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Comparative analysis between the active components presented in patented *Polypodium leucotomos* and *Pinus pinaster* botanical extracts (Fernblock ® and Pycnogenol ®) and in products available for pharmacy's compounding and preparations

Análise comparativa entre ativos farmacológicos dos extratos botânicos de Polypodium leucotomos e Pinus pinaster *patenteados (Fernblock® e Pycnogenol®) e produtos disponíveis para uso em manipulação*

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ABSTRACT

Introduction: The use of botanical extracts in dermatological treatments has received special attention in worldwide literature, especially for its antioxidant action. Different products containing *Polypodium leucotomos* and *Pinus pinaste*rs extracts are commercially available in industrialized presentations or compounding. However, in the second case, there is no standardization in the extraction process and there is a lack of data regarding the quantitative and qualitative content in the referred formulations that demonstrate its effectiveness and safety.

Purposes: to assess the different concentrations of actives in standardized and patented botanical extracts of *Polypodium leucotomos* (Fernblock®) and *Pinus pinaste*r (Pycnogenol®), respectively, comparing them to products offered by compounding pharmacies.

Methodology: The products were assessed by high-performance liquid chromatography, and pharmacologically active markers were identified in peaks by retention time.

Results: the concentrations of identified actives varied in the different products analyzed, being undetectable in many of them. Only industrialized products with standardized and patented extracts showed all the expected markers.

Conclusion: consistency was observed in specific products containing the patented extracts of *Polypodium leucotomos* (Fernblock®) and *Pinus pinaste*r (Pycnogenol®), demonstrating the importance of standardization in extraction processes and studies on the efficacy and safety profiles.

Keywords: Antioxidants; Plant extracts; Flavonoids

RESUMO

Introdução: *o uso de extratos botânicos nos tratamentos dermatológicos tem recebido especial atenção na literatura mundial, especialmente pela sua ação antioxidante. Diferentes produtos contendo extratos de Polypodium leucotomos e Pinus pinaste*r *estão disponíveis comercialmente em apresentações industrializadas ou para manipulação. Entretanto, no segundo caso, nem sempre existe uma padronização no processo de extração e faltam dados quantitativos e qualitativos nas referidas formulações que demonstrem sua efetividade e segurança.*

Objetivos: *avaliar as diferentes concentrações de ativos em extratos botânicos padronizados e patenteados de Polypodium leucotomos* (Fernblock®) e *Pinus pinaste*r (Pycnogenol®) *comparando-os, respectivamente, a produtos diversos oferecidos para uso em farmácia de manipulação.*

Metodologia: *os produtos foram avaliados por cromatografia líquida de alta eficiência, sendo os marcadores farmacologicamente ativos identificados em picos pelo tempo de retenção.*

Resultados: *as concentrações dos ativos identificados variaram nos diferentes produtos analisados, sendo indetectáveis em muitos deles. Somente os produtos industrializados com extratos padronizados e patenteados apresentaram todos os marcadores esperados.*

Conclusão: *a consistência foi observada em produtos específicos contendo os extratos patenteados de Polypodium leucotomos* (Fernblock®) e *Pinus pinaste*r (Pycnogenol®), *demonstrando a importância da padronização nos processos de extração bem como de estudos que demonstrem os perfis de eficácia e segurança dos produtos oferecidos.*

Palavras-chave: *Antioxidantes; Extratos vegetais; Compostos fitoquímicos*

Original Article

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INTRODUCTION

Extracts from plants, herbs, and spices containing compounds with natural bioactives have been administered for different diseases for thousands of years.[1] The scientific community has increasingly recognized the use of botanical extracts, and currently, studies using plant polyphenols include numerous clinical trials approved by the National Institutes of Health (NIH).[1] Botanical extracts with antioxidant activity have aroused great interest, especially when aiming at reducing the risk of skin diseases induced by UV radiation.[2] Polyphenols and phenolic derivatives have been considered a group of natural bioactive products with potential health benefits and are present in Polypodium leucotomos and *Pinus pinaste*r extracts.[3]

