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REVIEW

Current knowledge in *Polypodium leucotomos* effect on skin protection

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Abstract This article provides an overview of pharmacology, toxicity, pharmacokinetics and clinical data of *Polypodium leucotomos* L. (PL). PL aerial part has proven to exert antioxidant, photoprotective and immunomodulatory activities; its mechanism of action is complex and includes several activities: (1) PL diminishes the production of reactive oxygen and nitrogen species (ROS, RNS); (2) PL inhibits the photoisomerization of *trans*-urocanic acid (*t*-UCA); (3) PL inhibits apoptosis induced by ultraviolet radiation; (4) PL prevents damage to genetic material and (5) PL enhances DNA repair. PL is not mutagenic and does not induce acute or chronic toxicity. Its biological effects have been proved in cell cultures, animal models, murine models and in human beings. Photoprotective activity has been assessed in healthy volunteers as well as in patients suffering from several cutaneous diseases such as vitiligo, psoriasis, idiopathic photodermatosis or melasma. PL results to be an efficient treatment especially for sensitive cutaneous phototypes and adds extra protection when ultraviolet radiation (UVR) exposure cannot be avoided, such as wide or narrow band UVB phototherapy or treatment with psoralens plus UVA exposure radiation.

Keywords *Polypodium leucotomos* · Photoprotection · UV radiation · Pharmacodynamics · Clinical studies

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Introduction

Several chromophores normally present in the skin absorb ultraviolet radiation (UVR). These include melanin, DNA, RNA, proteins, lipids, aromatic aminoacids such as tyrosine or tryptophan, or *trans*-urocanic acid (*t*-UCA) [16]. As a result of their absorption, different photochemical reactions and secondary interactions take place that imply generation of reactive oxygen species (ROS) and result in cell damage.

DNA is the main target of UVR; pyrimidines suffer from photochemical modifications that lead to the formation of cyclobutane dimers, 8-deoxy-guanosine and other compounds that need to be repaired by specific cellular enzymatic systems [46]. Photoprotective effect towards this radiation damage on skin is an essential element in prophylaxis and therapy of skin diseases.

UVR is one of the main factors involved in skin cancer development [1, 9, 28, 29]. Moreover, continuous exposure of skin to sunlight induces other harmful effects such as sun-burn, immunosuppression, pigmentation changes or photoaging [10]. The mechanism that induces cutaneous damage is complex but falls under two main mechanisms: (1) an oxygen-independent direct damage mediated by photons absorption that is characteristic of UVB exposure radiation and (2) an oxidative damage caused by UVA exposure radiation mediated by free radicals and ROS [34].

The damage caused on skin by UVR is cumulative and may induce skin cancer. In this sense, the Cancer Council Victoria (Australia) made public the risks and benefits of solar exposure and recommended the need of reaching an appropriate and healthy balance that makes it necessary the use of antioxidant agents such as those that inhibit or scavenge the cutaneous damage induced by UVR [15, 48].

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Indigenous from Central and South America use the fern *Polypodium leucotomos* in traditional medicine for the relief of inflammatory diseases of the skin [22]. Oral administration of the hydrophilic extract obtained from aerial parts of PL decreases cutaneous sensitivity against UVR, prevents sun-burn, decreases immunosuppression induced by UVR, as well as mitochondrial and DNA damage and decreases tumour development [33, 34].

Preliminary studies in healthy patients showed that oral administration of PL decreases the cutaneous reaction induced by solar irradiation, together with cutaneous phototoxicity, erythema (MED-B) and persistent pigment darkening (MED-A) derived from UVB exposure radiation and UVA exposure radiation, respectively [1, 18, 19, 40, 47].

This review includes an updated overview on the knowledge on chemical composition, pharmacodynamics and clinical efficacy of PL. Molecular mechanisms that are implied in UVR—induced damage as well as the photoprotective effect of PL in cellular models, animal and murine models, and healthy humans—is analysed. The main therapeutic applications for oral use of PL such as vitiligo, psoriasis, idiopathic photodermatosis and melasma are included.

UVR effect on skin

Sun radiation and mainly UVR provokes several damaging effects on skin through induction of reactive oxygen species (ROS) and other active metabolites, inducing inflammation, DNA damage and immunosuppression (Table 1).

ROS generation

Skin exposure to UVR induces ROS that are physiologically eliminated by cellular antioxidant systems to prevent cell injury; a natural pro-oxidant/anti-oxidant balance thus exists to control these processes. Oxidant agents induce cellular membrane damage through peroxidation of the fatty acids present in it; this effect generates radicals such as lipid peroxide, hydroperoxide and other related products that not only keep but amplify oxidative damage. Chronic exposure to high UVR doses induces free radical accumulation that provokes deterioration of the cellular integrity and functionality; this consequently damages nuclear and mitochondrial DNA and can lead to extrinsic aging and tumour formation [46].

Inflammation

Erythema induced by UVR is due to an increase in blood flow and vasodilation, a process in which nitric oxide and prostaglandins play a crucial role. Other cells with an important role in skin homeostasis, such as Langerhans cells (LC) or mastocytes are also affected. UVR induces a selective recruitment of macrophages and neutrophils, ROS generation and cytokine secretion that contributes to the inflammatory process and cause tissue damage [19, 36, 39].

DNA damage

The heterocyclic bases in DNA chain are the main cromophores with a photon scavenger function in skin. UVB radiation absorption results in pyrimidine dimers formation (mainly thymine–thymine) and pyrimidine–pyrimidone photoproducts. UVA radiation induces formation of 8-deoxyguanosine and also induces common deletions in mitochondrial DNA. All these intermediate compounds act as early steps in mutagenesis and tumour formation. DNA damage is also related to systemic immunosuppression [27].

