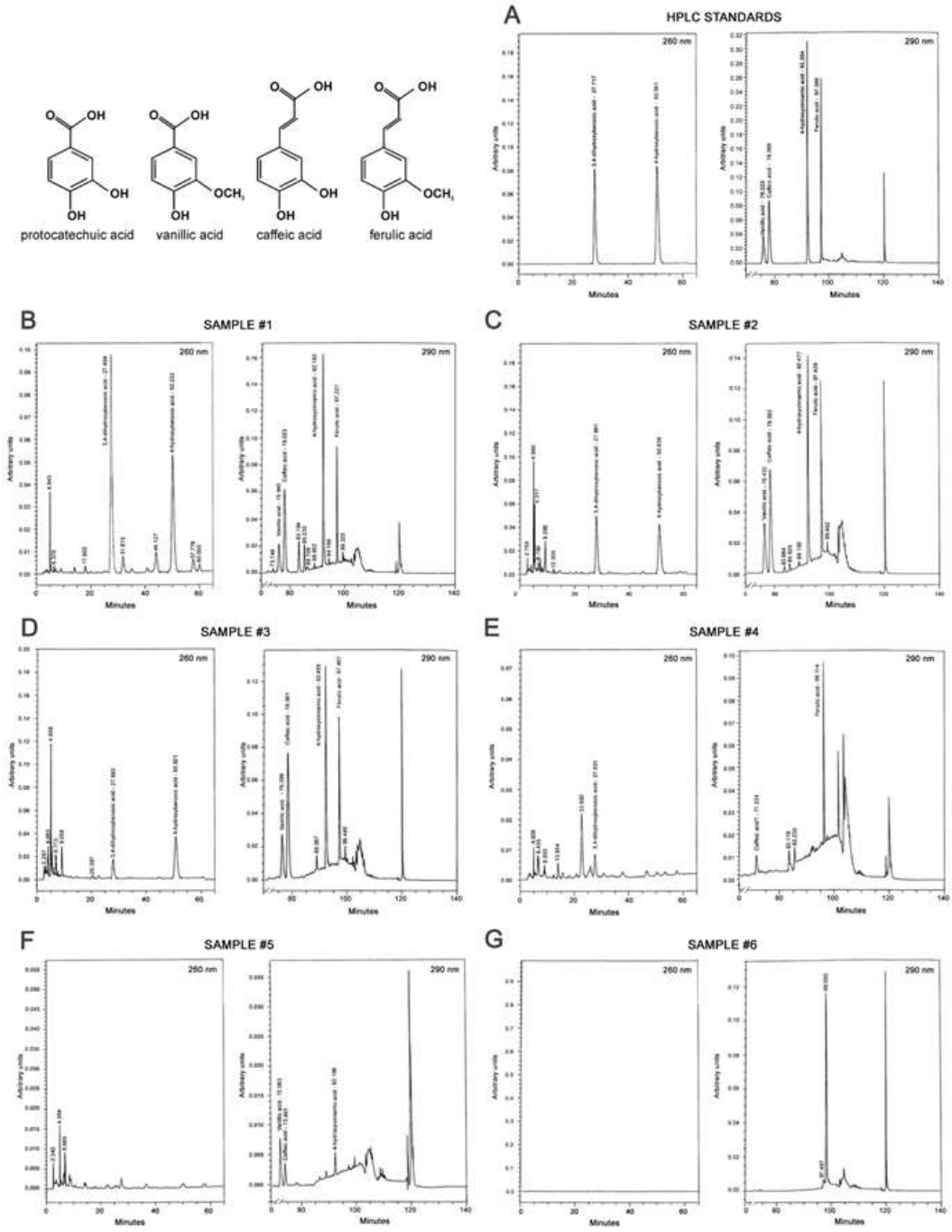


**Figure 1.** HPLC profiles of the *P. leucotomos* extracts reveal different levels of known antioxidant moieties. Top left, structures of some of the antioxidant moieties analyzed throughout. Panels depict HPLC profiles of control antioxidants (A) and the *P. leucotomos* extracts (B-G). In all cases, graphs represent arbitrary units of relative abundance in the Y axis and elution time in the X axis. Numbers in the images represent the elution time of each peak. (A) HPLC standards used in the experiment, 0.1 mL of each standard at 1 mg/mL. (B-G) HPLC curves of the extracts, labelled as samples #1-6. Identified moieties are indicated next to their retention times. Graphs are shown at 260 nm from times 0-65 min and at 290 nm from 70-140 min for clarity. A representative curve out of three performed is shown for each extract.

