

# Photoprotective Effect of the Hydroalcoholic Extract from Red and Black Peruvian Varieties of *Phaseolus vulgaris* L.

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## Abstract

**Introduction:** Ultraviolet radiation can be harmful to the skin and can create serious problems when a protective substance or equipment is not used regularly, for this reason the present study set out to evaluate the photoprotective effect of the hydroalcoholic extracts of the integument of the red and black varieties of pop beans (*Phaseolus vulgaris*). **Materials and Method:** The extracts were obtained by macerating the tegument of the two varieties of pop beans, black and red, in an ethanol/water (80:20) solution. The thin-layer chromatographic method was used to observe anti-tyrosinase activity, using the 0.05% kojic acid standard as a positive control. The photoprotective effect was evaluated in female mice of seven-week-old, *Mus musculus* Balb/c, with shaved back and exposed to UV-C radiation for 30 minutes once a day for thirty days to induce photo-damage in the skin. The treatments consisted in the application of extracts at 2% and 5% W/V in gel on the shaved area. Only the gel formulation, sunscreen or no treatment at all was used for the control group. Acute photodamage was quantified using the Draize scale, measuring the degree of erythema and edema; and the degree of photoaging using the Glogau scale at 7 days and 30 days. **Results:** It was found that the ethanolic extracts of the integument of both varieties of *Phaseolus vulgaris*, red and black, are practically non-toxic in mice by oral and dermal routes at a limit dose of 5000 mg/kg. The anti-tyrosinase effect at a qualitative level, evaluated by thin layer chromatography, showed the black variety were more active than the red one and both can be compared to the activity of the kojic acid. The two extracts of the two varieties showed photoprotective effect, however the extracts in 5% gel to a greater degree. **Conclusions:** It was concluded that the hydroalcoholic extract of the pop black beans tegument at 5% in gel has photoprotective effect.

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## Keywords

Anti-Tyrosinase Effect, Photoprotective Effect, *Phaseolus vulgaris*

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### 1. Introduction

The solar radiation that reaches the Earth can be classified as: visible, infrared and ultraviolet light, which can also be subdivided into three bands: UVA, UVB and UVC. This type of radiation is known to generate health problems on the skin such as premature wrinkles, blemishes and even cancer [1]. According to the World Health Organization (WHO), since the early 1970s the incidence of skin cancer has increased in the world [2] and in Peru the National Meteorology and Hydrology Service (SENAMHI) reported that in 2019 the levels of ultraviolet UVB and UVA radiation has increased between 12 and 18 W/m<sup>2</sup>, considered extremely high.

For this, the defense mechanisms of the skin in a natural way are not enough, so it becomes necessary to take protective measures, using photoprotectors [3], which have encouraged the study of various plant extracts that contain phenolic compounds and flavonoids [4] as substances that can replace chemical sunscreens [5] due to their photoprotective activity without the outcome of second effects like cytotoxicity. That is the case of hydroquinone, a broad used compound with anti-tyrosinase effect which has been banned in Ghana since 2016 because of its cytotoxicity [6].

The pop bean is a species originated in the Andean central region, their seeds have high protein contain and are consumed toasted [7]. In Peru, the pop beans (*Phaseolus vulgaris* L.) is one of the valuable legacies of Andean agriculture, in addition, its shell is rich in polyphenolic compounds [8].

There are no antecedents that demonstrate any medicinal properties at the ethnobotanical or pre-clinical level of the pop bean; however, some of the currently recognized phytochemicals in beans are: fiber, polyphenols, phytic acid, tannins, trypsin inhibitors, and lectins. Trypsin inhibitors inhibit carcinogenesis and can be used as chemoprotective agents. Bean lectins slow the growth of non-Hodgking lymphomas. Phytic acid has been shown to reduce the risk of cancer, probably due to its antioxidant power. Tannins function as effective antioxidants, anticancer, and antimutagens [9]. Then, being able to take advantage of all the phytochemical elements of the bean led us to think about the peel and its various colors, which give an indication of its richness in antioxidants and molecules with probable biological activity on the skin.

To all this, it motivates the study of the anti-tyrosinase activity and the photoprotective effect of the hydroalcoholic extract of two varieties of pop beans, aiming to offer an efficient plant nutritional base that can also be used for protection against skin-related disorders associated with ultraviolet rays.

