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STUDY ON WILD BLACK RASPBERRY (*RUBUS NIVEUS*) FRUIT: VALORIZATION OF ITS POTENTIAL TO NEUTRALIZE FREE-RADICALS AND REACTIVE OXYGEN SPECIES (ROS) AND ITS PHOTOSTABILITY UNDER SOLAR LIGHT

Miguel LEÓN[1*], Johany VELÁSQUEZ[1], Franklin VARGAS[1]

[1] Laboratorio de Fotoquímica, Centro de Química, Instituto Venezolano de Investigaciones Científicas - IVIC

*Correspondence:
Miguel LEÓN
migueldleonr@gmail.com

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Abstract: The present study evaluated the photostability of *Rubus niveus* fruit using a solar simulator. Since degradation under solar irradiation is a key indicator of stability capacity, photostability assays are fundamental to evaluating the preponderance of *R. niveus* extract for biological applications involving light. Additionally, the quenching and scavenging capacity against different types of free radicals was assessed. Ethanolic extracts were prepared from frozen black raspberries. Quenching and scavenging activity were measured using a series of analytical procedures. These included UV-Vis spectroscopic analysis, photostability assessment using solar radiation spectrum (with solar simulator), chemiluminescence, and DPPH assays. Reactions with singlet oxygen ($^1O_2$) and galvinoxyl radical were also employed to evaluate scavenging capacity. The results revealed that *R. niveus* is a source of anthocyanins (128.68±0.85 mg/lt and 48.19±0.31 mg/100 g of fresh weight) and exhibits remarkable photostability under solar light, with minimal degradation at its absorption wavelength. Its photostability was comparable to that of common sunscreen filters (Neo Heliopan Type Ma and Neo Heliopan Type OS). Furthermore, a noteworthy antioxidant capacity was demonstrated, effectively reducing reactive oxygen species (ROS) and free radicals, with significant scavenging activity comparable to that of vitamin C in some assays. Particularly, the extract displayed superior scavenging activity against highly reactive hydroxyl radicals compared to vitamin C (86.82% vs. 55.13%). These results suggest that the ethanolic extract of *R. niveus* is photostable and has the capacity to scavenge and neutralize different types of dark- and light-induced reactive oxygen species. Further *in vitro* and *in vivo* research is recommended to validate the potential of *R. niveus* compounds as natural ingredients in cosmetic and nutraceutical products.

Keywords: antioxidant, photostability, fruit, raspberry, light, free-radicals

Introduction

The *Rubus niveus*, also known as the Mysore raspberry or wild black raspberry, belongs to the Rosaceae family. Originally from Asia, it has spread to various parts of the world due to its ability to adapt to different climates and altitudes. It is considered an invasive species because it grows rapidly in regions where it is cultivated due to its ability to spread easily through various reproduction mechanisms. In addition, the fruits of *R. niveus* are edible and contain many seeds that contribute to the reproduction of this plant

(Pancholi and Rana). The fruits are also used for human consumption and have high medicinal value (Li et al., 2015; Ahmad et al., 2015).

Research in phytochemistry has shown that phenolic compounds have significant anti-inflammatory and antioxidant activity. This activity is essential for reducing oxidative stress and its consequences, such as the development of cancerous tumors, heart problems, and other chronic diseases (a.Hertog et al., 1993; b. Hertog et al., 1993). Thus, scientific research in this field is particularly relevant because it provides *in vitro* tests that can establish possible links between the antioxidant activity of different plant sources and the prevention of common human diseases. It is well known that some natural antioxidant compounds are involved in photoprotection mechanisms, reducing skin damage and aging (Silva et al., 2016; Khoo et al., 2017).

Various scientific studies have reported on the pharmacological value of the *R. niveus* plant in both *in vitro* and *in vivo* settings (Chiluisa et al., 2017; Parimelazh et al., 2013; Pancholi and Rana, 2020; Badhani et al., 2015; Nesello et al., 2017). For instance, extracts from this species have been found to prevent diseases such as diabetes, obesity, hypertension, cancer, and other conditions, as well as common microbial infections. For this reason, antibacterial, anti-inflammatory, and antioxidant properties have been attributed to *R. niveus*, due to the wide variety of polyphenolic compounds, such as anthocyanins and catechins, which are beneficial to human health. Thus, *R. niveus* fruits have been shown to be relevant not only to the biomedical field, but also to nutrition and ethnomedicine (Pancholi and Rana, 2020; Ahmad et al., 2015; Muniyandi et al., 2019).

However, there is a lack of information regarding the quenching and scavenging activity of the ethanolic extract of black raspberry fruit (*R. niveus*), as well as its behavior under visible and ultraviolet irradiation. It is important to acknowledge the scarcity of research focused on the quenching, scavenging and photostability potential of *R. niveus* fruit, given the emphasis placed on the properties of other plant parts, such as roots and stems (Parimelazh et al., 2013; Badhani et al., 2015; Nesello et al., 2017). Consequently, emphasizing the protective capacity and nutritional and health-promoting potential of *R. niveus* fruit is fundamental, regardless of preconceived notions about its origin and biological characteristics (Bachheti et al., 2023). Furthermore, it should be noted that chemiluminescence assays have not been utilized in previous research on the antioxidant and scavenging activity of *R. niveus*. This is a notable observation, given the reliability and high performance of these techniques in assessing antioxidant activity of foods and raw materials (Zvereva and Zhmurova, 2023; Da Silva Mendonça et al., 2022).

This research offers a comprehensive understanding of the photochemical behaviour (photostability) exhibited by the compounds present in *R. niveu*s extract and their ability to neutralize reactive oxygen species (ROS) and free-radicals. The experimental approach utilizes UV-Vis and fluorescence studies, as well as DPPH (2,2-diphenyl-1-picrylhydrazyl) and chemiluminescence methods (which have not previously been employed for *R. niveus*). The study also examines the reactions with the galvinoxyl radical and singlet oxygen. In this sense, the objective of this study is to highlight the photostability and antioxidant properties of *R. niveus* fruit.

Materials and Methods

Reagents and equipment

A Luzchem L2C-4V solar simulator, Perkin Elmer Lambda 35 spectrometer, FL 6500 Fluorescence Spectrophotometer, Luminoskan Ascent Luminometer, Ethanol

75% (Sigma-Aldrich, HPLC grade), Neolipan type Ma (menthyl anthranilate), Neoheliopan type OS (Octisalate, 2-Ethylhexyl salicylate), DPPH radical (2,2-diphenyl-1-picrylhydrazyl, Sigma-Aldrich), Ascorbic acid (Sigma-Aldrid), Galvinoxyl radical (Sigma), Rose Bengal (Aldrich), sodium acetate (CH_3COONa), potassium chloride (KCl), hydrogen peroxide (H_2O_2, Aldrich), Potassium ferricyanide ($K_3[Fe(CN)_6]$).

Plant material

The *Rubus niveus* fruit was picked in Los Teques, Miranda State, Venezuela, at an elevation of 1,200 meters. Mature, dark purple fruits were collected and stored at -10°C.