Immunosuppression

DNA damage is related to skin immunosuppression that is partially mediated by *cis*-urocanic acid (*c*-UCA), which is

Table 1 Harmful effects of UVR on skin

Mechanism	Responsible	Short-term effects	Long-term effects
Free radicals generation	ROS, RNS		Photoaging, cancer, immunosuppresion
Other damaging metabolites	UCA photoisomerization, *t*-decomposition		Photoaging, immunosuppresion
Inflammation	Cell death, apoptosis	Sun-burn eritema. Ache, itching	Photoaging, cancer
DNA damage	Pyrimidine dimers and pyrimidine-pyrimidona dimers		Tumorigenesis, cancer
Immunosuppression	Epidermal LCs depletion	Susceptibility to infections	Chronic inflammatory diseases, cancer

ROS reactive oxygen species, *RNS* reactive nitrogen species, *UCA* urocanic acid, *LCs* Langerhans cells

made by photoisomerization of *trans*-urocanic acid (*t*-UCA); *t*-UCA is obtained from hystidine deamination and exerts a natural photoprotective effect due to photons absorption. When large quantities of *c*-UCA are formed, it acts on epidermal LC and induces abnormal degranulation of mastocytes which are implied in immunomodulation [21, 28].

Ultraviolet radiation is known to induce a wide range of immunosuppressive responses that include the transition of competent LC to tolerogenic cells leading to a clonal anergy of Th1 cells [47].

In summary, effects of UVR on skin are not only acute effects such as sun-burn, inflammation and local and systemic immunosuppression, but also chronic or long-term effects such as hyperpigmentation, extrinsic aging and tumour development. These effects strongly support the need of photoprotective measures.

Polypodium leucotomos

Current development of photoprotective measures follows two main routes: (1) development of more efficient sunscreens that do not harm the environment, and (2) search of non-toxic substances that could fight UVR damaging effects [17, 26].

PL may be useful from both points of view: due to its chemical composition, it provides topical and systemic protection; it also inhibits some of the molecular mechanisms involved in cutaneous damage.

Chemical composition

The chemical composition of PL leaves include phenolic compounds, such as benzoates and cinnamates, together with quinic, shikimic, glucuronic and malic acids. 4-hydroxycinnamic acid (*p*-coumaric), 3-methoxy-4-hydroxycinnamic acid (ferulic), 3-hdihydroxycinnamic acid (caffeic), 3-methoxy-4-hydroxybenzoic acid (vanillic) and 3-caffeoilquinic acid (chlorogenic) are the most abundant among them [12]. These polyphenols are endowed with antiinflammatory, antimutagenic and antitumoral effects mainly due to their antioxidant ability [11, 50, 51]. Some of them, such as caffeic and ferulic acids bind a sugar moiety for increasing stability; both acids prevent peroxidation mediated by UVR by inhibiting the chain reaction of lipid peroxidation and scavenging nitrogen oxides [43]. Moreover, ferulic acid strongly absorbs UV-photons; together with caffeic acid, it protects human skin from UVB-induced erythema, which justifies their inclusion in the formulation of several topical solutions and sunscreens [44, 45].

Pharmacodynamics

The photoprotective effects of PL are well-documented in different in vivo and in vitro studies (Table 2).

Direct antioxidant activity

This activity is attributed to the polyphenolic compounds, mainly from the hydroxycinnamic acid family [12]. Polyphenols prevent peroxidation induced by UVR by inhibiting the lipidic peroxidation change reaction [43, 45]. Moreover, PL acts as a direct ROS scavenger against superoxide anion, hydroxyl, oxygen and oxygen peroxide [13].

The antioxidant capacity of PL isolated component increases in a concentration-dependent manner, ferulic and caffeic acid being the most powerful antioxidant when tested by the luminol/H_2O_2 assay [12]. The structural

Table 2 Beneficial effects of *Polypodium leucotomos* on skin

	Molecular effect	Short-term effect	Long-term effect
ROS and RNS generation	Inhibition Increase in antioxidant plasma capacity		Photoaging relief and tumour prevention
Other metabolites generation	Inhibits *t*-UCA photoismerisation and photodecomposition	Diminishes erythema and sun-burn	Photoaging relief and tumour prevention
Inflammation	Inhibits apoptosis and cell death	Reduces SOS response	
DNA damage	Pyrimidine dimers formation		
Immunosuppression	Prevents LCs depletion		
Proliferation	↓ proliferating cells ↑ p53 cells		Anticarcinogenic properties

↓ decrease

↑ increase

ROS reactive oxygen species, *RNS* reactive nitrogen species, *LCs* Langerhans cells, *SOS* SOS response is the depression of several genes that codify for proteins directed to DNA restoration

characteristics of these compounds are associated to their antioxidant capacities, the incorporation of a hydroxyl group at the *para*-position of a monophenol enhancing the antioxidant ability. Vanillic acid shows the weakest activity, probably due to the lack of a–CH = CH–COOH chain, which acts as an electron donor; the stabilisation of the resulting radical might be increased by the electron delocalization following hydrogen donation by the hydroxyl group. Meanwhile, the glycosylation of the carboxylate group does not influence the antioxidant potential of the molecule [12].

Also the antioxidant ability of PL is proven through the reduction of the oxidative damage on macromolecules such as lipid peroxidation in non-irradiated and irradiated fibroblasts and keratinocytes. This effect indicates a protective effect on cell membrane [37].

In vivo tests show that PL inhibits glutathione peroxidation and prevents LC depletion induced by UVR in a nude rat model [36]. One study describes the effect of the oral administration of a hydrophilic extract of PL on epidermal antioxidant levels in nude rats. Irradiation induces an increase in GST and CAT enzymes activities in erythrocytes (14 and 25 %, respectively), with a decrease in the plasmatic levels of GSH about 25 %. Pre-treatment with PL decreases in an effective manner glutathione oxidation in epidermis and peripheral tissues (erythrocytes, plasma) with an increase in GSH/GSSG ratio. These changes in oxidative damage could reflect that activation of antioxidant systems and increased expenditure of GSH are two main actions to neutralize ROS increase induced by radiation. De novo synthesis is ruled out as erythrocytes are anucleated cells; therefore, the observed changes are due to catabolic changes of these implied enzymatic systems. On the contrary, the most evident effect of UVR on epidermis is a consistent depletion of LCs, this effect being the main cause of UV-dependent immunosuppression [36].

Another study confirms the antioxidant and scavenging properties of PL in hairless mice [42] after oral administration of a hydrophilic extract of the fern *Polypodium leucotomos* (300 mg/kg during 5 days before UVR and for two additional days after irradiation). This study found a reduction in the number of proliferating cells by 13 %, an increase in the number of p53 cells and antioxidant plasma capacity (63 and 30 %, respectively) and reinforcement in the network of dermal elastic fibres. The study revealed no significant changes to endogenous antioxidant systems (antioxidant-related enzymes) indicating that the beneficial effect of PL is probably due to its antioxidant and anti-ROS properties.