Polypodium leucotomos is a botanical extract from a tropical fern that grows in Central and South America. It has been described since the 18th century in botanical expeditions, and the natives used it in infusions due to its antiphlogistic and antitumor action.[4-5] From the 1990s, studies with the standardized extract from the leaves of Polypodium leucotomos emerged, patented as Fernblock®. It contained several pharmacological markers, including phenolic derivatives such as hydroxybenzoic acid, protocatechuic acid, vanillic acid, caffeic acid, ferulic acid, p-coumaric acid, and isomers of chlorogenic acid.[6-7] Since then, a series of scientific studies have been published demonstrating the different mechanisms of action of this phytoextract, such as antioxidant and immunoprotective action, DNA protection, and reorganization of skin architecture.[6-11]

In 1996, a study demonstrated the Polypodium leucotomos antioxidant action through its ability to reduce 55% of superoxide anion, 50% of lipid peroxidation, and 10% of singlet oxygen. More recently, the ability of caffeic acid and ferulic acid to inhibit the lipid peroxidation chain was evidenced. Ferulic acid also proved to be a potent absorber of UV photons.[4-6] In 2004, a study with ten human volunteers showed that the presence of Polypodium leucotomos reduced the depletion of Langerhans cells. The study also assessed its action in inhibiting the urocanic acid photoisomerization, demonstrating the immunomodulatory action.[8-9] Polypodium leucotomos reduces inflammation, prevents immunosuppression, activates the tumor suppression factor p53, and inhibits UV-induced enzyme cyclooxygenase-2 (COX-2) expression.[10] The ability to inhibit the formation of sunburn cells and the formation of thymine dimers demonstrated the DNA protection potential exerted by Polypodium leucotomos. A study conducted with cultured fibroblasts and keratinocytes suggested that Polypodium leucotomos would also have a protective effect on fibroblasts.[11] It prevents DNA damage induced by UV and ROS, inhibiting AP1 and NF-κB and protecting natural antioxidant enzyme systems, common deletions of UVA-induced mitochondrial DNA damage, and MMP-1 expression induced by visible light and infrared radiation.[1]

Pycnogenol® is a plant extract, a source of flavonoids, extracted from the bark of the French maritime pine *Pinus pinaste*r. It attracted attention due to its potent antioxidant action, demonstrating its ability to modulate melanogenesis, UV-induced erythema, and the expression of nuclear factor κB (NF-κB, nuclear factor kappa B).[12] The therapeutic use of this active ingredient dates back to the 16th century, when infusions with the bark of *Pinus pinaste*r were used to treat scurvy. Phytoextracts with antioxidant action were identified, stimulating the development of extraction techniques, whose standardization and validation resulted in the extract patented as Pycnogenol®. The technique's improvement allowed obtaining extracts with even greater antioxidant activity, and the evaluation by chromatography could identify its main pharmacological components.[13] Pycnogenol® is a complex mixture of flavonoids that contains 72.5% of polyphenols, including procyanidin B1 (5%), catechins (2.98%), epicatechins (0.23%), and about 60% oligomeric proanthocyanidins.[12;14-17]

Several articles have shown positive and encouraging results, suggesting the use of Pycnogenol® in different indications, especially those related to healthy aging. In the skin, in addition to studies involving its antioxidant action, evidence shows that its supplementation promotes photoprotection and improves hyperpigmentation and the skin barrier, in addition to the homeostasis of the extracellular matrix.[18-19] Possible effects on aging have been suggested by protection against UV damage induced and by the inhibition of metalloproteinases 1 (MMP-1), metalloproteinases 2 (MMP-2), and metalloproteinases 9 (MMP-9).[12] Ni et al. (2002) investigated the effectiveness of Pycnogenol® in treating melasma and were the first to demonstrate that the substance could reduce skin pigmentation.[20] The results of a recent in vivo study demonstrated molecular evidence that oral ingestion of Pycnogenol® could down-regulate the expression of genes involved in melanin synthesis.[19]