It should be noted that the *R. niveus* plant used in this study was botanically certified by the Ecology Center of the Venezuelan Institute of Scientific Research (IVIC).

UV-Vis Spectrum

For the UV-Vis spectrum, 1 ml of the concentrated extract was dissolved in 3 ml of ethanol. The spectrum was recorded using a Perkin Elmer Lambda 35 spectrometer.

Monomeric anthocyanins quantification

The pH-differential method employed by (Lee et al., 2005) was used to quantify the total monomeric anthocyanins in black raspberry extract. One milliliter of the extract was diluted in three milliliters of the respective buffer (sodium acetate or potassium chloride), and three consecutive measurements of the solution were taken at the corresponding wavelength. ΔAbs values were obtained for each measurement, and the total anthocyanin values in milligrams per liter (mg/l) were calculated using the following formula:

$$Anthocyanin\ content\ (mg/l) = \frac{\Delta Abs\ x\ MW\ x\ DF\ x\ 1000}{\varepsilon\ x\ l}$$

(1)

Where ΔAbs = absorbance difference; MW is the molecular weight of cyanidin-3-glucoside (449.2 g/mol); DF is the dilution factor (in this case 4); l is the optical path length of the UV-Vis quartz cell; ε is the molar extinction coefficient of cyanidin-3-glucoside (26.900). These measurements were taken by triplicate. Note that the expression "ε x l" is the molar extinction coefficient multiplied by the path length of the UV-Vis quartz cell (1 cm).

On the other hand, in order to compare anthocyanin content values reported in previous research, the total anthocyanin value was calculated in milligrams per 100 grams of fresh weight (mg/100 g) using the following formula:

$$Anthocyanin\ content\ (mg/100\ g) = \frac{\Delta Abs\ x\ MW\ x\ DF\ x\ V\ (lts)\ x\ 1000}{\varepsilon\ x\ l\ x\ m(grams\ of\ sample)} x\ 100$$

(2)

Where: ΔAbs = Absorbance difference; MW= Molecular weight of cyanidin-3-glucoside (449.2 g/mol); V = Final volume of the extract (liters); m = Mass of the sample (grams); l= Path length of the UV-Vis quartz cell (1 cm); DF = Dilution factor (4 in this case); ε = Molar extinction coefficient of cyanidin-3-glucoside (26,900). These measurements were taken by triplicate. Note that "ε x l x m" is the molar extinction coefficient multiplied by the path length of the UV-Vis quartz cell (1 cm) and the mass of the sample.

Photostability assays

To evaluate the photostability of the black raspberry extract, 2 ml of the concentrated

ethanolic extract was mixed with 4 ml of distilled water. Absorbance changes were measured using a spectrophotometer after irradiating the mixture for 15 minutes with a Luzchem L2C-4V solar simulator (visible light range). The extract was irradiated in a quartz flask, and the respective changes in absorbance were measured using quartz cells for UV-visible spectrophotometry

Reactions with singlet oxygen ($^1O_2$)

The reaction of black raspberry ethanolic extract with singlet oxygen was measured by observing the decrease in absorbance of the extract under visible irradiation (227 LUX; 1.88 W/m²) and in the presence of a type II photosensitizer (Rose Bengal). To perform this experiment, a solution was prepared with 2 ml of black raspberry extract and 2 ml of distilled water, to which 50 microliters of Rose Bengal (~$2x10^{-4}$ M) were added. The total irradiation time was 3 hours, and changes in absorbance were measured in the UV-Vis spectrophotometer after 1 hour and 2 hours of irradiation.

Chemiluminescence assays

To assess the neutralization activity of hydroxyl radicals and peroxides by *Rubus niveus* fruit, chemiluminescence experiments were conducted, based on the reaction of luminol with ROS (Vargas et al., 2004). For this purpose, 10 microliters of the antioxidant (ethanolic extract of black raspberry and vitamin C for comparison) and 6 microliters of hydrogen peroxide (~3.5 mM) were taken. The intensity of chemiluminescent light was measured in a Luminoskan Ascent Luminometer every 10 seconds for a total time of 40 seconds, after the automatic injection of luminol (15 microliters). Subsequently, these same tests were carried out but in the presence of ferrous iron with the addition of 15 microliters of $K_3[Fe(CN)_6]$ (~40 nM), in order to generate hydroxyl radicals and study the scavenging capacity of these radicals by the *R. niveus* extract.

The percentage of inhibition of H_2O_2 and OH radicals was calculated using the following formula:

$$\%\ inhibition = \frac{1\ control - 1\ sample}{1\ control}\ x\ 100 \quad (3)$$

The total percentage of inhibition was obtained by comparing the samples studied to the control by measuring the intensity of the light of the samples and the control at every point and applying formula 3). The final percentage of inhibition reported was the mean of all inhibition percentages calculated at each point.

Radical scavenging activity

The radical scavenging capacity was studied using galvinoxyl and DPPH radicals by UV-Vis spectrophotometry, using the methods of (Bobinaitė et al., 2011; Gulcin and Alwasel, 2023) as references. For the DPPH radical assays, the free radical scavenging potential (RSC) was calculated 30 minutes after reaction with *Rubus niveus* extract (100 microliters) with 3 milliliters of DPPH radical (~10^{-4} M). Three consecutive measurements (triplicate) were taken and compared with the RSC_{30} value of vitamin C. The RSC of berry extracts was expressed as a percentage of inhibition of the DPPH radical, according to the following formula:

$$RSC = \frac{Absorbance\ of\ Blank\ Solution\ -\ Absorbance\ of\ Sample}{Absorbance\ of\ Blank\ Solution} x100\% \quad (4)$$

On the other hand, the reaction of black raspberry extract with the galvinoxyl radical was carried out using a solution of the same at a concentration of approximately 10^{-4} M. The titrations were performed with 3 ml of this solution, with 3 successive additions of 15

microliters of pure *R. niveus* extract. The reaction was carried out using UV-Vis spectrophotometry and fluorescence (using excitation spectrum).

Note that RSC was calculated after 30 minutes of reaction because it is a standardized method stipulated in the bibliography. Most RSC values reported in previous research were calculated using this time frame.

Statistical Analysis

To validate the results and distinguish the neutralizing effects in the chemiluminescence and DPPH assays, statistical analyses were performed using unpaired t-test with statistical analysis free-software (GraphPad). This analysis determined if there were significant differences between the compounds being compared, in this case, black raspberry extract and vitamin C.

Results and Discussions

Figure 1 shows a characteristic absorption spectrum of black raspberry ethanol extracts with a band at 543 nm, which is the typical wavelength of anthocyanin molecules according to references (Dangles and Fenger, 2018; Aguilera-Otíz et al., 2011). Meanwhile, experiments to quantify the anthocyanins in *Rubus niveus* extract using the differential pH method yielded a result of 128.68±0.85 mg/L of total anthocyanins (0.85 being the standard deviation of the measurements taken in triplicate). **Figure 2** show the photostability tests of the ethanolic extract of *R. niveus* solar irradiation (using solar simulator). This value is equivalent to 48.19±0.31 mg/100 g of fresh weight.