## 2. Materials and Method

### 2.1. Extract Preparation

The two varieties of *P. vulgaris* seeds were obtained in September 2019 in “La Parada” market in the city of Lima. Two plants of each variety were germinated for its botanic identification in the Herbarium of the Universidad Nacional Mayor de San Marcos. To obtain the extract, the seeds were soaked in 7 L of distilled water for 7 hours, the cotyledons were manually separated from the tegument and dried for 7 days at 40 °C. Once dry, the teguments of each variety were ground and macerated for 6 hours in a solution of ethanol/water (80:20) v/v. The extract obtained was dried, evaporating the solvent in an extractor hood and then in an oven for 3 days at 40 °C.

### 2.2. Determination of Anti-Tyrosinase Activity

The anti-tyrosinase activity was evaluated qualitatively *in vitro* using thin layer chromatography test (TLC), where the standard (kojic acid) and the samples of the hydroalcoholic extracts (diluted in Dimethyl sulfoxide) of the integument of the red and black varieties of pop beans was spotted in the plate. A mixture of methanol-ethyl acetate (60:40) was used as the mobile phase. The development was carried out with tyrosinase and tyrosine solutions sprayed to the plates. Colorless spots were observed on a gray-purple background [10].

### 2.3. Preparation of the Extract of the Integument of the Red and Black Varieties of Pop Beans Gel for the Photoprotective Evaluation

A 1% Carbopol 940 colloidal suspension was prepared in distilled water for 24 hours to achieve optimal hydration, this solution had a pH of 3. A Nipagin/Nipasol in a 2:1 ratio (0.05% Nipagin + 0.01% Nipasol) solution was added as a preservative [11]. Then the dry extract of the two varieties of pop beans were weighted and incorporated so the final concentration was 2% and 5% respectively. For the incorporation of the dry extract of red pop beans, 0.6 g of the solid was dissolved in 4 drops of propylene glycol and incorporated into the 30 g of base gel, stirred until a homogeneous mixture was obtained (pH 6.5).

### 2.4. Implementation of UV-C Irradiation System

A UV-C irradiation system was implemented, consisting of a Philips brand fluorescent lamp of 90 cm in length and 40 watts with an emission spectrum of 100 - 280 nm with a peak of 253.7 nm, placed in a camera 70 × 128 × 173 cm hermetically sealed with a black plastic cover to avoid contamination by UVC radiation.

### 2.5. Acute Oral and Dermal Toxicity of the Hydroalcoholic Extract of the Integument of the Red and Black Varieties of Pop Beans

All hair on the back of the *Mus musculus* individuals was shaved, and waited 24 hours to avoid skin irritation [12]. The extract diluted in water at a maximum

dose of 5000 mg/kg was administered orally and topically on the shaved back of the animal, it was covered with gauze and sealed with tape for complete penetration. After four hours the gauze was removed and the animals were observed for 14 days, at the end of this period the biological samples were killed with an overdose of sodium pentobarbital (40 mg/kg) by intraperitoneal route. Immediately the individuals of control and exposed to the extracts of both black and red pop beans were dissected the skin of the loin exposed to the gel (portion of 2 cm × 2 cm) as well as the liver, stomach, kidney and lung so they were deposited in a 10% buffered formaldehyde solution. The tissues were taken to the Central Laboratory of Clinical Pathology of the Hospital Nacional Arzobispo Loayza for histological analysis.

### **2.6. Photoprotective Effect of the Red and Black Varieties of Pop Beans Hydroalcoholic Extract of the Integument in Mice Exposed to UVC Radiation**

The experimental mice (*M. musculus* Balb/ c) were acquired in the Cayetano Heredia University animal facility, they were housed in metal cages within the Norbert Wiener University animal facility, for adaptation for a week prior to the experiments, with free access to water and food, air-conditioned environment of 20°C ± 1°C temperature, with 55% ± 5% relative humidity with 12 hours of light and 12 hours of darkness. Seven working groups were formed consisting of five mice each group: the control group (C-) without gel, the control group (C) that was applied only the skin vehicle made up of hydrophilic gel without the extract, the control group (C+) with sunscreen with SPF 70 and the experimental groups with 2% w/v and 5% w/v of the extract of red and black pop beans, respectively.