In vitro studies on the photoprotective effect of PL showed that PL efficiently blocked cellular death induced by UVA-R in fibroblasts and keratinocytes and restored their proliferation. At cellular level, PL inhibited disorganisation of the actin cytoskeleton and cell–cell adhesive contacts and cell matrix induced by UVR [2]. PL also inhibited proteases secretion induced by UVR and improved membrane integrity of irradiated cells [37].

Inhibition of photoisomerization and photodecomposition of t-UCA

As outlined the previous section, *t*-UCA is the main product from histidine metabolism. It possesses photoprotective and ROS scavenging properties. Photons absorption induces isomerization to *c*-UCA as the way to avoid damage to the other cellular structures. PL inhibits *t*-UCA photoisomerisation resulting in a dose-dependent decrease in the levels of *c*-UCA in the presence of hydrogen peroxide. Moreover, when ROS and a catalyzer such as TiO_2 are present, UV photons lead to the formation of hydroxyl and other oxygen radicals. They are responsible for the inactivation of several enzymatic systems that are required for skin homeostasis. PL inhibits *t*-UCA degradation and improves peroxidase inactivation when incubated together with TiO_2 under UVR. These results assess PL efficacy as a *t*-UCA photoisomerization inhibitor, and also suggest its use to prevent the generation of oxidative metabolites catalyzed by TiO2 [7].

Molecular immunoregulation

PL extract inhibits TNF-alpha and nitric oxide synthase (iNOS) expression, together with transcriptional activation of nuclear factors, apoptosis and MMPs production induced by sun radiation. Pretreatment of human keratinocytes with PL followed by solar simulated radiation (SSR) inhibits the increase of TNF-α induced by SSR and decreases nitric oxide (NO) production. PL also blocks the induction of iNOS and peroxinitrites related to NO degradation. In relation with the main inflammatory ways of signal transduction, PL inhibits the transcriptional activation of nuclear factors NF-κB and AP1 [24]. These effects are related to an increase in cell survival and a concomitant decrease in apoptosis. Therefore, PL modulates the production of inflammatory mediators and regulates the signaling pathways implicated in inflammation.

UVR exposure increases deposition of elastotic material and matrix metalloproteinase expression (MMPs) and it decreases collagen levels. A study in cell culture was carried on with the irradiation of UVA (0.6, 1.8 or 3.61 J) or UVB (0.75, 2.5 or 7.5 mJ) followed by an incubation period with or without PL (0.01, 0.1 and 1 %) in order to assess its effects on membrane integrity, lipid peroxidation and expression of elastin and MMP-1 in fibroblasts and keratinocytes [37]. UVR did not significantly alter MMP-1 expression but did increase elastin expression in

fibroblasts. PL effects were more prominent in the presence of UVA or UVB for both cell types. Results show that even at low concentrations (<0.1 %), PL is beneficial for the prevention of photoaging and improves the membrane integrity; higher concentrations (>0.1 %) could revert the normal elastic fibre loss associated to intrinsic aging.

Cell immunoregulation

PL also modulates inflammatory activity by acting on the Th1/Th2 response. PL blocks some of the harmful effects of UV radiation on skin, such as LCs depletion, direct apoptosis, inflammation, inhibition of the expression of adhesive molecules needed for the migration of LCs towards skin [39, 41]. PL not only prevents LCs depletion but also inhibits changes in their morphology, maintaining their functionality [35]. PL induces the expression of cellular mediators such as IL-10, IL-12 and TGF-β, which regulates other cellular responses such as macrophages. Gonzalez et al. [14] demonstrated that PL extract partially inhibits cytokine production in lymphocytes with a Th1 pattern (IL-2, IFN-γ and -α) in PHA-stimulated peripheral human nuclear cells. The levels observed were 24 % for IL-2, 72 % for IFN-γ and 53 % for TNF-α. In relation to Th2 cytokines, the authors found that PL significantly increased (33 %) IL-10 production and completely inhibited IL-6 production, the latter being a pro-inflammatory cytokine. PL preserves the skin immune system (morphology, number and functionality of Langerhans cells) and thus exerts a protective role against infective diseases and tumour development.

PL also exerts regulation on different molecules and cells which are implied in the inflammatory immune system. Topical application of PL on the skin of healthy albine hairless mice (Skh-1) significantly decreases mastocyte infiltration and the number of vascular vessels induced by UVR. In an oxazolone hypersensitive murine model, PL also exerted an immunoprotective effect [49].

Taken all together, these results show a multifactorial protective mechanism that is based not only on the antioxidant ability of PL; it induces suppressive/anti-inflammatory cytokines such as IL-10 or TGF-β; it moderately inhibits Th1 responses, which underlines the observed immunoregulating, anti-inflammatory and antioxidant activities. The marked inhibitory effect on TNF-α and IL-6 production may be responsible of in vivo angiogenesis inhibition and the prevention of LCs depletion caused by sun radiation in humans.

PL significantly inhibits the contact hypersensitivity response; therefore, it prevents immunosuppression caused by UVB radiation in mice (local and systemic effects); this activity could explain, at least in part, the decrease in the skin cancer development induced by UVBR in mice. PL preserves the skin immune system and its functionality, which leads to improve skin protection against viral infections and tumour development.

Pharmacokinetics

Doses-response and pharmacokinetic studies reveal that the phenolic compounds from PL are rapidly absorbed and undergo complete metabolism 24 h after oral administration; absorption is highly efficient (70–100 %) [12]. Coumaric, phenolic and vanillic acids are metabolised by monooxygenase enzymes dependent on cytochrome P450 (CYP450) in the liver, suffering partial conjugation to glucuronic sulphate and glucuronic acid (plasmatic half-life, 4–6 h) [53]. These conjugates are present in plasma and demonstrate the metabolism and absorption of phenolics through intestinal barrier [3].