antioxidant assays were performed, FRAP and ABTS. Although both assays measure the antioxidant activity of the extracts, FRAP requires acidic pH, whereas ABTS requires a pH close to 7. Results are represented as the

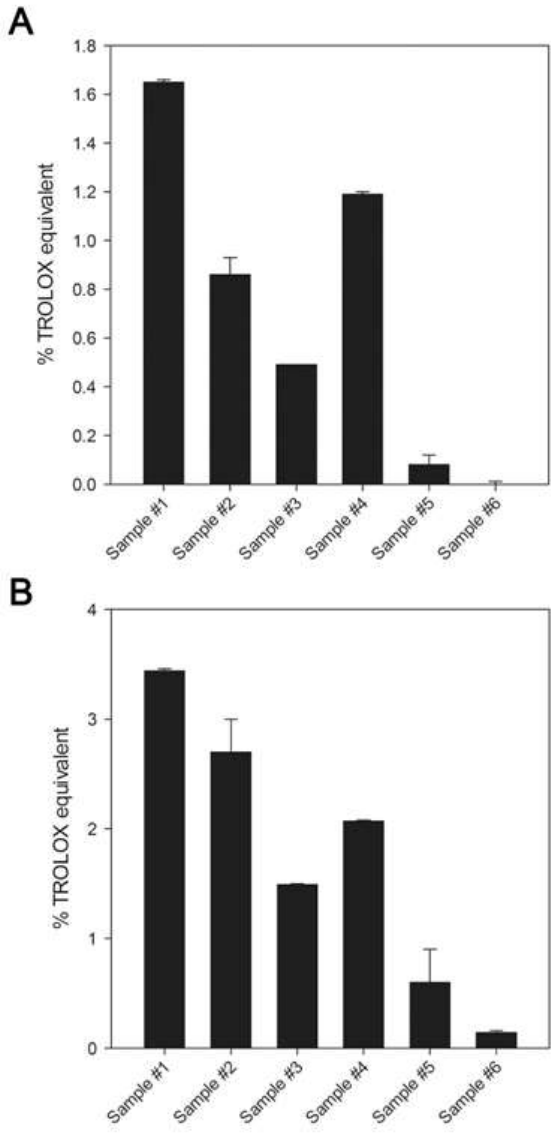


**Figure 2.** Antioxidant determinations in cell-free systems reveal the different antioxidant potential of the assayed *P. leucotomos* extracts. FRAP (A) and ABTS (B) antioxidant assays were performed as indicated in the Material and Methods section for each sample (#1-6). Vehicle was water and Trolox C was used as reference in each case to set up 100% on each curve. For FRAP, data represents the antioxidant potential of 0.15 mg of each extract (150 µL of 1 mg/mL solution) compared to 30 nmol of Trolox C (150 µL of a 0.2 mM solution of Trolox C), which was arbitrarily set up as 100%. For ABTS, data represents the antioxidant potential of 0.3 mg of each extract (300 µL of 1 mg/mL solution) compared to 60 nmol of Trolox C (300 µL of a 0.2 mM solution of Trolox C), which was arbitrarily set up as 100%. In both graphs, data is the mean percentage ± SD of three independent experiments performed in triplicates.

percentage of the antioxidant ability of a well-characterized antioxidant, Trolox. Data are as shown in Figure 2 (FRAP) and Figure 2B (ABTS). These results indicate that Samples #1 to 4 were endowed with significant antioxidant activity (a value ≥0.3% is considered an active antioxidant in these assays),