A dose of 5 mg of the gel by square centimeter of skin, was applied to each of the specimens with the treatments in the shaved dorsal region and after 10 minutes they were exposed to UV-C radiation, for an interval of 30 minutes, at a 20 cm distance from UV-C lamp. This procedure was repeated daily for one month [13]. The photoprotective effect was evaluated based on the degrees of erythema, edema and photoaging at 7 and 30 days respectively.

### **2.7. Statistical Analysis**

The values of each case were collected according to the experimental model proposed for each experimental unit (rodents). The data obtained in the trials were subjected to the analysis of variance (ANOVA), after verifying their normality and homogeneity of variances, using the Statistical Package Social Science version 24 program and at a 95% confidence level.

### **2.8. Ethic Aspects**

The experimental animals were treated appropriately following the guidelines of the manual for the handling of experimental animals of the National Institute of Health of Peru. "Guide for the handling and care of laboratory animals: mice" [14].

### 3. Results

#### 3.1. Acute Oral and Dermal Toxicity of the Hydroalcoholic Extract of the Integument of the Red and Black Pop Beans Varieties

It was found that the hydroalcoholic extracts of pop beans were “practically non-toxic” according to the Trevan scale, as no dead specimens were found at 5000 mg/kg and the damage of the evaluated organs was slight (**Table 1**).

In the qualitative phytochemical study of the hydroalcoholic extract [15], it was observed the presence of: quinones, flavonoids, tannins, reducing compounds, lipids, alkaloids, coumarins, leucoanthocyanidins, saponins and polysaccharides. Also, the absence of steroids, catechins and proteins was observed (**Table 2**).

#### 3.2. Anti-Tyrosinase Activity of the Red and Black Varieties of Pop Beans Hydroalcoholic Extract of the Integument by Thin Layer Chromatography

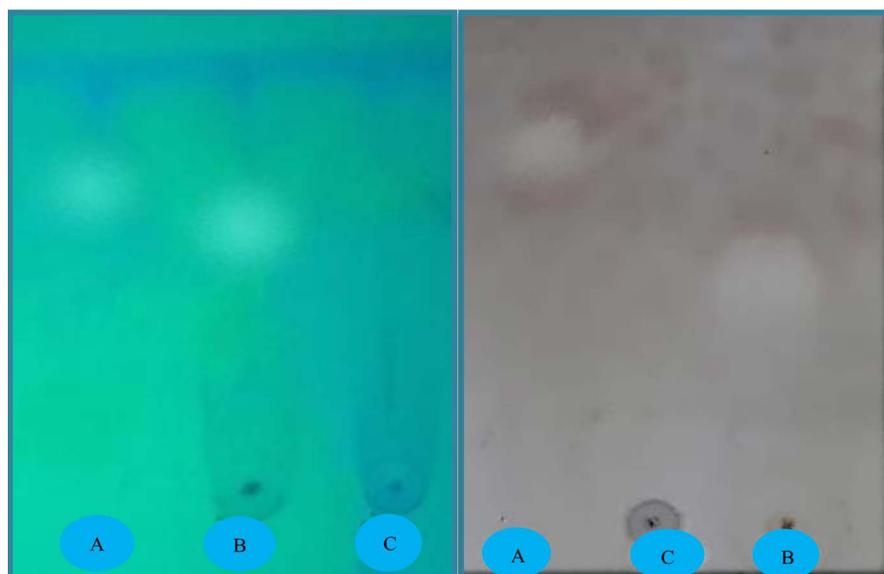
The anti-tyrosinase activity was evaluated qualitatively by thin layer chromatography (**Figure 1**). On a chromatographic plate previously spotted with the extracts of red pop beans (B), black pop beans (C) and kojic acid as standard (A). Was eluted in a mixture of methanol:ethyl acetate (60:40), then a tyrosinase solution was sprayed over the entire plate and immediately the same amount of L-tyrosine solution was sprayed on the plate and allowed to incubate at temperature ambient. The inhibition of tyrosinase was observed as the appearance of colorless areas on a gray-violet background of the plate. Colorless areas were observed in the kojic acid standard (inhibition of the effect of tyrosinase by chelation of its copper ion), in the same way with the ethanolic extract of black pop beans and to a lesser extent in the ethanolic extract red pop beans. This indicates that the ethanolic extract of the black variety has a greater inhibitory capacity of the activity of the anti-tyrosinase.