An in vitro study addresses the intestinal absorption and metabolism of the main phenolics in PL extract [12]: *p*-coumaric acid (4-hydroxycinnamic), ferulic acid (3-methoxy-4-hydroxycinnamic), caffeic acid (3,4-dihydroxycinnamic), vanillic acid (3-methoxy-4-hydroxybenzoic) and chlorogenic acid (3-caffeoil-quinic). Cell lines from human colorectal cancer line (Caco-2) were used; rat hepatocytes were extracted from Sprague–Dawley rats and were seeded and then exposed to different concentrations of *p*-coumaric, ferulic, caffeic, vanillic and chlorogenic acids (50 μM, 200 μM). Aliquot samples were taken along 8 h for HPLC analysis. Results show a high absorption percentage, between 70 and 100 % of apparent permeability. Every phenolic acid is stable in the culture conditions assayed and is metabolized at hepatic level: coumaric, ferulic, and vanillic acids are conjugated to glucuronic acid and sulphate (35, 50 and 10 %, respectively). Plasmatic esterases do not participate in this metabolism. No binding to cellular proteins is observed [3].

Topical use of PL also shows favourable effects [19].

Clinical studies

First studies on photoprotection were carried on experimental animals (hamsters) with exposure to UVB radiation. Animals which had been topically treated with a PL extract achieved a nearly complete photoprotection. These results led to the first clinical studies which also proved PL efficacy on human volunteers exposed to limited UVA radiation [20].

Clinical studies performed with PL may be classified according to the target population. (a) healthy subjects; (b) vitiligo patients; (c) psoriasis patients; (d) idiopathic photodermatosis patients; (e) melasma patients and (f) other studies. Table 3 shows a summary of the clinical studies performed with *Polypodium leucotomos* and their main outcomes.

Table 3 Main outcomes of the clinical studies carried out with *Polypodium leucotomos*

Indication	Mechanism of action	Dose		Reference
Photoprotection	Antioxidant, immunoprotective	480 mg/day		Middelkamp-Hup et al. 2004a, 2004b [33, 34] Gonzalez et al. 2011 [17]
Photoaging and skin cancer	Antioxidant, immunoprotective. Reduction in chronic elastosis and expression of metalloproteinases from the matrix	480 mg/day		Philips et al. 2003 [37] Gonzalez et al. 2011 [17]
Vitiligo	Photoprotection: adjuvant therapy to PUVA	720 mg/day		Reyes et al. 2006 [40]
	Photoprotection: adjuvant therapy to NB-UVB	250 mg three times daily		Middelkamp-Hup et al. 2007 [32]
Polymorphous light eruption	Antioxidant, immunoprotective	During phototest: ≤55 kg body weight: 720 mg/day 56–70 kg body weight: 960 mg/day >70 kg body weight: 1200 mg/day Maintenance 7.5 mg/kg body weight/day or 480 mg/day	during summer months	Caccialanza et al. 2007 [5] Caccialanza et al. 2011 [6] Tanew et al. 2012 [52]
Melasma	Photoprotection: adjuvant therapy to sun protection	240 mg twice daily	3 months	Martin et al. 2012 [abstract, unpublished]
Atopic dermatosis	Antioxidant, immunoprotective	age < 6 years: 240 mg/day 6–12 years: 360 mg/day age > 12 years: 480 mg/day		Ramírez-Bosca et al. 2012 [38]
Lupus erythematosus	Antioxidant, immunoprotective	240 mg/day		Breithaupt et al. 2012 [4]

The first clinical study with PL tried to prove its potential antitumoral activity [23], although the mechanism of action was not deeply investigated. Later on, studies focused on anti-inflammatory activity, mainly related to skin diseases such as psoriasis [32], vitiligo [35] and atopic dermatitis [25]. The molecular basis of these effects is, at least in part, the immunomodulating ability of PL.

PL effect on healthy subjects

PL significantly decreases acute phototoxicity due to UVR exposition after psoralens ingestion [8]. This effect was shown in an open study carried out to determine the beneficial effect of PL co-administration in patients receiving psoralens-UVA-R therapy [8] which is recommended for the treatment of several skin disorders such as psoriasis. Treatment with psoralens plus UVA-R is effective in psoriasis management but it could promote skin tumours development, especially in skin phototypes I and II [30]. Oral PL administration diminished sun burning and mastocytes infiltration in skin and so decreased loss of LCs [33].

Clinical observations with PL are related to its histological effects on skin. PL decreases histological damage induced by UVR characterized by profound changes to keratinocytes maturation, microvesicle formation and vacuolization. When compared to control (UV-radiated skin), skin that had been previously treated with PL showed a less quantity in sunburn cells, decreased levels of pyrimidine cyclobutane dimers (an indicator of DNA induced damage) and epidermal cellular proliferation, together with a decrease in mastocyte and neutrophil infiltration [9].

A clinical study was designed to in vivo evaluate the usefulness of the photoprotective effect of a PL extract after oral or topical use. This study took into account all the previous pharmacological studies on PL immunomodulating and antioxidant properties, together with its photoprotective effect on vitiligo treatment [18]. 21 healthy volunteers with cutaneous phototypes III and IV were included in a randomised clinical trial. The following clinical parameters were evaluated, before and after topical and oral PL administration: immediate pigment darkening (IPD), minimal erythema dose (MED), minimal melanogenic dose (MMD), and minimal phototoxic dose (MPD). Immunohistochemistry was also performed. PL-treated groups received a total dose of 1,080 mg of dry standardised extract (Difur, IFC): 240 mg, three times daily during the day before the study, followed by a 360 mg dose on the day of the study. The obtained results proved the photoprotective effect of PL after topical or oral use: the treatment induced an increase in the UVR dose necessary for IPD ($p < 0.01$), MED ($p < 0.001$) and MPD ($p < 0.002$) was observed. Following oral administration of PL, MED increased up to 2.8 ± 0.59 folds whereas MPD increased up to 2.75 ± 0.5 and 6.8 ± 1.3 folds, depending on the type of psoralen applied. Moreover, immunohistochemical examination demonstrated the protection of PL treatment (oral or topical) on LCs.

Another study [33] proved the protective effect of the oral administration of PL on UVR-induced damage. The aim of this study was to evaluate the photoprotective effect of orally administered PL extract when artificial UVR was applied. Nine healthy volunteers with II and III skin phototypes were exposed to variable doses of UVR following oral administration of PL (7.5 mg/kg body weight) or placebo in an open study. Twenty-four hours after UVR exposure, the erythematogenic response was measured and paired samples from treated and non-treated skin were taken. Results show a significant decrease in erythema in treated skin ($p < 0.001$). Histological results show a lower quantity of burn cells in PL-treated skin ($p < 0.05$), cyclobutanopyrimidine dimers ($p < 0.001$), proliferative epidermal cells ($p < 0.001$) and mastocyte infiltration ($p < 0.05$). The study also showed a non-significant trend to preserve LCs. All the above results demonstrate the efficacy of the oral administration of PL as a systemic chemopreventive agent that exerts a significant protection on UV radiated skin.