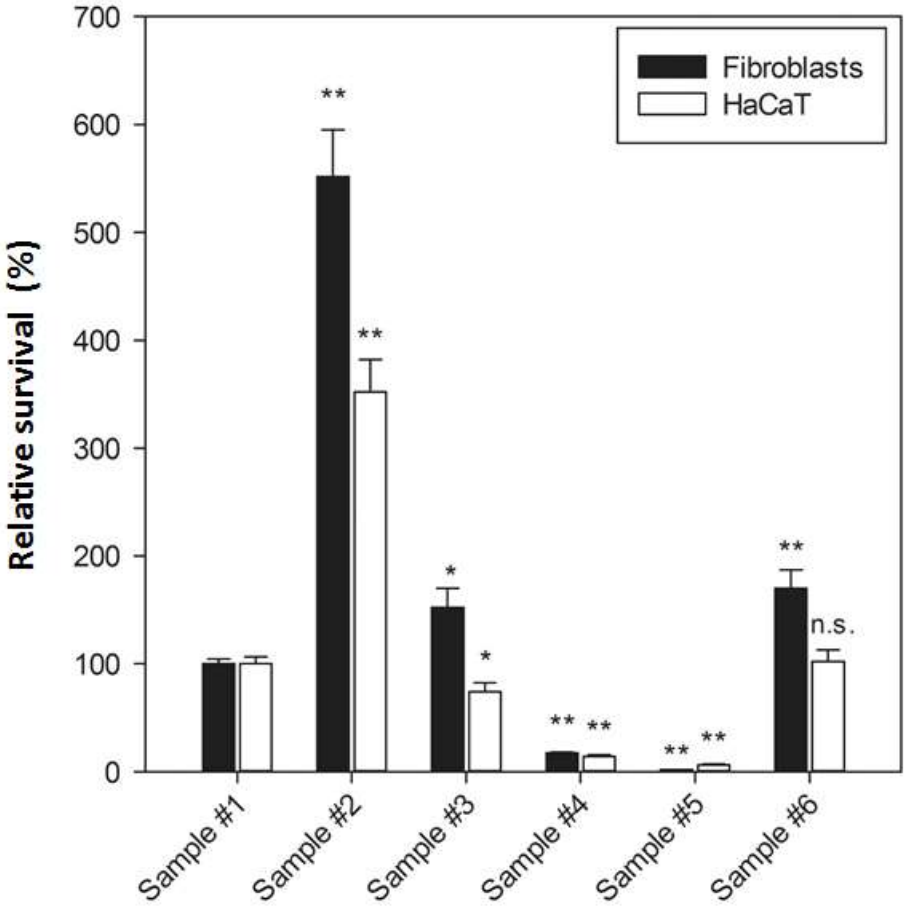


**Figure 3.** The assayed *P. leucotomos* extracts have a different effect on cell survival upon UV insult. Dermal fibroblasts (black bars) and HaCaT transformed keratinocytes (white bars) were treated with 1 mg/mL of each extract and irradiated. Cell survival was determined by staining with crystal violet by absorbance at 543 nm. Data was relativized to the survival induced by sample #1 (first pair of bars). Data is the mean ± SD of three independent experiments performed in triplicate. Significance was determined using Student's t test. * $p<0.05$; ** $p<0.01$; n.s., not significant.

whereas Samples #5 and #6 displayed much lower, almost negligible antioxidant capability.

### *In vitro* photoprotective activity of the *P. leucotomos* extracts

The actual photoprotective activity of the different *P. leucotomos* extracts was then measured in *in vitro* assays using HaCaT cells (to represent skin keratinocytes) and dermal fibroblasts, which are the two most abundant cell populations in the skin (Fernandez et al., 2014). Cells were subjected to a dose of UV (280 to 400 nm) corresponding to 2.2 J/cm$^2$. Figure 3 shows the survival data relative to the survival induced by sample #2, as reported previously (Alonso-Lebrero et al., 2003). The data indicates that, compared to Sample #1, Sample #2 was much more active (>5 times on fibroblasts and >3 times on HaCaT). Samples #3 and #6 exerted a certain degree of photoprotection, but none of these reach a 2-fold threshold. Finally, Samples #4 and #5 were almost inactive in terms of UV-induced photoprotection.