Figure 3 shows the activity of *R. niveus* extract against H_2O_2 and its comparison with vitamin C. **Figure 3.a** shows the decrease in chemiluminescent light intensity when *R. niveus* extract and vitamin C are added, compared to the H_2O_2 control. **Figure 3.b** shows the percentage inhibition of H_2O_2 according to the results shown in **figure 3.a**.

To evaluate the activity of the ethanolic extract of *R. niveus* against ·OH radicals, a chemiluminescence experiment was carried out, induced by the addition of ferrous iron. The results in **figure 4** show the intensity of chemiluminescent light detected after the addition of the antioxidants studied for comparison.

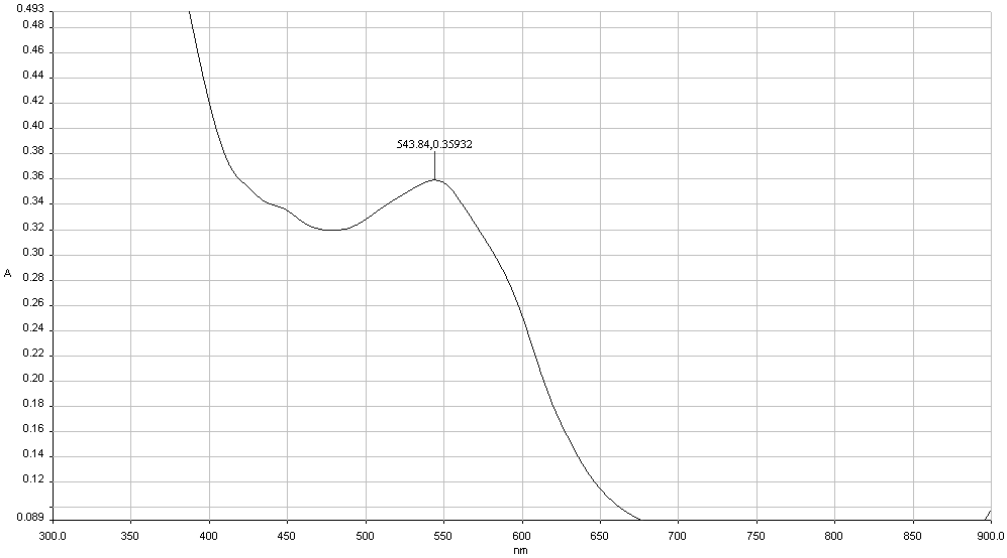


Fig. 1. Absorption spectra of black raspberry extracts in ethanol (1 ml concentrated extract + 3 ml ethanol)

A higher intensity of chemiluminescent light was observed after the addition of vitamin C than with the *R. niveus* extract, demonstrating the scavenging capacity of these antioxidants against the ·OH radical.

The RSC for *R. niveus* extract was 59.98±3.78, and 70.56±1.26 for vitamin C (where 3.78 and 1.26 represent the standard deviation of the triplicate measurements made for each respective case). **Figure 5** shows the scavenging activity of the *R. niveus* extract and its comparison with vitamin C in graphical form.

Figure 6 shows the reaction of black raspberry ethanolic extract with the galvinoxyl radical. It can be seen that the absorption spectrum of this radical has two major absorption points: one at ~380 nm and another at ~430 nm. Furthermore, it is evident that, with the addition of *R. niveus* extract, the absorption intensity at ~380 nm increases, while that at ~430 nm decreases.

On the other hand, **figure 7** shows the course of the reaction of *R. niveus* extract with the galvinoxyl radical using a fluorescence spectrum (with excitation wavelength at 350). It can be seen that the intensity of the excitation light from galvinoxyl radical decreases after addition of black raspberry extract.

Figure 8 shows the reaction of black raspberry extract with singlet oxygen in the presence of Rose Bengal (a type II photosensitizer). The reaction shows that, after irradiation for 2 hours, the wavelength decreases in intensity, with a shift from 544 nm to 549 nm. A similar situation occurs after irradiating for an additional hour, as the wavelength remains at ~549 nm with a corresponding decrease in intensity.