The same authors carried out another study with healthy volunteers with skin phototypes II and III to evaluate the effect of the oral administration of PL extract on the psoralen-UVA (PUVA)-induced toxicity in human skin [34]. The application of PUVA in patients with light skin phototype is limited by the adverse effects such as acute phototoxicity and possible long-term carcinogenesis. In this study, 10 volunteers received PUVA alone (oral administration of 8-methoxypsoralen, 0.6 mg/kg) or PUVA plus PL (7.5 mg PL extract/Kg body weight, per os). After 48 and 72 h, phototoxicity was decreased in subjects receiving PL ($p < 0.005$) with a decrease in pigmentation after 4 months. Histological analysis revealed a significant reduction in sun-burn cells ($p = 0.05$), LCs preservation ($p \leq 0.01$), decrease in the infiltration of tryptase-positive mastocytes ($p < 0.05$) and decrease in vasodilation ($p \leq 0.01$). The study also showed no significant differences in Ki-67 proliferative cells. These results prove that PL acts as an efficient chemophotoprotective agent against acute skin phototoxicity induced by PUVA and is endowed with important benefits in skin protection, as proved by histological data.

These results demonstrate the photoprotective role of PL after topical and oral administration.

Vitiligo patients

The effect of concomitant treatment with PL and narrow band UVBR on skin repigmentation in patients suffering

from vitiligo vulgaris was studied [32]. 50 patients were recruited for a randomised, double blind, placebo-controlled trial. The treated group received 750 mg standardised PL extract for 25–26 weeks (250 mg, three times daily). After the treatment period, repigmentation had improved in head and neck areas (44 % versus 27 %, $p = 0.06$); no significant differences were found in the trunk (6 % increase), extremities (4 %), feet and hands (5 %). Patients who attended 80 % or more of the narrow band UVB sessions showed an increased repigmentation in the head and trunk in the treated group (50 % *versus* 19 %, $p < 0.002$); the researchers found no significant differences within other body areas. Patients with skin phototypes II and III in the PL-treated group showed a better repigmentation in head and neck (47 % versus 21 %, $p = 0.01$). This study concludes that there exists a marked tendency to repigmentation in vitiligo vulgaris patients when oral PL is associated to narrow band UVB therapy, mainly in head and neck areas, this effect being more pronounced in light skin phototypes.

A pilot study was designed to detect the immunomodulatory systemic effects of PL as an adjuvant therapy of PUVA in vitiligo treatment [40]. 19 patients were included in a randomised, double-blind, placebo-controlled trial. Blood samples were collected a t_0 (before the first PUVA session) and $t_{12\ weeks}$ (before the last PUVA session). Response to repigmentation was assessed by independent dermatologists after 12 weeks and was classified as none or minimal (<25 %), moderate (25–50 %) and moderate to excellent (>50 %). Studies on isolation of peripheral mononuclear cells, immunofluorescence response and proliferation were also performed.

Clinical evaluation showed a significant better repigmentation for PL-treated group (720 mg/day plus another 720 mg dose 1 h before irradiation) than placebo. A significant correlation was observed between the clinical response and the drop on mononuclear cells percentage (correlation coefficient = −0.493).

Psoriasis

Combined psoralen with UVAR therapy is effective for psoriasis treatment, but its use is limited because of the possibility of increasing skin cancer risk, especially in light skin phototypes (I and II) with a more marked tendency to redness, irritation and photoaging [31]. Moreover, this treatment may induce hyperpigmentation that makes it necessary to increase UVAR dose and thus its toxicity.

Idiopatic photodermatosis

Idiopatic photodermatosis (IP) are abnormal skin reactions to ordinary light exposure and constitute a group of photosensitive skin diseases which cause is unknown. The pharmacological treatment includes therapy for the acute phase and prophylactic therapy to prevent symptoms before exposure to UVR.

The study conducted by Caccialanza et al. [5] included 28 patients diagnosed with IP (26 with polymorphic light eruption and 2 with solar urticaria) who had not experienced any benefit from habitual treatment. Patients were advised to diminish solar exposition during summer time and received 480 mg PL extract (7.5 mg/kg body weight/day divided in two doses) since day 15 before solar exposition along the whole exposition. Clinical outcomes were evaluated according to normalisation (absence of skin reactions and subjective symptoms), improvement (light skin and subjective improvement) or no improvement (no changes in any parameter).

25 within the 28 recruited patients were eligible. 80 % of them ($n = 20$) found PL treatment as beneficial. The improvement in punctuation after PL administration was significant ($p < 0.05$). Results revealed that 49 % of patients improved and 31 % were back to normal levels after photoexposition with no adverse effects.

A recent study performed by the same authors [6] included 57 patients affected by IP (53 with polymorphic light eruption and 4 with solar urticaria). Patients received a normal sunlight exposition while taking 480 mg of PL extract (7.5 mg/kg body weight/day divided in two doses) starting 15 days before solar exposure and continuing throughout the period of exposure. A statistical evaluation of the basal clinical conditions compared to those after sunlight exposure was performed (normalisation, clear improvement, slight improvement or no improvement).

Results showed that 73.68 % of the patients obtained benefit from PL treatment during sunlight exposure (43.86 % had an improvement and 29.82 % were completely normal), with a significant reduction of skin reaction and subjective symptoms ($p < 0.005$). No adverse effects were observed. Authors concluded that the multifactorial protection exerted by PL, together with its lack of toxicity makes it an effective photoprotective treatment for IP.

A more recent study [52] evaluated the effect of a concentrated hydrophilic extract of PL (Fernblock, IFC, Madrid, Spain) on the prevention or delay of the photoinduction of polymorphic light eruption (PLE) lesions by artificial UVR. 35 patients with long-standing PLE were recruited for this open, non-controlled bicenter study; they were induced PLE by artificial UV and UVA light and all patients with positive phototest reaction received a daily dose of PL extract according to body weight (720 mg PL for body weight ≤55 kg; 960 mg for 56–70 kg; 1,200 mg/day for ≥70 kg). After 2 weeks of daily treatment with PL extract, a second photoprovocation was performed. After

the summer time, a follow-up assessment recorded the efficacy of PL extract in protecting from PLE episodes.