Ayres et al. (2015) assessed the bleaching activity of Pycnogenol® in an ex vivo experimental model after exposure to ultraviolet radiation A (UVA) and B (UVB), infrared radiation (IVA), visible light (VL), and association UVA/UVB/IVA/VL (ASS). Fragments of human skin obtained from elective plastic surgery were treated with Pycnogenol® and subsequently subjected to the radiation described above, and histological evaluation of the melanic pigmentation was performed using the Fontana-Masson technique. In control fragments not treated with Pycnogenol®, there was an increase in melanin deposition in all irradiated groups. However, in fragments previously incubated with Pycnogenol®, the study observed a decrease in pigment deposition after all irradiations. Thus, a reduction in melanin deposition was demonstrated in ex vivo skin treated with Pycnogenol® after irradiation with ultraviolet A and B, infrared-A, and visible light, suggesting its antimelanogenic activity.[21]

In 2021, Zhao et al. published a double-blind, randomized, placebo-controlled, clinical trial with crossover intervention, analyzing the action of oral Pycnogenol® in a Chinese population of workers exposed to urban air pollution and seasonal climate changes. The authors observed an improvement in skin pigmentation during autumn when there is still a high level of

ultraviolet radiation associated with high air pollution. In this context, the action of pollution as an aggravating factor of cutaneous hyperpigmentation is discussed.[22]

Recently, a study conducted by Ayres et al. (2022) investigated the bleaching activity of Pycnogenol® in cultures of human melanocytes, quantifying the synthesis of melanin, tyrosinase, endothelin-1, and PPAR, under the action of ultraviolet radiation A (UVA) and B (UVB), infrared-A (IVA), visible light (VL), and the association of all radiation (ASS). Human melanocytes were seeded and incubated with a dry extract solution of *Pinus pinaste*r (Pycnogenol®) and, after determining the non-cytotoxic concentrations, they were exposed to isolated UVA/UVB, IVA, and VL radiation, and to the combination of radiation. After, the study quantified melanin concentration, tyrosinase activity, and endothelin-1 and PPAR mediators. The results indicate that Pycnogenol®, compared to the control without the extract, promoted a reduction in melanin synthesis by 7.66%, 5.14%, and 4.05% when cultures were exposed to UVA/UVB, IVA, and ASS radiation, respectively. Regarding the activity of the tyrosinase enzyme, Pycnogenol®, compared to the control without the extract, promoted a reduction of approximately 66.5% in the enzyme activity and presented a decrease in the synthesis of endothelin-1 in up to 56.47%, 59.33%, 58.00%, and 73.03% when cultures were exposed to UVA/UVB, IVA, VL, and ASS radiation, respectively. PPAR synthesis was reduced to 38.41%, 26.39%, 19.51%, and 56.44% compared to non-incubated controls submitted to UVA/UVB, IVA, VL, and ASS radiation, respectively. The authors concluded that Pycnogenol® reduces the production of melanin pigmentation by regulating the synthesis of tyrosinase, as well as reducing the synthesis of endothelin-1 and PPAR mediators, which are possibly involved in this process.[23]

The results show evidence of the benefits of Polypodium leucotomos and *Pinus pinaste*r in different indications. However, most studies used patented and commercially available extracts (Fernblock® and Pycnogenol®). On the other hand, compounded products are becoming more common, and Brazil is the largest market worldwide, according to data from the National Association of Master Pharmacists (ANFARMAG). Nevertheless, despite this significant growth, there are no regulations with the same rigor and requirements for good handling practices as for industrialized drugs. The consequence is handling errors and falsifications, which can lead to incorrect doses in the preparations and, therefore, to serious impacts on the patient's health. [24-25]

Thus, to assess the possibility of using the various products offered on the market with efficacy and safety, it is necessary to demonstrate the activity of their pharmacologically active markers.

The present study aims to evaluate the presence and concentration of bioactive pharmacological markers in commercial products available for manipulation containing Polypodium leucotomos and *Pinus pinaste*r extracts as described below.