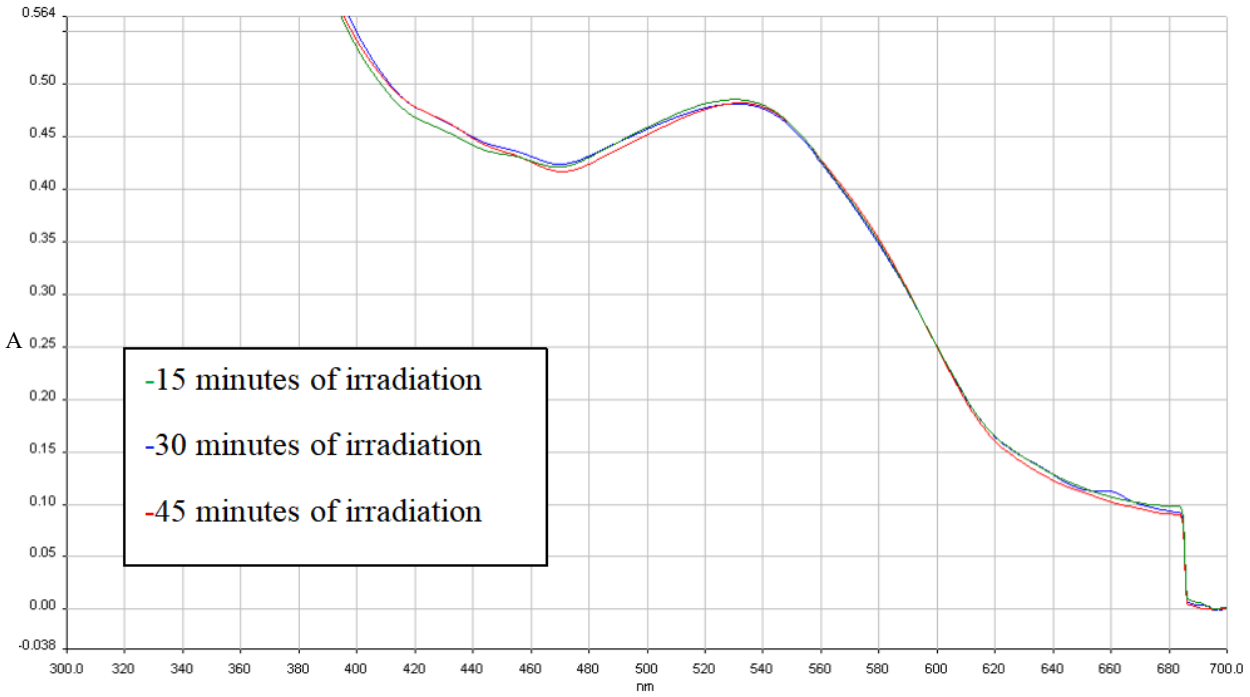
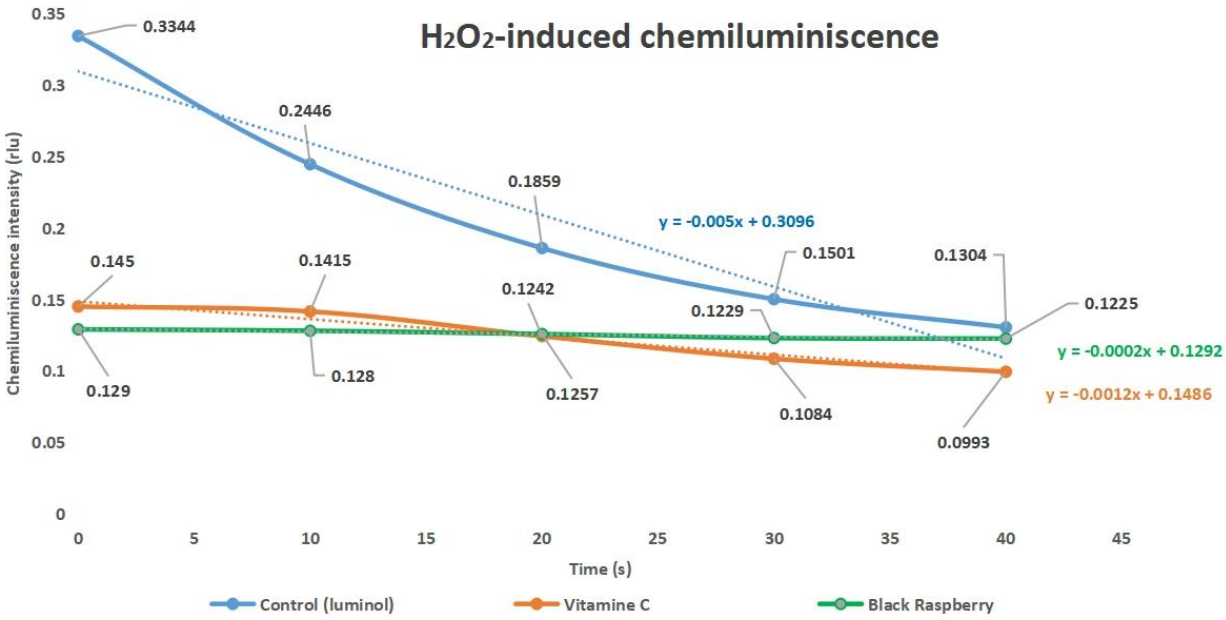
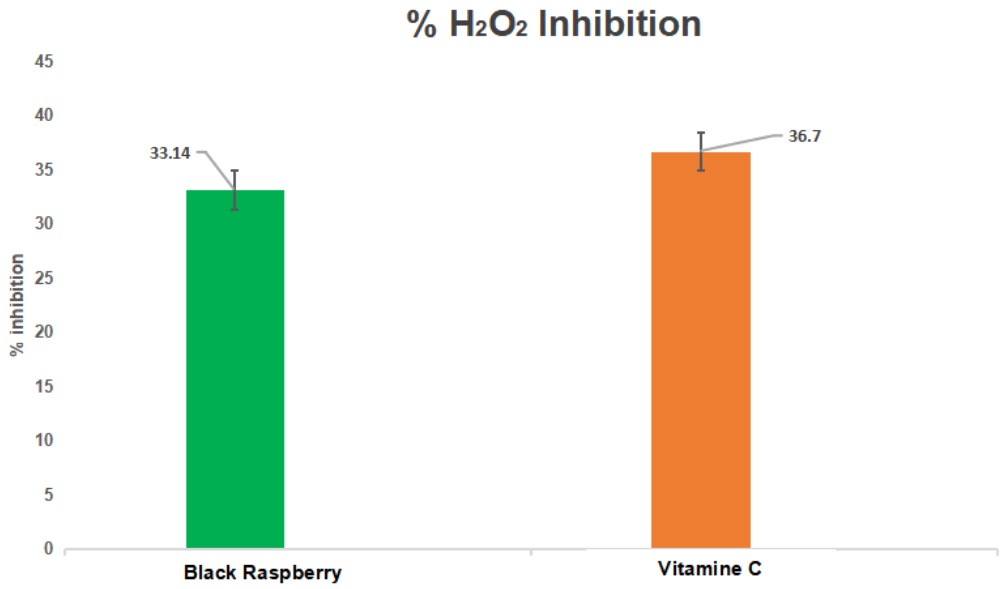


Fig. 2. Photostability tests of raspberry extract under irradiation with a solar simulator

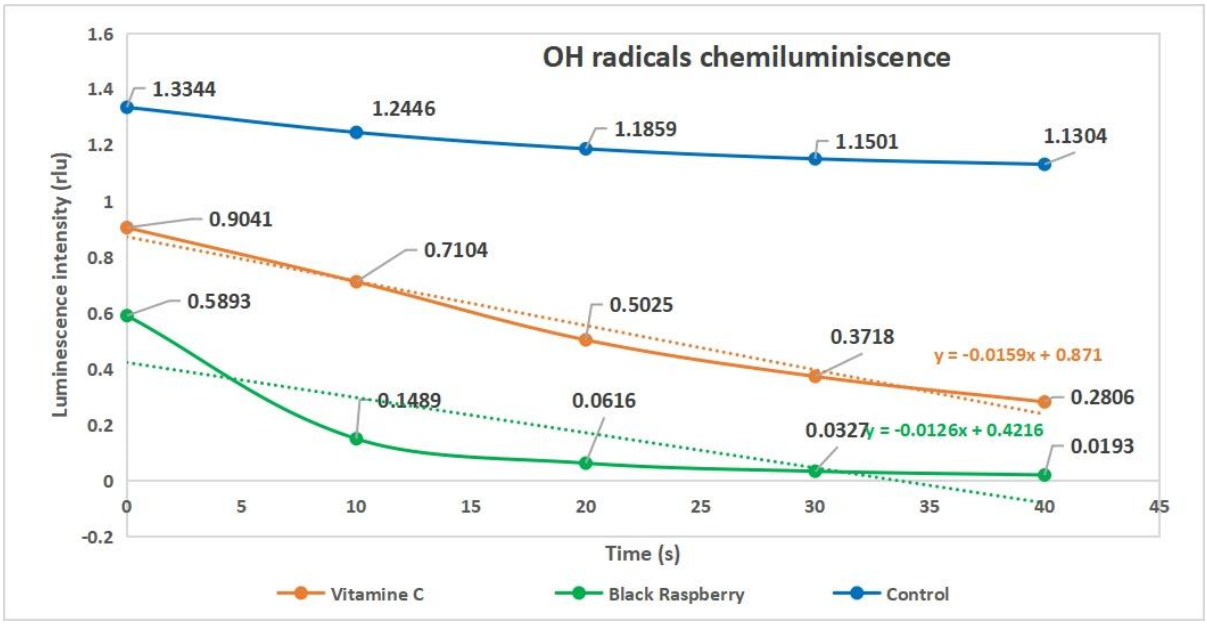


(a)