30 patients showed typical PLE lesions after repeated irradiation with UVB or UVA exposure radiations; all of them were sensitive to UVA exposure radiation. 18 patients also reacted to UVB exposure radiation. After two weeks of continuous PL treatment, a significant increase in the threshold for induction of PLE lesions by UVA was observed (negative phototest for 30 % of all UVA-sensitive patients); the remaining 21 patients increased the mean number of UVA exposures required to induce PLE (from 1.95 ± 1.07 to 2.62 ± 2.922, $p = 0.047$). Again, tolerance to treatment was excellent and no adverse events were recorded.

The authors concluded that oral PL treatment may be beneficial in the prevention of PLE in severely affected patients, although studies on treatment duration and the most effective dose are to be conducted.

Others

Melasma Melasma is defined as an acquired hyperpigmentation appearing mainly in the forehead and cheeks areas with significant impact on health-related quality of life.

A recent randomised double-blind placebo controlled study evaluated the effectiveness and tolerability of oral PL in patients with melasma [Martin LK, Caperton C, Woolery-Lloyd H, Avashia N. (2012). A randomised double-blind placebo controlled study evaluating the effectiveness and tolerability of oral *Polypodium leucotomos* in patients with melasma. In: Poster abstracts of the 70th Annual Meeting of the American Academy of Dermatology; March 16–20; San Diego, California: JAAD; 2012. Vol 66(4). Abstract nr AB21]. Patient improvement and tolerability was measured through the melasma quality of life scale (MELASQOL), which had been developed as valid instrument to evaluate the response to melasma treatment, regarding both clinical severity and quality of life. In this study, twenty-one healthy female subjects aged 18–50 years with epidermal melasma were randomised to receive oral PL or placebo twice daily plus sunscreen SPF 45 for 12 weeks. After treatment period, no significant differences in compliance or adverse effects between both groups were found. At the 12 week follow-up, the PL group had significantly improved mean Melasma Area and Severity Index (MASI) scores (5.7–3.3; $p < 0.05$), while the placebo group did not (4.7–5.7; $p < 0.05$). Photographic assessment revealed mild improvement in 43 % of subjects receiving PL compared to 17 % of placebo, and marked improvement in 14 % of subjects receiving PL compared to 0 % of placebo. Patient self-assessment indicated a 50 % of PL subjects reporting mild improvement and 13 % reporting marked improvement, compared with 17 % and none of placebo subjects, respectively. Some MELASQOL parameters worsened in 50 % of placebo compared to 20 % of PL subjects, while parameters improved in 59 % of PL subjects and only 27 % of placebo ($p < 0.05$).

In conclusion, PL extract is well-tolerated and effective in patients with melasma and may be a useful adjunctive topical or systemic treatment to prevent further hyperpigmentation disorders which can be exacerbated by UV light.

Atopic dermatosis Atopic dermatosis is a chronic inflammatory disease from skin which is characterized by pruritus, dry skin, inflammation and exudate. It is frequently associated to asthma, allergic rhinitis, food allergy and secondary infections in skin. Pharmacological treatment focuses on the reduction of pruritus, control of inflammation and prevention of new episodes.

A clinical trial has recently studied the effect of a PL extract on patients with atopic dermatosis [38]. It is a well-designed study (multicenter, double-blind, randomised versus placebo) with 105 patients aged between 2 and 17 years and diagnosed with atopic dermatosis of moderate intensity who were treated with topical corticosteroids. Treatment duration was 6 months (240–480 mg/day, orally, depending on patients' age); the use of topical corticosteroids during that period was evaluated. At the end of the treatment period, the group that received PL showed a no significant decrease in the use of topical corticosteroids, together with a significant decrease in the use of oral antihistaminic drugs. These results show the beneficial effect of PL on paediatric treatment of atopic dermatitis by reducing the inflammatory lesion and itching.

Conclusions

In this work, the evidence of the beneficial effect of *Polypodium leucotomos* fern in the prevention and treatment of skin diseases induced by UV radiation exposition is shown. Its beneficial effects are assessed through several pharmacological and clinical studies and rely on three main different and complementary mechanisms of action: direct antioxidant activity, inhibition of photoisomerization and photodecomposition of *t*-UCA and immunomodulation. These activities make *Polypodium leucotomos* an effective and safe treatment for those patients who need an efficient protection against ultraviolet radiation when sun exposition or phototherapy is required.