**Table 1.** Histopathological analysis of rodent organs subjected to an acute dose of pop beans integument extract.

Group	Skin	Liver	Kidney	Stomach	Lung
Control	Preserved skin without signs of toxicity	Has congestion, but no toxicity is seen	Glomeruli with a low degree of congestion are seen	No lesions on the mucous glands	No toxicity observed
Pop red beans	Normal histopathologic characteristics	Presence of erythrocytes without hepatocyte damage	Congested glomeruli	Mild inflammation of the mucous glands	Presence of erythrocytes and minor lesions
Pop black beans	Thick skin with increased collagen and hair follicle	Increased numbers of red blood cells and hepatocyte congestion	Glomeruli with low degree of congestion and presence of erythrocytes	Presence of erythrocytes and lesions in the mucous glands	Presence of erythrocytes and minor lesions

**Table 2.** Results of the phytochemical study of the hydroalcoholic extract of the integument of the red and black varieties of *P. vulgaris*.

Test/Reagent	Phytochemical	Red pop beans	Black pop beans
Liebermann-Burchard	Steroid	–	–
Bortranger	Quinones	+	+
Assay for Catequins	Catechins	–	–
Shinoda	Flavonoids	+	+
Ninhydrin reaction	Proteins	–	–
FeCl <sub>3</sub>	Polyphenols	+	+
Fehling	Reducing sugars	+	+
Assay for resins	Resins	+	+
Assay with gelatin	Tannins	+	+
Sudán	Lipids	+	+
Dragendorff	Alkaloids	+	+
Baljet	Coumarins	+	+
Mayer	Alkaloids	–	–
Wagner	Alkaloids	+	+
Rosenhein	Leucoanthocyanidins	+	+
Foam test	Saponins	+	+
Mucilage assay	Polisaccharides	+	+

**Figure 1.** Anti-tyrosinase activity of red and black pop beans extracts in thin layer chromatography. A (kojic acid), B (Red pop beans extract), C (Black pop beans extract) plate chromatography eluted with methanol: ethyl acetate (60:40), observed in full light and at 366 nm (UV light). Discolorations on dark background.

### 3.3. Photoprotective Effect of the Hydroalcoholic Integument Extract of the Two Varieties of Pop Beans in Mice Exposed to UVC Radiation 7 Days after Treatment

Seven days after exposure to UV radiation and application of the treatments, it

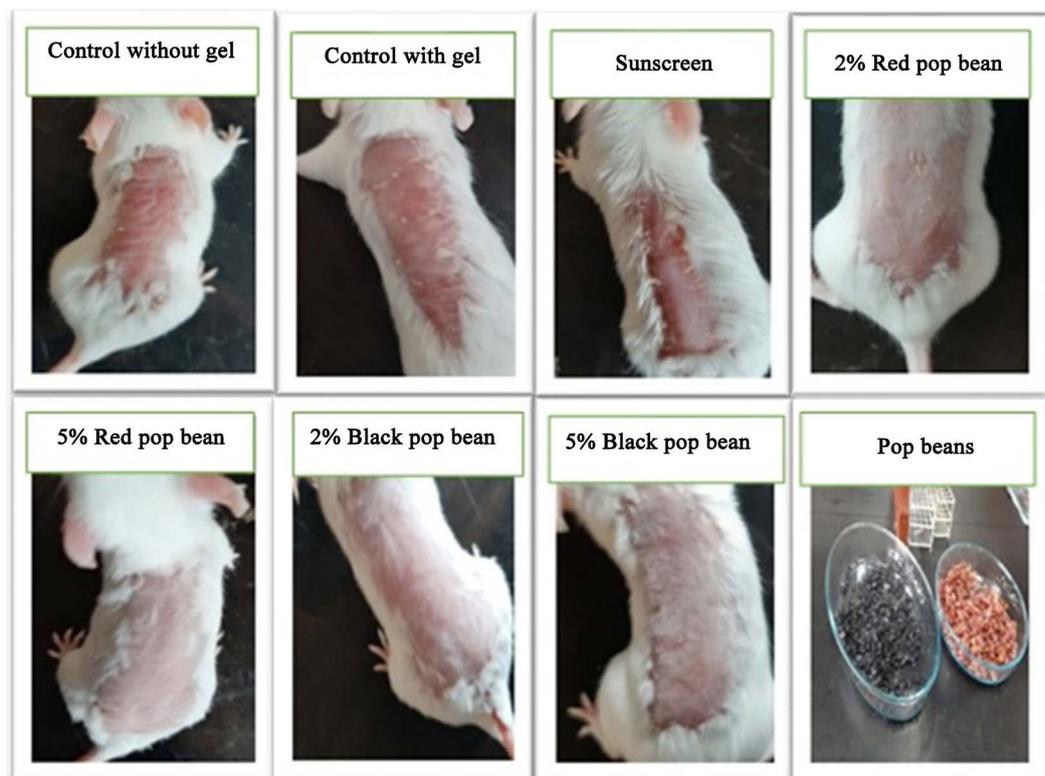
was found that the extracts of black pop beans (2% and 5%) and 5% red pop beans had a lower degree of erythema and edema even compared to the control group to which sunscreen was applied. For the evaluation of photoaging, the extract of black variety presented a lower degree of statistical similarity with the control group (**Table 3**).