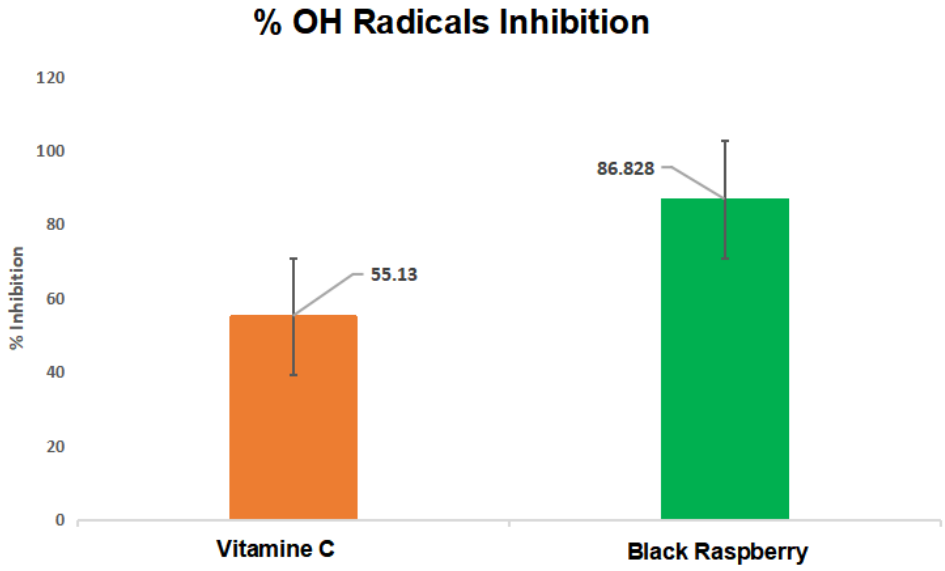


(b)

Fig. 3. H_2O_2-induced chemiluminescence assays



(a)



(b)

Fig. 4. Ferrous iron-induced chemiluminiscence

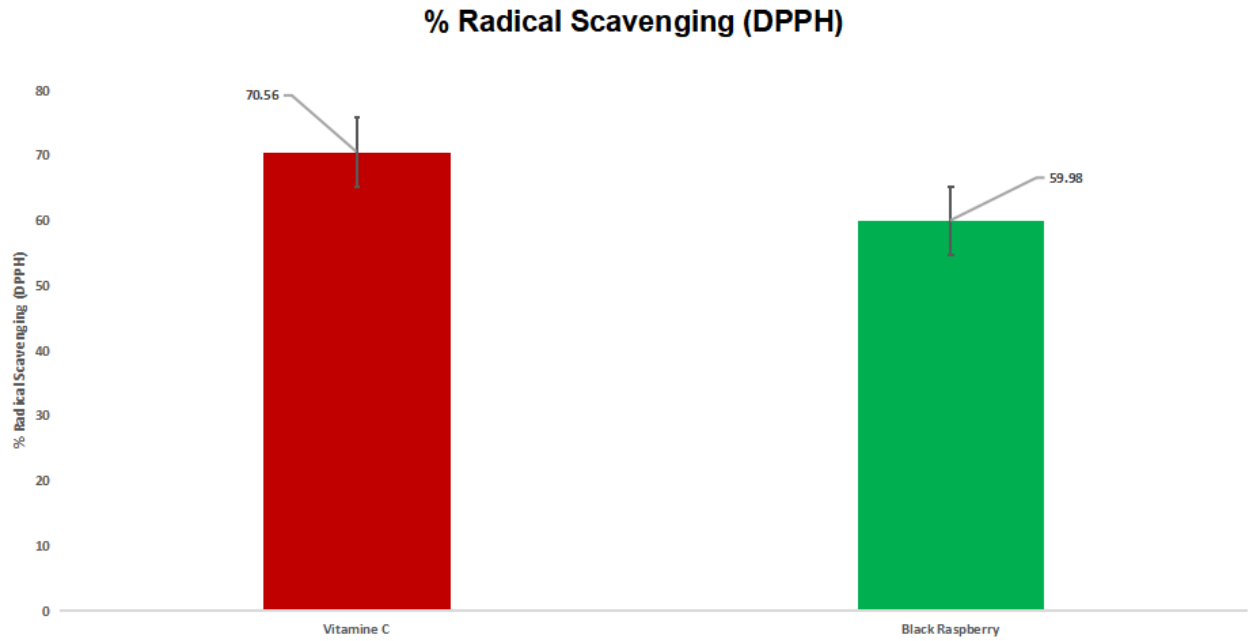


Fig. 5. Radical scavenging (RSC) from black raspberry extract and vitamin C

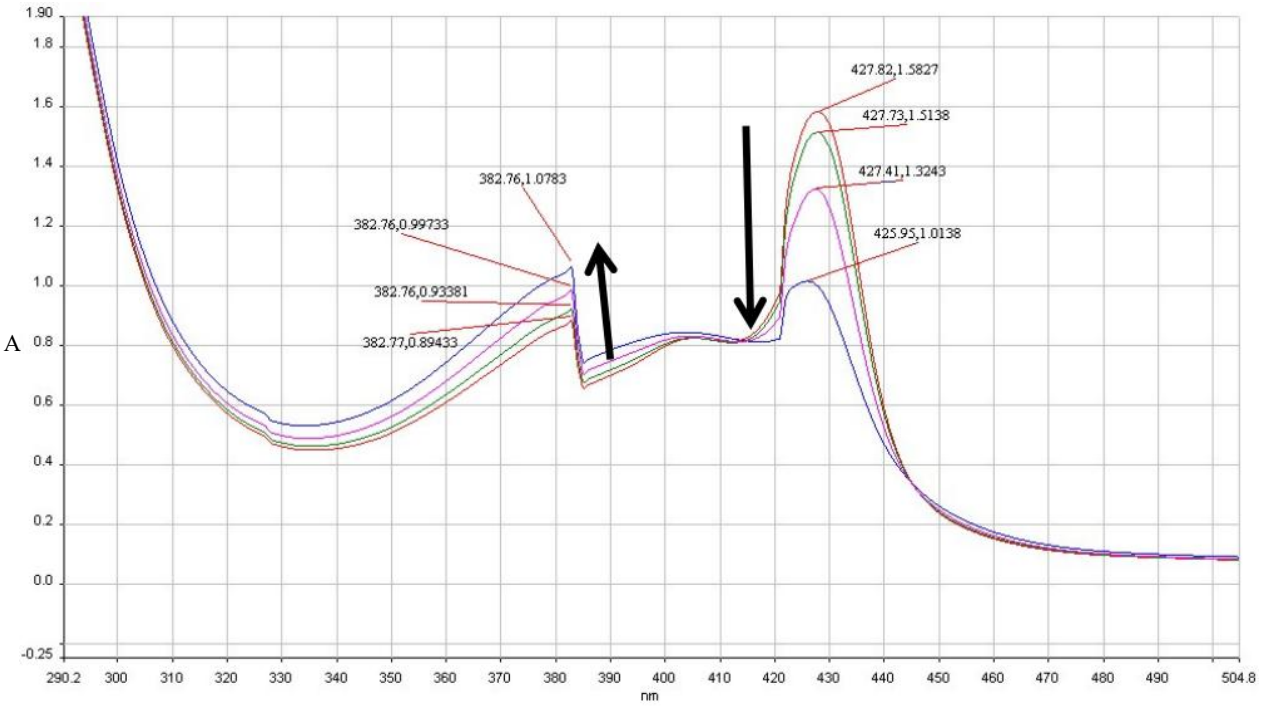


Fig. 6. Reaction of black raspberry extract with galvinoxyl radical followed by UV-VIS spectroscopy