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References

1. Aguilera P, Carrera C, Puig-Butille JA, Badenas C, Lecha M, Gonzalez S, Malvehy J, Puig S (2013) Benefits of oral *Polypodium leucotomos* extract in MM high-risk patients. J Eur Acad Dermatol Venereol 27(9):1095–1100
2. Alonso-Lebrero JL, Domínguez-Jiménez C, Tejedor R, Brieva A, Pivel JP (2003) Photoprotective properties of a hydrophilic extract of the fern *Polypodium leucotomos* on human skin cells. J Photochem Photobiol B 70:31–37
3. Azuma K, Ippoushi K, Nakayama M, Ito H, Higashio H, Terao J (2000) Absorption of chlorogenic acid and caffeic acid in rats after oral administration. J Agric Food Chem 48:5496–5500
4. Breithaupt AD, Jacob SE (2012) Subacute Cutaneous Lupus Erythematosus: a case report of *Polypodium leucotomos* as an adjuvant therapy. Cutis 89:183–184
5. Caccialanza M, Percivalle S, Piccinno R, Brambilla R (2007) Photoprotective activity of oral *Polypodium leucotomos* extract in 25 patients with idiopathic photodermatoses. Photodermatol Photoimmunol Photomed 23(1):46–47
6. Caccialanza M, Recalti S, Piccinno R (2011) Oral *Polypodium leucotomos* extract photoprotective activity in 57 patients with idiopathic photodermatoses. G Ital Dermatol Venereol 146:85–87
7. Capote R, Alonso-Lebrero J, García F, Brieva A, Pivel JP, Gonzalez S (2006) *Polypodium leuctomos* extract inhibits trans-urocanic acid photoisomerization and photodecomposition. J Phytochem Photobiol 82:173–179
8. De las Heras ME, Ledo E, Gonzalez S, Rocamora A, Ledo A (1997) An extract of *Polypodium leucotomos* (Difur) as an a-dyuvant to PUVA therapy. Med Cutan Iber 25:103–107
9. Dummer R, Maier T (2002) UV protection and skin cancer. Recent Results Canc Res 160:7–12
10. Enta T, Kwan TY (1998) Melanocytic nevi in sun-protected Canadian Hutterite children. Arch Dermatol 134:379–381
11. Garcia F, Pivel JP, Guerrero A et al (2006) Phenolic components and antioxidant activity of Fernblock, an aqueous extract of the aerial parts of the fern *Polypodium leucotomos*. Methods Find Exp Clin Pharmacol 28:157–160
12. Gombau L, García F, Lahoz A, Fabre M, Roda-Navarro P, Majano P, Alonso-Lebrero JL, Pivel JP, Castell JV, Gómez-Lechon MJ, Gonzalez S (2006) *Polypodium leucotomos* extract: antioxidant activity and disposition. Toxicol In Vitro 20:464–471
13. Gomes AJ, Lunardi CN, Gonzalez S, Tudesco AC (2001) The antioxidant action of *Polypodium leucotomos* extract and kojic acid: reactions with reactive oxygen species. Braz J Med Biol Res 34:1487–1494
14. Gonzalez S, Alcaraz MV, Cuevas J, Pérez M, Jaén P, Alvarez-Mon M, Villarrubia VG (2000) An extract of the fern *Polypodium leucotomos* (Difur®) modulates Th1/Th2 cytokines balance in vitro and appears to exhibit anti-angiogenic activities in vivo: pathogenic relationships and therapeutic indications. Anticancer Res 20:1567–1576
15. Gonzalez S, Alonso-Lebrero JL, Del Río R, Jaén P (2007) *Polypodium leucotomos* extract: a nutraceutical with photoprotective properties. Drugs Today 43(7):475–485
16. Gonzalez S, Fernández-Lorente M, Gilaberte-Calzada Y (2008) The latest on skin photoprotection. Clin Dermatol 26(6):614–626
17. Gonzalez S, Gilaberte Y, Philips N, Juarranz A (2011) Current Trends in Photoprotection – A new generation of oral photoprotectors. Open Dermatol J 5:1–9
18. Gonzalez S, Joshi PC, Pathak MA (1994) *Polypodium leucotomos* extract as an antioxidant agent in the therapy of skin disorders. J Invest Dermatol 102(4):651–659
19. Gonzalez S, Pathak MA, Cuevas J, Villarubia VG, Fitzpatrick TB (1997) Topical or oral administration with an extract of *Polypodium leucotomos* prevents acute sunburn and psolaren-induced phototoxic reactions as well as depletion of Langerhans cells in human skin. Photodermatol Photoimmunol Photomed 13:50–60
20. Gonzalez S, Pathak MA (1996) Inhibition of ultraviolet-induced formation of reactive oxygen species, lipid peroxidation, erythema and skin photosensitization by *Polypodium leucotomos*. Photodermatol Photoimmunol Photomed 12:45–56
21. Hart PH, Grimbaldeston MA, Swift GJ et al (1999) A critical role for dermal mast cells in cis-urocanic acid-induced systemic suppression of contact hypersensitivity responses in mice. Photochem Photobiol 70:807–812
22. Hassler M, Swale B. (2001). Checklist of ferns and fern allies. Available at http://homepages.caverock.net.nz/~bj/fern/phlebodium.htm. Accessed 14 Jan 2014
23. Horvath A, Alvarado F, Szocs J, de Alvardo ZA, Padilla G (1967) Metabolic effects of calagualine, an antitumoral saponin of *Polypodium leucotomos*. Nature 214:1256–1258
24. Janczyk A, García-López MA, Fernández-Peñas P, Alonso-Lebrero JL, Benedicto I, López-Cabrera M, Gonzalez S (2007) A *Polypodium leucotomos* extract inhibits solar-simulated radiation-induced TF-α and iNOS expression, transcriptional activation and apoptosis. Experimental Dermatol 16:823–829
25. Jiménez D, Naranjo R, Doblare E, Munoz C, Vargas JF (1987) Anapsos, an antipsoriatic drug in atopic dermatitis. Allergol Immunopathol (Madr) 15:185–189
26. Konda S, Geria AN, Halder RM (2012) New horizons in treating disorders of hyperpigmentation in skin of color. Semin Cutan Med Surg 31:133–139
27. Kripke ML, Cox PA, Alas LG, Yarosh DB (1992) Pyrimidine dimers in DNA initiate systemic immunosupression in UV-irradiated mice. Proc Natl Acad Sci USA 89:7516–7520
28. Kurimoto I, Streilein JW (1992) Deleterious effects of cis-urocanic acid and UVB radiation on Langerhans cells and on induction of contact hypersensitivity are mediated by tumor necrosis factor-alpha. J Invest Dermatol 99:69S–70S
29. Leffell DJ (2000) The scientific basis of skin cancer. J Am Acad Dermatol 42(1 Pt 2):18–22
30. Melski JW, Tanenbaum L, Parrish JA, Fitzpatrick TB, Bleich HL (1977) Oral methoxsalen photochemotherapy for the treatment of psoriasis: a cooperative clinical trial. J Invest Dermatol 68:328–335
31. Mercadal Peyri O, Maesci Cappitanio F (1981) Preliminary communication on the treatment of psoriasis with anapsos. Actas Dermosifiliogr 72:65–68
32. Middelkamp-Hup MA, Bos JD, Rius-Diaz F, Gonzalez S, Westerhof W (2007) Treatment of vitiligo vulgaris with narrow-band UVB and oral *Polypodium leucotomos* extract: a randomized double-blind placebo-controlled study. J Eur Acad Dermatol Venereol 21:942–950
33. Middelkamp-Hup MA, Pathak MA, Parrado C, Garcia-Caballero T, Rius-Diaz F, Fitzpatrick TB, Gonzalez S (2004) Orally administered *Polypodium leucotomos* extract decreases psoralen-UVA-induced phototoxicity, pigmentation, and damage of human skin. J Am Acad Dermatol 50:41–49
34. Middelkamp-Hup MA, Pathak MA, Parrado C, Goukassian D, Rius-Diaz F, Mihm MC, Fitzpatrick TB, Gonzalez S (2004) Oral *Polypodium leucotomos* extract decreases ultraviolet-induced damage of human skin. J Am Acad Dermatol 51(6):910–918
35. Mohammad A (1989) Vitiligo repigmentation with Anapsos (*Polypodium leucotomos*). Int J Dermatol 28(7):479
36. Mulero M, Rodríguez-Yanes E, Nogués MR, Giralt M, Romeu M, Gonzalez S, Mallo J (2008) *Polypodium leucotomos* extract inhibits glutathione oxidation and prevents Langerhans cell depletion induced by UVB/UVA radiation in a hairless rat model. Exp Dermatol 17:653–658