The degrees of erythema and edema were also evaluated daily during the seven days. Show that the treatment with 5% black variety not only shows a lower degree of them, but also delays the appearance of photodamage (**Figure 2**).

**Table 3.** Photoprotective effect of the extracts of red and black pop beans after seven days.

Group	Erythema degree*	Edema degree*	Photoaging degree*
Sunscreen	2b	2b	1a
Control gel base	4d	4d	3c
Control without gel	3c	3c	3c
2% Black pop beans	1a	1a	2b
5% Black pop beans	1a	1a	1a
2% Red pop beans	2b	2b	2b
5% Red pop beans	1a	1a	2b

\*From the average of 5 repetitions, the same letters are associated with statistically similar treatments according to the Mann Whittney test  $p < 0.05$ .



**Figure 2.** Macroscopic view of photodamage in *Mus musculus* according to experimental group at seven days and red and black varieties of pop beans.

### 3.4. Photoprotective Effect of the Integument Hydroalcoholic Extract of the Red and Black Pop Beans in Mice Exposed to UVC Radiation 30 Days after Treatment

The degrees of erythema, edema and photoaging were analyzed in mice exposed to UVC radiation. A degree of erythema equal to zero was found in all experimental groups, which therefore was not subjected to statistical analysis. For the degrees of edema and photoaging (**Table 4**), it was found that in the case of edema, the extracts of black pop beans were just as efficient as the sunscreen SPF 70; while in the case of photoaging in this long period of 30 days, only the 5% extracts of both varieties of pop beans resulted in a lower degree, even less than the treatment with sunscreen (**Figure 3**).

In **Figure 3** the microscopic view of the photodamage in *Mus musculus* is shown. In the control group without gel, the skin presented grade 3 edema and parakeratosis. In the group with sunscreen, there was slight edema at the skin level. In the treatment groups with the extract of 2% and 5% red and black pop beans, slight edema was observed and also presents hyperactivity of the hair follicle, which is why the presence of hair is observed, this is an unexpected reaction regarding its hair growth promoting effect, in general the extract showed a photoprotective effect on UVC radiation.