METHODS

Samples of 250 mg of Polypodium leucotomos extract (Helioral® FARMOQUÍMICA SA, composed of the standardized extract Fernblock® and six products available for manipulation) were collected to evaluate the amount of actives 3,4-dihydroxybenzoic acid, vanillic acid, caffeic acid, coumaric acid, and ferulic acid. Likewise, extract samples of *Pinus pinaste*r (Flebon®, FARMOQUÍMICA SA, composed of the standardized extract Pycnogenol®, and six products available for manipulation) were evaluated to detect the amounts of the actives 3,4-dihydroxybenzoic acid, catechin, acid caffeic, taxofolin, and ferulic acid. The studies were conducted at Chemyunion, Sorocaba (SP), Brazil.

The products were assessed by high-performance liquid chromatography (HPLC) in an Agilent model 1200 equipment composed of a vacuum degasser, binary pump, automatic sampler, thermostated column compartment, and diode array detector. The identification of the peaks was made by the retention time and using the standards described in Table 1.

RESULTS

Seven products containing 250 mg of Polypodium leucotomos were analyzed. One product was Helioral®, composed of the standardized extract Fernblock®, commercially available in several countries worldwide, and six other products from different compounding pharmacies. Table 2 lists the results of the analysis.

Similarly, we analyzed seven products containing 50 mg of *Pinus pinaste*r extract. One product was Flebon®, composed of the standardized extract Pycnogenol®, commercially available in Brazil, and six other products from different compounding pharmacies. Table 3 lists the results of the analysis.

Table 1: Standards used with respective batches, suppliers and validity

Standard	Batch	Supplier	Validity
3,4-dihydroxybenzoic	BCBR5849V BCBW5738	Sigma	20/01/2019
Vanillic acid	BCBT1791	Sigma	10/11/2022
Caffeic acid	SLBW6830	Sigma	08/01/2024
P-coumaric acid	BCBV5232	Sigma	19/07/2023
Ferulic acid	STBH3067	Sigma	16/11/2023
Catechin	WXBC6250V	Sigma	30/11/2021
Taxofolin	BCBQ3955V	Sigma	07/07/2021

Table 2: Analysis results of the seven collected products containing 250 mg of *Polypodium leucotomos*

Material	3,4-dihydroxy-dobenzoic acid (ppm)	Vanillic acid (ppm)	Caffeic acid (ppm)	P-coumaric acid (ppm)	Ferulic acid (ppm)
Product 1 (Helioral®)	74.6	32.8	41.8	48.7	22.5
Product 2	457.0	13.9	40.3	20.8	Not detected
Product 3	21.3	Not detected	Not detected	Not detected	15.5
Product 4	136.5	Not detected	54.4	2.8	Not detected
Product 5	202.1	18.3	46.5	3.4	Not detected
Product 6	337.6	152.9	Not detected	16.2	59.8
Product 7	18.7	Not detected	Not detected	Not detected	16.2

Table 3: Analysis results of the seven collected products containing 50 mg of *Pinus pinaster* extract

Material	3,4-dihydroxy-benzoic acid (ppm)	Catechin (ppm)	Caffeic acid (ppm)	Ferulic acid (ppm)	Taxofolin (ppm)
Product 1 (Flebon®)	351.4	3029.0	490.5	608.0	3365.0
Product 2	Not detected	804.2	Not detected	1958.7	941.9
Product 3	155.8	1070.0	Not detected	Not detected	Not detected
Product 4	Not detected	351.0	Not detected	Not detected	Not detected
Product 5	Not detected	634.7	Not detected	Not detected	413.7
Product 6	Not detected	606.6	Not detected	Not detected	Not detected
Product 7	Not detected	509.4	Not detected	Not detected	315.9

DISCUSSION

Phenolic compounds are secondary metabolites present in plants. In the human diet, polyphenols are the most abundant antioxidants and are present in drinks and foods of botanical origin. Also, they have many beneficial effects on human health, including antioxidant activity, gene expression modulation, and carcinogenesis inhibition.26 High-performance liquid chromatography (HPLC) identified that Polypodium leucotomos contains several phenolic compounds in its chemical composition that were separated according to their retention time. The most abundant were phenolic acids (cinnamic acid), specifically 3-methoxy-4-hydroxycinnamic acid (ferulic), 4-hydroxycinnamic acid (p-coumaric), 3,4-dihydroxycinnamic acid (caffeic), 3-methoxy-4-hydroxybenzoic acid (vanillic), 3,4-dihydroxycinnamic acid (caffeic), and 3-caffeoylquinic acid (chlorogenic).[27]