### Effect of the *P. leucotomos* extracts on the appearance of DNA damage markers upon UV irradiation

To gain further insight into the photoprotective mechanism of the extracts, the phosphorylation of H2AX (γH2AX) in Ser139 was assessed by flow cytometry and immunofluorescence. This is a non-exclusive marker of the appearance of double-strand breaks in DNA (Kuo and Yang, 2008). These experiments revealed that, upon UV

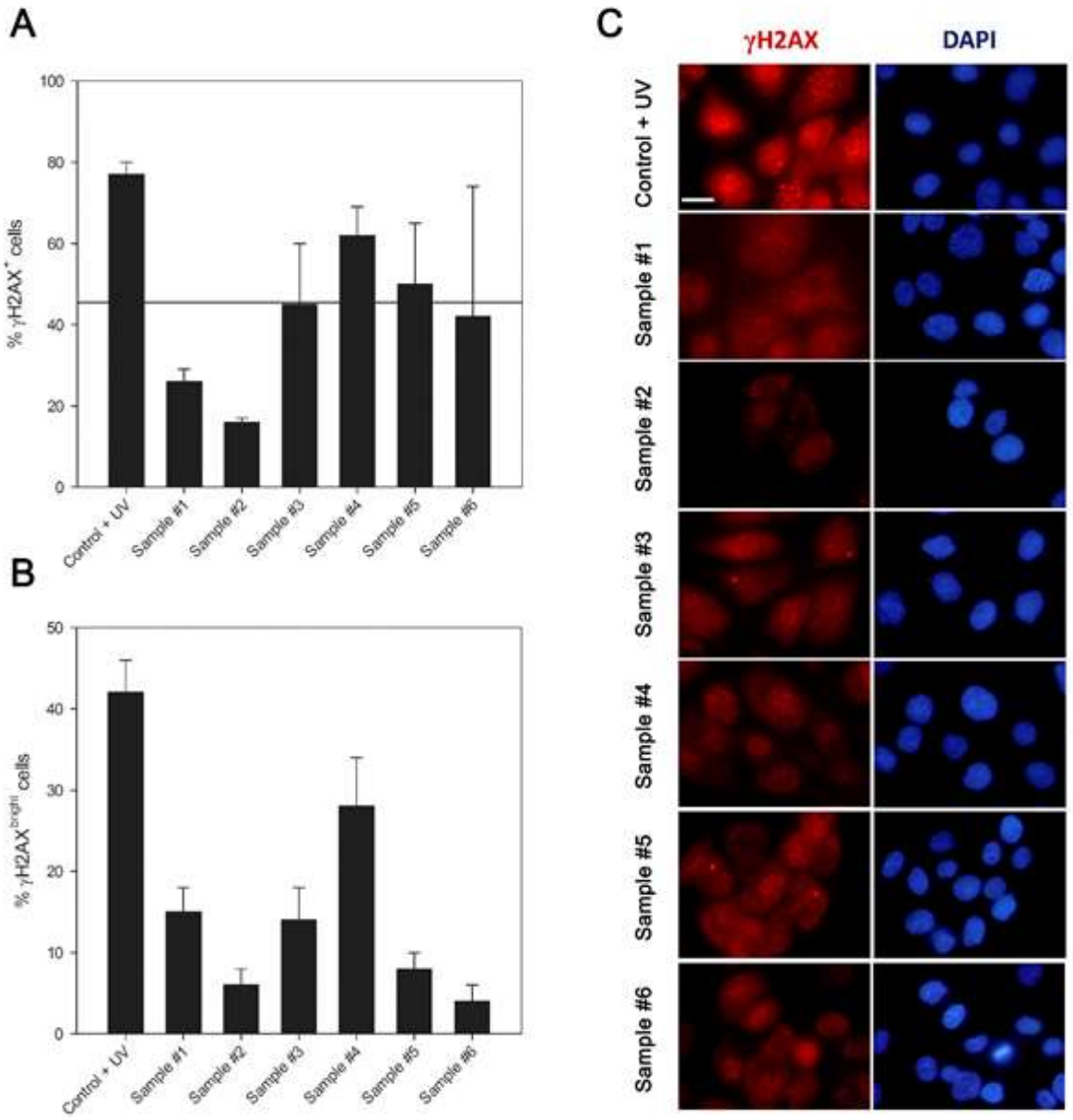


**Figure 4.** The assayed *P. leucotomos* extracts have a different effect on the phosphorylation of H2AX upon UV irradiation. (A-B) HaCaT transformed keratinocytes (white bars) were treated with 1mg/mL of each extract and irradiated as indicated in Material and Methods. Cells were then fixed, stained for gH2AX and measured by flow cytometry. Data in (A) represents the percentage of positive cells (γH2AX$^{+}$), whereas data in (B) represents the percentage of very positive cells (γH2AX$^{bright}$). (C) Representative immunofluorescence images of irradiated cells in the presence of the different extracts. Briefly, cells were allowed to adhere for 24 h to glass coverslips, medium was replaced with colorless medium containing the extracts at 1 mg/mL, irradiated, allowed to settle for 2.5 h, fixed and stained as in (A). Fields are representative of >40 examined per condition. Bar= 20µm.

irradiation, almost 80% of the cells became positive for γH2AX (γH2AX$^{+}$, Figure 4A). Of these, over 40% contained high levels of γH2AX (γH2AX $^{bright}$) as determined by setting an arbitrary threshold, MFI ≥ 30,000 (Figure 4B). As before, the extracts showed important differences in terms of numbers of γH2AX $^{+}$ and γH2AX $^{bright}$ cells. Sample #1 decreased the number of γH2AX $^{+}$ cells to just over 20% and of γH2AX $^{bright}$ cells to around 15%. Sample #2 was even more effective, decreasing the levels to less than 20% and 5%, respectively. On the other hand, samples #3-5 were less effective, although the latter decreased the number of γH2AX $^{bright}$ cells than the other two. Finally, sample #6 was not very effective reducing the number of γH2AX $^{+}$