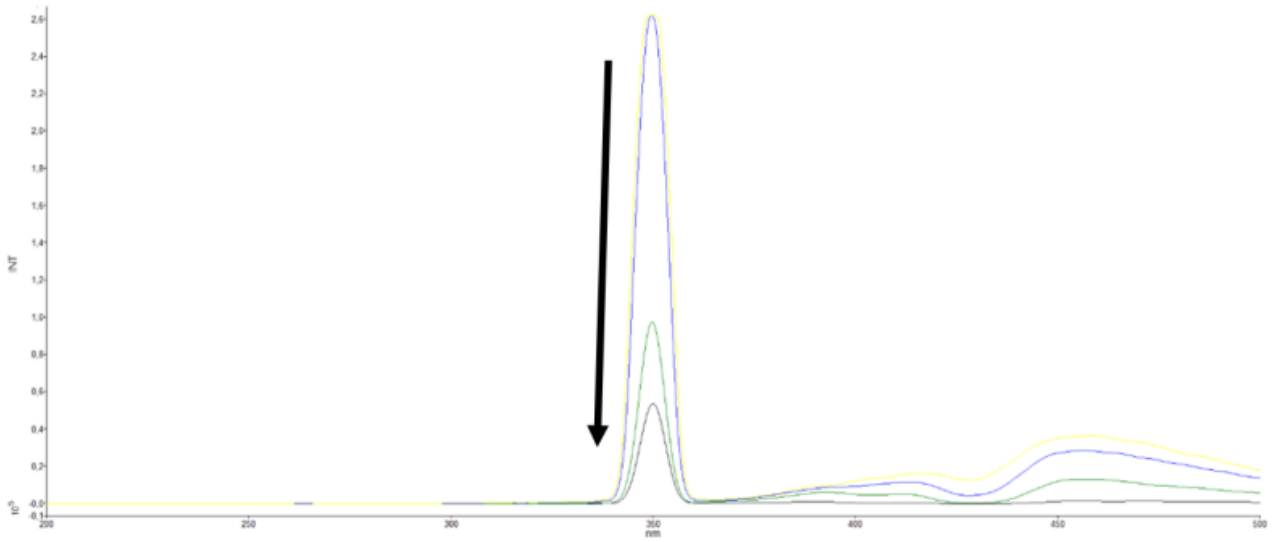


Fig. 7. Reaction of black raspberry extract with galvinoxyl radical using excitation band

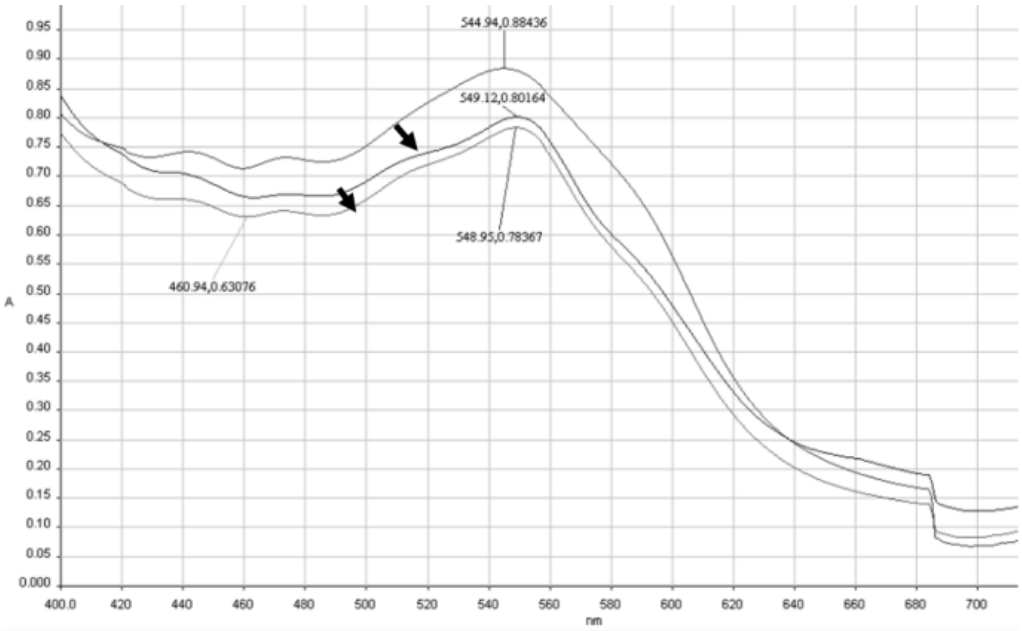
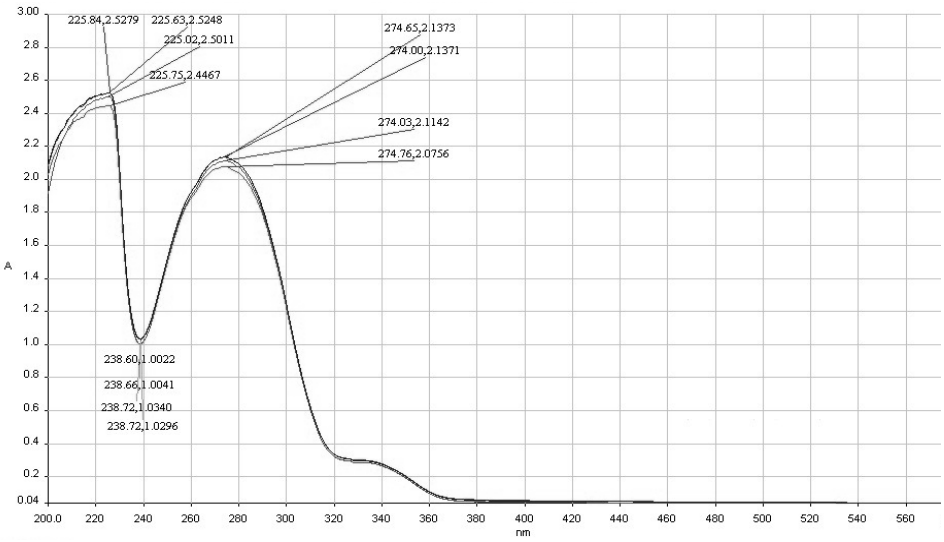