The literature already documented the mechanisms of action and benefits of Polypodium leucotomos and *Pinus pinaste*r extracts. However, most published studies use the patented extract Fernblock® and Pycnogenol®, although using brand names is often restricted to avoid creating bias or promoting a particular product. Thus, generic products use the same nomenclature, confounding the consumer - who does not always understand possible differences between the presentations.28

This study illustrates the different concentrations of bioactive markers between industrialized products, with standardization of extraction, and products offered in compounding pharmacies under prescription. We emphasize that markers are essential predictors for quality control of products derived from botanical extracts to guarantee their therapeutic action and safety.[29]

According to the tests performed on samples containing Polypodium leucotomos, described in Table 2, we can see that:

- Regarding the content of 3,4-dihydroxybenzoic acid, product 2 presented more the active with 457.0 ppm, while product 7 presented the lowest value with 18.7 ppm.

- Regarding the content of vanillic acid, product 6 was the one that presented more the active with 152.9 ppm, while product 2 presented the lowest value with 13.9 ppm. The tests did not detect the presence of this active in products 3, 4, and 7.

- Regarding the caffeic acid content, product 4 presented more the active, with 54.4 ppm, while product 2 had the lowest value, with 40.3 ppm. The tests did not detect the presence of

this active in products 3, 6, and 7.

- Regarding the content of coumaric acid, product 1 (Helioral® – FARMOQUÍMICA SA) presented more the active with 48.7 ppm, while product 4 presented the lowest value with 2.8 ppm. The tests did not detect the presence of this active in products 3 and 7.

- Regarding the ferulic acid content, product 6 presented more the active, with 59.8 ppm, while product 3 had the lowest value, with 15.5 ppm. The tests did not detect the presence of this active in products 2, 4, and 5.

- We can observe that only product 1 (Helioral® – FQM) presented all the actives characteristic of the Polypodium leutocomos extract.

Similarly, the results from the tests with the samples containing *Pinus pinaste*r, described in Table 3, allow us to observe that:

- Regarding the content of 3,4-dihydroxybenzoic acid, product 1 (Flebon®) presented more the active, with 351.4 ppm, while product 3 presented the lowest value, with 155.8 ppm. The tests did not detect the presence of this active in the other products - 2, 4, 5, 6, and 7.

- Regarding the catechin content, product 1 (Flebon®) presented more the active, with 3029.0 ppm, while product 4 presented the lowest value, with 351.0 ppm.

- Regarding the caffeic acid content, product 1 (Flebon®) was the only one that presented the active, with 490.5 ppm. The tests did not detect the presence of this active in the other products.

- Regarding the ferulic acid content, product 2 presented more the active, with 1958.7 ppm, while product 1 (Flebon®) presented a lower value, with 608.0 ppm. The tests did not detect the presence of this active in products 3, 4, 5, 6, and 7.

- Regarding the taxolin content, product 1 (Flebon®) presented more the active, with 3365.0 ppm, while product 7 had the lowest value, with 315.9 ppm. The tests did not detect the presence of this active in products 3, 4, and 6.

We can observe that only product 1 (Flebon®) presented all the actives characteristic of Pycnogenol®.

CONCLUSION

Polypodium leucotomos and *Pinus pinaste*r are available as oral herbal medicines and have demonstrated antioxidant and photoprotective properties with adjuvant skincare benefits. However, there is a lack of data regarding the quantitative and qualitative content of polyphenols in most formulations. The present study demonstrated that only the Helioral® product had all the characteristic actives of the standardized and patented Polypodium leucotomos extracts. Similarly, it showed that only Flebon® presented all the actives described in the literature on Pycnogenol®, a fact corroborated by the large number of published studies that demonstrate the effectiveness and safety of both extracts and confirm the importance of controlling quality and regulations of products on the market. ●

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