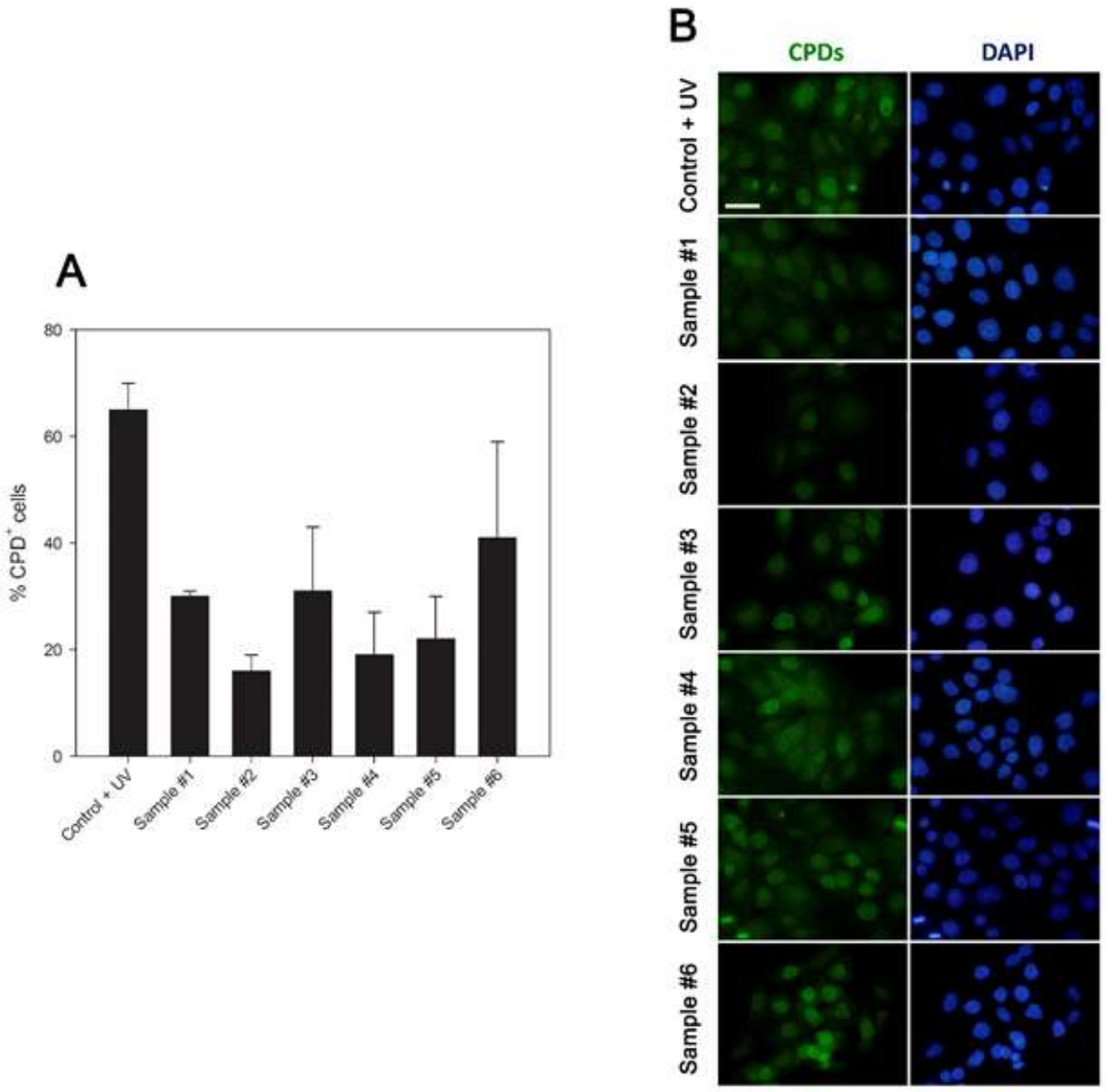


**Figure 5.** The assayed *P. leucotomos* extracts have a different effect on the appearance of CPDs. (A) HaCaT transformed keratinocytes (white bars) were treated with 1mg/mL of each extract and irradiated. Cells were then fixed, stained with the CPD detection kit as indicated in Material and Methods, and measured by flow cytometry. Data represents the percentage of CPD-positive cells. (C) Representative immunofluorescence images of irradiated cells in the presence of the different extracts. Briefly, cells were treated as in Fig. 4C, then stained with the kit as in (A). Fields are representative of >40 examined per condition. Bar= 20µm.

cells, but it reduced the number of γH2AX $^{bright}$ cells to levels similar to those elicited by Sample #2. These observations were consistent with the qualitative assessment of the cells by immunofluorescence, in which Sample #2 reduced the γH2AX signal most efficiently as compared to the other extracts (Figure 4C).

Also, formation of cyclobutane pyrimidine dimers was determined. CPDs constitute an additional marker of UV-induced DNA damage (Nikitaki et al., 2015). These experiments showed that UV irradiation induced the appearance of CPDs in almost 70% of the cells (Figure 5A). This phenomenon was quenched by all the extracts except Sample #6, but most efficiently by Sample #2 as determined by flow cytometry (Figure 5A) and immunofluorescence (Figure 5B).

## DISCUSSION

In this study, a direct, unbiased comparison of the different extracts of the fern *P. leucotomos* has revealed

important molecular information regarding the reported beneficial effects of these extracts. HPLC analysis revealed different amounts of aromatic acids bearing antioxidant properties. In general, the three formulations made from the leaves of *P. leucotomos* are richer in antioxidant aromatic acids than the extracts obtained using the rhizome. This is particularly noticeable in the case of 3,4-dihydroxybenzoic and 4-hydroxybenzoic acids, which are absent from two of the rhizome formulations and present in a very small amount in the third (Sample #4). This is consistent with observations made in other plant species, e.g. *Drynaria* ferns. The fronds of *Drynaria* are endowed with higher anti-oxidant potential and tyrosinase activity than the rhizomes (Tan and Lim, 2015). Likewise, the leaves of ferns of selected highland ferns of Malaysia contain 10 to 20 higher levels of anti-oxidant moieties (flavonoids, hydroxycinnamic acid and proanthocyanidin) than the corresponding rhizomes of the same plants (Chai et al., 2013).