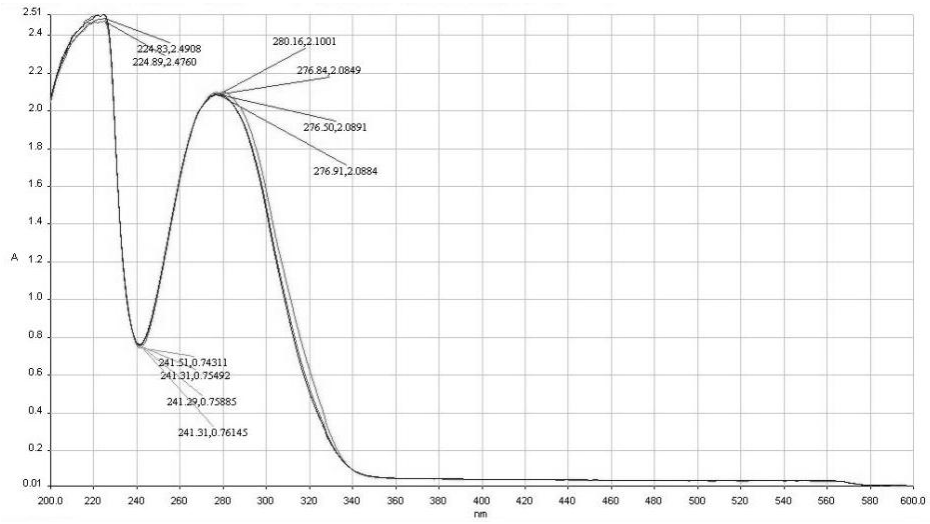
Fig. 8. Decrease in the maximum absorbance of black raspberry extract in the presence of Rose Bengal (~2x10^{-4} M) after irradiation for 2 hours + 1 hour with white light.

It should be noted that the results presented in **figure 2** offer compelling evidence for the photostability of black raspberry extract (*R. niveus*), as evidenced by the minimal degradation observed at the maximum wavelength following irradiation with solar simulator, indicating that there is not photodegration processes during irradition (Ahdmad et al., 2016), suggesting the stability capacity of *R. niveus* extract under solar irradiation.

As illustrated in **figures 9.a** and **9.b**, the photostability behavior of common sunscreen filters under visible radiation (i.e., solar simulator) is evident. The filters in question include Neo Heliopan type Ma (menthyl anthranilate) and Neo Heliopan type OS (Octisalate, 2-Ethylhexyl salicylate).



(a)



(b)

Fig. 9. Photostability under Visible radiation of (a) Neoheliopan Type Ma and (b) Neoheliopan Type OS

The response of each constituent, dissolved in dichloromethane due to the lack of solubility of these compounds in water-ethanolic mixtures, to visible light was minimal following irradiation, as demonstrated by negligible shifts in the maximum absorption.

A comparison of the results obtained with the irradiation of black raspberry extract reveals that the polyphenols present in *R. niveus* fruit exhibit a photostability against solar simulator radiation analogous to that observed for sunscreen filters (**Figure 9**).

The latter suggests the potential of ethanolic *R. niveus* extract to provide photostability *in vitro*.

On the other hand, the antioxidant potential of the *R. niveus* ethanolic extract was evidenced by its capacity to diminish the presence and intensity of reactive oxygen species, specifically H_2O_2 and OH radicals. In reactions against these radicals, a comparison with vitamin C reveals discrepancies in their efficacy. The *R. niveus* extract demonstrated a lower percentage of inhibition for H_2O_2 (33.14%) in comparison to vitamin C (36.7%), as illustrated in Figure 3.b. However, while the

capacity to neutralize H_2O_2 is reduced, *R. niveus* has been observed to have a greater potential to inhibit ·OH radicals, as shown in figure 4.b **(**86.82% of inhibition for *R. niveus* and 55.13% of inhibition for vitamin C**).** This phenomenon can be attributed to the observation that the chemiluminescent light intensity in the presence of the extract is lower in comparison to the reaction with vitamin C (**Figure 3.a**). These findings suggest that the phenolic compounds present in the *R. niveus* fruit have an exclusive affinity for highly reactive radicals, which may be fundamental to preventing damage caused by oxidative stress (Speer et al., 2020).

In addition, the scavenging capacity of *R. niveus* extract against the galvinoxyl radical is significant, since the absorbance of the radical decreases at 430 nm and increases at 370 nm. This suggests that a transformation of a more reactive radical (galvinoxyl) to a less reactive one does indeed occur, promoted by the ·OH functional groups commonly found in phenolic compounds, which contribute to the antioxidant potential of *R. niveus* extract. Additionally, assays with the DPPH radical show that the RSC was 59.98%, which, although lower than the 70.56% for vitamin C, indicates significant potential for free radical scavenging (Bobinaitė et al., 2012; Gulcin and Alwasel, 2023).

The significance of the previous results is evident in **Table 1**. The differences in the results can be explained by analyzing the p-value; $p<0.05$ indicates a statistically significant difference in the experimental results. In this case, there was no significant difference in the H_2O_2 assay. Thus, despite the different percentages of H_2O_2 inhibition (33.14% for black raspberry and 36.7% for vitamin C), vitamin C and black raspberry exhibit similar behavior in neutralizing peroxides. However, according to the slopes of the equations in **figure 3a**, vitamin C has a more stable capacity to neutralize peroxides over time. Black raspberry, on the other hand, exhibits faster inhibition at the initial stage of the chemiluminescent reaction. This suggests that the inhibitory effect of vitamin C is more sustained than that of black raspberries in neutralizing. In other words, black raspberries tend to have a faster initial effect on neutralizing peroxides than vitamin C, although the neutralization capacity of black raspberry decays over time. However, significant differences were observed in the OH radical assays, confirming that black raspberry extract has a stronger capacity to scavenge OH free radicals *in vitro*. The calculated difference is statistically significant: 86.828% for black raspberry versus 55.13% for vitamin C. Therefore, black raspberry can be assumed to be a potent antioxidant capable of quickly and effectively neutralizing a large number of OH radicals (see the equation of lines in **figure 4.a**). However, the DPPH assay results indicated that vitamin C (70.56% of inhibition) has stronger scavenging activity against DPPH radicals than black raspberry extract (59.98% of inhibition).

Table 1. Unpaired t-test results analysis

	T-test	
	T value	**P value**
H_2O_2 assay	0.3090	0.7652
OH assay	2.8350	0.0220
DPPH assay	6.2586	0.0033

These differences can be explained on a structural basis (Kim and Lee, 2004; Yamauchi et al., 2024). While discussing these contradictory results is beyond the scope of this research, they can be attributed to the specificity of polyphenols and vitamin C against DPPH molecules and free OH radicals. This assumes greater specificity of vitamin C and DPPH radicals than the polyphenolic components of black raspberry fruit.