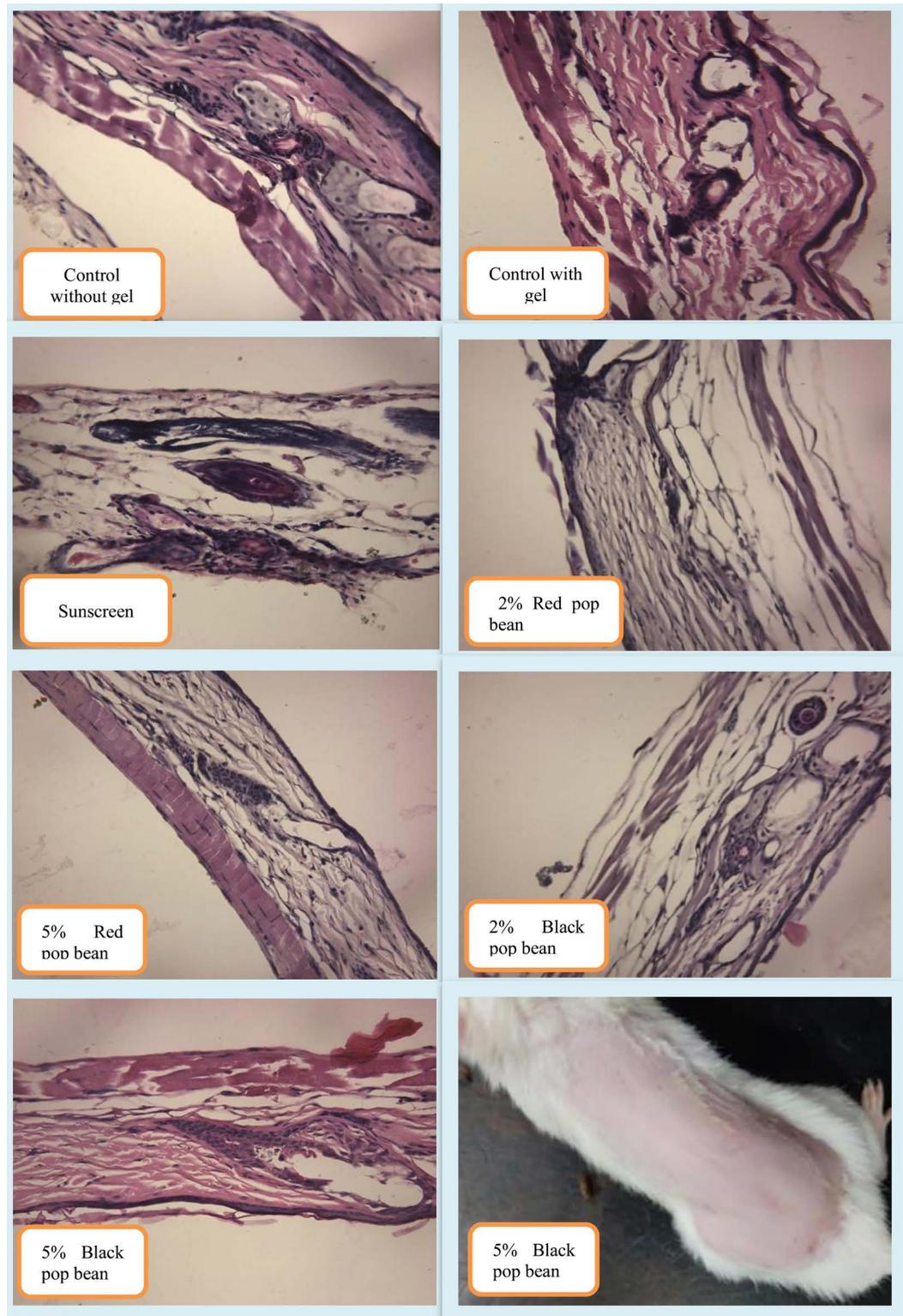
## 4. Discussion

Among the secondary metabolites present in various plants, polyphenolic compounds such as those found in *P. vulgaris* (**Table 2**) present antioxidant activity which counteract the harmful effects of oxidative stress, which is the source of several problems at the cellular level, among them damage associated with UV radiation [16] [17]. Polyphenols were not the only compounds evidenced in the pop beans integument, others such as flavonoids, tannins, alkaloids, were observed. This agree with qualitative analysis in other types of beans, specifically in cowpea bean flour (*Vigna unguiculata* L.), where is suggested that it can be used for the treatment of physiological disorders, to prevent cell oxidation and

**Table 4.** Degrees of edema and photoaging of the integument hydroalcoholic extract of the red and black varieties of *P. vulgaris* in radiation-induced mice.

GROUP	EDEMA DEGREE*	PHOTOAGING DEGREE*
Sunscreen	0.6a	2.4c
Control gel base	2.0b	3.0d
Control without gel	2.2b	3.0d
2% black pop bean	0.2a	2.0b
5% black pop bean	0.6a	1.0a
2% red pop bean	0.2a	2.0b
5% red pop bean	2.0b	1.0a

\*From the average of 5 repetitions, the same letters are associated with statistically similar treatments according to the Mann d Whittney test  $p < 0.05$ .



**Figure 3.** Microscopic view of photodamage in *Mus musculus* according to experimental group in histopathological sections of skin.

protecting cells from carcinogenesis [18] [19].