It is interesting to note that the overall antioxidant capability of the extracts seemed to depend directly on the presence of ferulic acid. Indeed, the only rhizome extract with significant antioxidant activity in the ABTS and FRAP assay (Sample #4) is also the only containing a significant amount of ferulic acid (Figure 1E to G).

Although caffeic acid is a more potent radical scavenger than ferulic acid (Kikuzaki et al., 2002), the peak of ferulic acid is much higher in Sample #4 than in the other two rhizome extracts. In addition, the small amount of caffeic acid present in Sample #5 could explain its low but significant antioxidant potential (Figure 1F). However, this does not seem to be the major factor in determining cellular photoprotection. Samples #4 and #5 do not protect cells from UV irradiation despite the presence of caffeic acid in both and ferulic acid in the former.

Conversely, Sample #6 does confer significant photoprotection. This is likely due to the presence of unidentified moieties in this extract, peptidic or otherwise. As for the leave extracts, the most obvious differences are the significant presence of 3,4-dihydroxybenzoic and 4-hydroxybenzoic acids as well as 4-hydroxycinnamic acid.

The concurrence of these three moieties could underlie the strong photoprotection conferred by the aerial extracts, although these experiments do not rule out the contribution of additional moieties of different molecular families, peptides, proteins or aromatic compounds.

It is interesting to note that survival correlates well with markers of DNA damage only at extreme positivity. Indeed, the basic leave extract (sample #2, Fernblock®) is the most efficient photoprotector considering all the metrics used in this study (survival, H2AX phosphorylation and CPD appearance). However, the rest of the extracts offered mixed effects. The acidic- and neutral pH-extracts (Samples #1 and #3) were very similar in all the photoprotection metrics, suggesting that the important moieties are those detected at 290 nm, e.g.4-hydroxycinnamic acid (the acidic extraction method decreases the recovery of 3,4-dihydroxybenzoic and 4-hydroxybenzoic acids, compare Figure 1B and D). Conversely, Sample #4 offered the worst results in terms of H2AX phosphorylation and CPD appearance. Sample #5 was slightly worse than Sample #2 in terms of overall numbers of H2AX-positive cells, but better in terms of H2AX-bright cells and appearance of CPDs. Finally, Sample #6 was worse in terms of overall numbers of H2AX-positive cells and appearance of CPDs, but much better in terms of H2AX-bright cells. There is no easy explanation for these results. It could be speculated that additional moieties present in Samples #4 and #6 could provide a higher threshold degree of resistance, preventing extensive damage (hence lower H2AX-bright cells). Such a mechanism could be explained by the ability of small amounts of undetermined moieties that could scavenge higher energy UV photons, likely responsible for deep damage. However, their lower levels of characterized antioxidants would not be sufficient to prevent the damage caused by the avalanche of lower energy UV photons. In this regard, it is worth noting that the bulk of UV illumination used here is UVA (315 to 400 nm), which contains lower energy than UVB, which is also used.

In conclusion, this work reveals profound differences among different extracts of *P. leucotomos*, which is likely linked to the part of the plant, the extraction method, and perhaps the geographical origin and growing conditions of the plants. In general, leaf extracts are more potent and yield results endowed with better significance.

However, this study also indicates that additional moieties, antioxidant or not, may also play fundamental roles in the function of these extracts as dietary supplements with antioxidant and antiaging properties.

## ACKNOWLEDGEMENTS

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## ABBREVIATIONS

**UV,** Ultraviolet; **PLE,** *Polypodium leucotomos* extract; **CPD,** cyclobutane pyrimidine dimers; **TNF,** tumor newcrosis factor; **iNOS,** inducible nitric oxide synthase; **HPLC,** high performance liquid chromatography; **HAX,** histone 2AX.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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