Thus, it is necessary to consider the reaction of black raspberry extract with the galvinoxyl radical. Fluorescence studies using excitation bands (in this case at 350 nm) indicate that the reaction follows both a scavenging mechanism and a quenching mechanism. This is due to the fact that the excitation band of the galvinoxyl radical, as detected by the instrument, decreases following the addition of the ethanolic extract of *R. niveus*. In this regard, it is proposed that the black raspberry extract may function as a quencher of the excited states of free radicals. These mechanisms may be analogous to those previously suggested in studies related to free radical reactions and quenching (Khudyakov, 2023; León and Tovar, 2025). It is important to note that the excitation spectrum of the galvinoxyl radical was selected because it is a radical species whose fluorescent emission is not detectable with conventional equipment, although fluorescent species derived from this radical, such as the galvinoxylate anion, may exist (Grilj et al., 2012).

Conversely, the reaction of the ethanolic extract with singlet oxygen demonstrate that *R. niveus* is susceptible to the presence of this free radical, as evidenced by a decrease in the maximum wavelength after each measurement. While this behavior may compromise the integrity of the ethanolic extract, it may be advantageous for biological applications. The presence of compounds in black raspberry fruit that react with $^1O_2$ suggests that this ROS may dissipate over time, and protect from damage that is usually attributed to this type of ROS (Tournaire et al., 1993; De Rosso et al., 2008).

A comparison of the results of this study with those of previous research reveals that the anthocyanin content was lower than the values reported in previous studies of *Rubus niveus* fruit. This study obtained values of 17.09 mg/100 g (León and Soledad, 2020) and 5.63 mg/100 g (Badhani et al., 2015), whereas the previous study obtained a value of 48.19 mg/100 g of fresh weight. These differences can be attributed to different solvents, calculated dilution factors, and extraction conditions. The ripeness of the selected fruits, as well as picking and conservation conditions (e.g., elevation of the cultivation area, climate, and storage), may also influence the reported anthocyanin content in *R. niveus* fruit (Maro et al., 2013).

Additionally, it is well established that *R. niveus* has a higher anthocyanin content and radical scavenging capacity than the wild yellow raspberry, *R. ellipticus*. For instance, Ahmad et al. (2015) demonstrated that the DPPH radical scavenging capacity of cultivated Himalayan *R. niveus* berries was 68.30%, while the capacity of wild *R. ellipticus* berries was 45.97%. However, inhibition of *R. ulmifolius* berries was higher (80.28%), and the scavenging potential of *R. niveus* was higher than the vitamin C standard (53.73%). The differences between this study and our research can be explained by the different cultivation and extraction procedures; the latter study used methanolic extracts. In our study, we employed ethanolic extracts of *R. niveus*, and the fruits were collected in an area at a lower altitude than the Himalayan mountains. This difference in altitude may significantly affect the content of polyphenolic components in the fruits (Maro et al., 2013).

A comparison of the properties of *R. niveus* and the commercially available black

raspberry, *Rubus occidentalis*, reveals similar levels of anthocyanin compounds in both species. Total amounts of these molecules have been reported to range from 20 to 216 mg/100 g of fresh fruit and 1,770 mg/100 g of freeze-dried fruit. Once again, it is evident that cultivation, storage, and extraction conditions significantly impact the availability of phenolic compounds (Maro et al., 2013). In addition, different RSC_{30} values were observed in black raspberry varieties (*R. occidentalis*) grown in Lithuania, ranging from 25 to 80%, depending on the variety (Viškelis et al., 2010). Despite the different locations where the fruit was grown and harvested, as well as the different extraction conditions used in each case, these tests were carried out with methanolic extracts and obtained values close to those reported in this study (59.98% RSC_{30} for *R. niveus*).

Additionally, ethanol extracts of Korean black raspberry (*R. coreanus*) exhibited RSC activity, demonstrating 60-80% inhibition. These differences are primarily due to the concentration of ethanol (EtOH) used: the extract with 100% EtOH exhibited the lowest activity, while the extract with 50% EtOH exhibited the highest activity. These results show that the type of solvent is closely related to the *in vitro* antioxidant activity of natural black raspberry extracts (Kim et al., 2014).

This study reports the values of wild *R. niveus* fruit and compares them to those of other berry sources. This comparison highlights the fruit's significant antioxidant potential, particularly its capacity to scavenge radicals like DPPH. However, this capacity is slightly lower than that of vitamin C in this specific assay (59.98% vs. 70.56%). Furthermore, despite the differences in methodology and cultivation conditions, the anthocyanin content is within the expected range and comparable to that of other blackberry sources. For example, *R. occidentalis* and *R. coreanus* have reported anthocyanin content values of 6.7, 165, and 312.2 mg/100 g of fresh weight, depending on the genotype of the fruit (Ku and Mun, 2008).

In summary, the ethanolic extract of *Rubus niveus* fruit consists primarily of anthocyanin molecules, as demonstrated by UV-Vis spectra and the differential pH method (which can only detect monomeric anthocyanins). These flavonoid compounds are well known for their high antioxidant capacity (especially against OH radicals, that was significantly higher than vitamin C), suggesting that the scavenging activity demonstrated in the results of this study is mainly due to this class of molecules, although other compounds present in black raspberries, such as catechins or tannins, may also play an important role in photostability and antioxidant potential (Ahmad et al., 2015).

Conclusions

The ethanolic extract of black raspberry fruit (*Rubus niveus*) is a source of flavonoid compounds, especially anthocyanins, as demonstrated by UV-Vis spectroscopy and the differential pH method. This property is crucial for its high antioxidant capacity against reactive oxygen species (ROS), including H_2O_2, OH, and $^1O_2$. This behavior is complemented by the scavenging and quenching potential demonstrated by the extract against galvinoxyl and DPPH radicals. In addition, the results provide a comprehensive explanation for the photostability of the *R. niveus* extract under solar light, after comparison with spectral changes of common sunscreen filters. Future research will be conducted in our laboratory to determine the different types of flavonoids and other natural components present in *R. niveus* fruit, which might be of photochemical and photobiological interest.

Despite these findings, it is important to acknowledge the limitations of this research. The extraction solvent, dilution factors, and environmental conditions (e.g., altitude,

climate, fruit ripeness, and storage conditions) in which the fruit was collected and processed may impact the specific anthocyanin content obtained, as well as the observed scavenging and quenching activities. These factors may also affect the generalization of quantitative comparisons with other studies. Additionally, although anthocyanins are recognized as significant contributors, providing a comprehensive description of all the flavonoid types and other bioactive substances present in the extract was beyond the scope of this study.

Finally, research on formulation studies, bioavailability, mechanistic pharmacology, dermatological models, and stability in pharmaceutically relevant matrices would be desirable. A deeper comparison with established photoprotective actives would provide mechanistic insights at the chemical, cellular, and molecular levels. Therefore, further *in vitro* and *in vivo* research is essential to corroborating the antioxidant and photoprotective mechanisms of *R. niveus* for biological and product applications.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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