Of all these compounds, the most active and abundant seem to be polyphenols

and the highest phenol contents were quantified in the black testa variety, followed by the red one, with statistical differences between them, and to a lesser extent the white testa variety [20]. Therefore, the results found when carrying out the preliminary phytochemical qualitative analysis bear some similarity with the secondary metabolites found in previous studies.

In this study, the anti-tyrosinase activity was evaluated and according to the results, the ethanolic extracts of the *Phaseolus vulgaris* variety used showed inhibitory capacity of the tyrosinase enzyme in a development by thin layer chromatography. At present it is known that the most important stimulus for melanogenesis is ultraviolet radiation [21] and that polyphenols of plant origin affect the biosynthesis of melanin [22] [23]. Apparently, with respect to polyphenols, among their many properties is the inhibitory activity of tyrosinase and one of the mechanisms is to competitively inhibit tyrosinase due to its ability to chelate copper in the active site, which leads to irreversible tyrosinase inactivation [24].

The various skin disorders including skin cancer are caused by overexposure to solar radiation, particularly the ultraviolet (UV) component [25]. It is known that 5% of the UV radiation received by the earth is composed of UVB radiation [26] and it is the most active component of solar radiation, responsible for a wide variety of skin diseases, including melanoma and non-melanoma skin cancer. Other damage known from the exposition to UV radiation can induce DNA damage, oxidative stress, premature skin aging and can act as a tumor initiator and co-carcinogen [27] [28].

On the other hand, UVA ultraviolet radiation constitutes between 90% and 95% of the solar radiation that reaches the surface of the earth and due to its longer wavelength, it has a high capacity to penetrate the skin, this leads to generation of reactive oxygen species (ROS) that can cause damage at the level of cellular proteins, lipids and DNA [27]. Both UVB and UVA ultraviolet radiation are responsible for up to 90% of the changes commonly attributable to aging [29].

The results obtained showed that the extracts of black pop beans (2% and 5%) and red pop beans 5% presented a lower degree of erythema and edema in the individuals studied. Regarding photoaging, the extract of black pop beans presented a lower degree of statistical similarity with the control group ( $p$  value < 0.05). The degrees of erythema and edema were also evaluated daily for seven days, in which it stands out that the treatment with 5% black pop beans not only shows a lower degree of skin damage, but also delays the appearance of photo-damage.

The results found agree with the evaluation of other vegetal extracts, such as the anthocyanic extract of *Zea mays* “purple corn”, where the evaluated acute and subacute photodamage and photoaging induced by UVC radiation were shown to have a photoprotective effect against the harmful activity of UVC radiation [7]. Various investigations have shown the correlation between the increase in UVR, visible and far infrared radiation and the production of anthocyanins (photo-induction) as a mechanism used by plants to prevent oxidative

damage by radiation [30] [31]. Like Tarquino [8] who evaluated *in vitro* the photoprotective activity of phenolic extracts from 4 varieties of quinoa in which he determined that the variety of quinoa Ajara (black) had a higher content of phenolic compounds that absorb in the range of UVA and UVB radiation.

The results also showed some damage in the group that applied the base cream free of the extracts. This can be justified according to Ishiwatari *et al.*, who mention that preservatives such as methylparaben and propylparaben intervene in the physiology of the skin by inhibiting the production of hyaluronic acid and type V collagen, which are found in the basal layer of the skin providing hydration, proliferation and differentiation of the epidermis and by decreasing their production, deterioration and aging of the skin occurs. The mechanism of these preservatives is to accumulate on the epidermis, affecting keratinocytes, which triggers a series of unfavorable changes on the skin [32].

Similarly, Osamu *et al.* determined that methylparaben can have harmful effects on the skin at 0.3% concentrations and can present a synergism of toxicity since radiation triggers a series of unfavorable biochemical reactions for the skin [33].

## 5. Conclusion

The present findings show that the pop bean extract provides excellent photoprotection of the skin, probably due to its antityrosinase and radical scavenging activity of its active components, which is why the importance of dietary phytochemical supplements to combat aging and skin disorders. The use of natural chemopreventive agents may be a new alternative for protection against exposure to ultraviolet radiation. This is particularly interesting regarding the extract of the black variety; Therefore, the shell as a by-product of the industrial processing of pop bean as a snack, could be used as a low-cost ingredient with a high added value in the cosmetic and pharmaceutical field for photoprotective applications. It is recommended to investigate the nature of the components of the pop bean extract.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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