37. Philips N, Smith J, Séller T, Gonzalez S (2003) Predominant effect of *Polypodium leucotomos* on membrane integrity, lipid peroxidation expression of elastin and matrixmetalloproteinase-1 in ultraviolet radiation exposed fibroblasts, and keratinocytes. J Dermatol Sci 32:1–9
38. Ramírez-Bosca A, Zapater P, Betlloch I, Albero F, Martinez A, Díaz-Alperi J, Horga JF (2012) Extracto de *Polypodium leucotomos* en dermatitis atópica: ensayo multicéntrico, aleatorizado, doble ciego y controlado con placebo. Actas Dermosifilogr 103:599–607
39. Rattis FM, Concha M, Dalbiez-Gauthier C, Courtellmont P, Scmitt D, Peguet-Navarrol J (1998) Effects of UVB radiation on human Langerhans cells: functional alteration of CD86 upregulation and induction of apoptotic cell death. J Invest Dermatol 111:373–379
40. Reyes E, Vitale MA. (2013) Advances in photoprotection. Molecular mechanisms. Piel 28(4): 235–247. Available at http://dx.doi.org/10.1016/j.piel.2012.04.011
41. Richters CD, Reits EA, Van Pelt AM, Hoekstra MJ, Van Baare J, Du Pont JS, Kamperdijk MJ (1996) Effect of low dose UVB radiation on the migratory properties and functional capacities of human skin dendritic cells. Clin Exp Immunol 1004:191–197
42. Rodríguez-Yanes E, Juarranz A, Cuevas J, Gonzalez S, Mallo J (2012) *Polypodium leucotomos* decreases UV-induced epidermal cell proliferation and enhances p53 expression and plasma antioxidant capacity in hairless mice. Exp Dermatol 21:630–642
43. Romeu M, Mulero M, Giralt M, Folch J, Nogués MR, Torres A, Sureda FX, Cabré M, Paternáin JL, Mallo J (2002) Parameters related to oxygen free radicals in erythrocytes, plasma and epidermis of the hairless rat. Life Sci 71:1739–1749
44. Saija A, Tomaino A, Lo Cascio R et al (1999) Ferulic and caffeic acids as potencial protective agents against photooxidative skin damage. J Sci Food Agric 79:476–480
45. Saija A, Tomaino A, Trombetta D et al (2000) In vitro e in vivo evaluation of caffeic and ferulic acids as topical photoprotective agents. Int J Pharm 199:39–47
46. Scharffetter-Kochanek K, Wlaschek M, Brenneisen P, Schauen M, Blaudschun R, Wenk J (1997) UV-induced reactive oxygen species in photocarcinogenesis and photoaging. Biol Chem 378(11):1247–1257
47. Simon JC, Tigelaar RE, Bergstresser PR et al (1991) Ultraviolet B radiation converts Langerhans cells from immunogenic to tolerogenic antigen-presenting cells. induction of specific clonal anergy in CD4 + T helper 1 cells. J Immunol. 146:485–491
48. Sinclair C (2006) Risks and benefits of sun exposure: implications for public health practice based on the Australian experience. Prog Biophys Mol Biol 92:173–178
49. Siscovick JR, Zapolanski T, Magro C, Carrington K, Prograis S, Nussbaum M, Gonzalez S, Ding W, Granstein R (2008) *Polypodium leucotomos* inhibits ultraviolet B radiation-induced immunosupression. Photodermatol Photoimmunol Photomed 24:134–141
50. Tanaka T, Kojima T, Kawamori T et al (1993) Inhibition of 4-nitroquinoline-1-oxide-induced rat tongue carcinogenesis by the naturally occurring plant phenolics caffeic, ellagic, chlorogenic and ferulic acids. Carcinogenesis 14:1231–1325
51. Tanaka T, Kojima T, Kawamori T, Yoshimi N, Mori H (1993) Chemoprevention of diethylnitrosamine-induced hepatocarcinogenesis by a simple phenolic acid protocatechuic acid in rats. Cancer Res 53:2775–2779
52. Tanew A, Radakovic S, Gonzalez S, Venturini M, Calzavara-Pinton P (2012) Oral administration of a hydrophilic extract of *Polypodium leucotomos* for the prevention of polymorphic light eruption. J Am Acad Dermatol 66:58–62
53. Williamson G, Day AJ, Pluma GW, Couteau D (2000) Human metabolic pathways of dietary flavonoids and cinnamates. Biochem Soc Trans 28:16–22

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54. Ishii Y, Sugimoto S, Izawa N, Sone T, Chiba K, Miyazaki K (2014) Oral administration of Bifidobacterium breve attenuates UV-induced barrier perturbation and oxidative stress in hairless mice skin. Arch Dermatol Res 306:467–473
55. Muthusamy V, Piva TJ (2013) A comparative study of UV-induced cell signalling pathways in human keratinocyte-derived cell lines. Arch Dermatol Res 305:817–833
56. Narbutt J, Olejniczak I, Sobolewska-Sztychny D, Sysa-Jedrzejowska A, Slowik-Kwiatkowska I, Hawro T, Lesiak A (2013) Narrow band ultraviolet B irradiations cause alteration in interleukin-31 serum level in psoriatic patients. Arch Dermatol Res 305:191–195
57. Xu D, Yuan R, Gu H, Liu T, Tu Y, Yang Z, He L (2013) The effect of ultraviolet radiation on the transforming growth factor beta 1/Smads pathway and p53 in actinic keratosis and normal skin. Arch Dermatol Res 305:777–786