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Radical Scavenging Activity of Polyphenolic Contained in the Black Raspberries. An Assay on Absorption and Emission Spectra of Galvinoxyl Radical †

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Abstract

Black raspberries exhibit polyphenolic radical scavenging activity. It was carried out through the assays on galvinoxyl radical absorption and emission spectra. Black raspberries are rich in polyphenols, such as anthocyanins and ellagic acid, which contribute to their potent antioxidant activity. These polyphenols effectively neutralize free radicals such as the galvinoxyl radical, as demonstrated by studies that have measured their neutralization capacity. Galvinoxyl radical absorption and emission spectra were used to evaluate the efficacy of these antioxidants in neutralizing the radical via quenching and/or scavenging. These spectra and also chemiluminescence assay provide information about the interaction between the antioxidant extracts and the reactive oxygen substances (ROS). The position and number of hydroxyl groups in polyphenols can influence their scavenging ability, with ortho-hydroxy positions often being more effective. Polyphenols in black raspberries can donate electrons or hydrogen atoms to free radicals, neutralizing them and preventing oxidative damage. Polyphenols, particularly flavonoids, can effectively scavenge free radicals due to their structural features, such as the presence of a 3′,4′-dihydroxy group (catechol structure). Black raspberries extract showed an efficient scavenging activity in darkness of galvanoxyl radical in ethanolic solutions. The reactivity of this extract toward galvanoxyl (a model phenoxyl radical) in ethanol solution was also investigated.

Keywords: radical scavenging; polyphenols; black raspberries; galvinoxyl radical; antioxidant

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1. Introduction

Raspberries are a widespread group of plants. They originate mainly from Asia, though they have been cultivated in North America for decades, and are highly resistant to pathogens and are characterized by their high dietary and commercial value [1]. These characteristics, together with their botanical features, make raspberries an important subject of scientific research [2,3].

Raspberry fruits can be red, purple, or black depending on the species. In any case, raspberries have been recognized for their medicinal properties due to the presence of bioactive components, such as anthocyanins, catechins, and flavonols, which can

neutralize reactive oxygen species (ROS). Therefore, various studies have suggested the antioxidant, anti-inflammatory and anti-carcinogenic potential of raspberries, which also play an important role in maintaining health and preventing disease [4,5].

In this context, in vitro assays are essential for studying and analyzing the ROS scavenging capacity. Galvanoxyl radical emission and absorption studies, in particular, are a viable alternative for estimating the behavior of natural extracts against ROS due to the high sensitivity and reactivity of this free radical, as well as the relative ease and speed with which these experiments can be carried out [6].

Although a great deal of research has been conducted on the antioxidant capacity of *Rubus niveus*, most of this has focused on parts of the plant other than the fruit, such as the roots and stems. In fact, the biological activity of this species (including its antipyretic and antidiabetic properties, among others) is attributed to the organic extracts of its stems and roots [7,8], and not to its fruit, which contains most of the polyphenols. Therefore, this study aims to analyse the ROS scavenging capacity of black raspberry fruit extract (*Rubus niveus Thumb*.) using galvinoxyl radical tests. UV-Vis and fluorescence equipment will be used to observe changes in galvinoxyl light intensity (absorbance and emission) in the presence of the extract. These experiments represent a first approach to the antioxidant potential of the fruit of this plant species and are an essential precursor to future research in our laboratory.

Notably, this study is novel since it shows the use of UV-Vis and fluorescence spectroscopic techniques to directly evaluate the scavenging potential against the galvinoxyl radical in the fruit of *Rubus niveus*. While the properties of polyphenolic compounds in Rubus plant genera are well understood, this research provides information on how these components interact with the galvinoxyl radical at a molecular level using spectroscopic evidence. This specific evaluation against galvinoxyl provides new experimental evidence that helps improve our understanding of the antioxidant mechanisms of *Rubus niveus*, especially in extracts of its ripe fruit.

2. Materials and Methods

2.1. Materials and Equipment

Perkin Elmer Lambda 35 UV-Vis Spectrophotometer, Perkin Elmer LS 45 Fluorescence Spectrometer, galvinoxyl radical, Black raspberries (Rubus niveus Thumb), Ethanol. Most of these materials and equipment were purchased from Sigma Aldrich (St. Louis, MO, USA) and Perkin Elmer (Waltham, MA, USA)

2.2. Methods

2.2.1. Black Raspberry Ethanolic Extract Preparation: Solid-Liquid Extraction

The solid-liquid extraction process involved combining 2–3 g of frozen black raspberries with 20 mL of ethanol in a mortar. After resting for 10 min, the mixture was filtered, yielding a raspberry extract of approximately 10 mL.

2.2.2. UV-VIS and Fluorescence Spectra of Ethanolic Extract

For the UV-Vis and fluorescence spectra, one milliliter of concentrated extract dissolved in three milliliters of ethanol was used. The fluorescence spectra were recorded using a PerkinElmer FL 6500 fluorescence spectrometer, and the UV-Vis spectra were recorded using a PerkinElmer Lambda 35 spectrometer.

2.2.3. Reaction with Galvinoxyl Radical: UV-VIS and Fluorescence Study

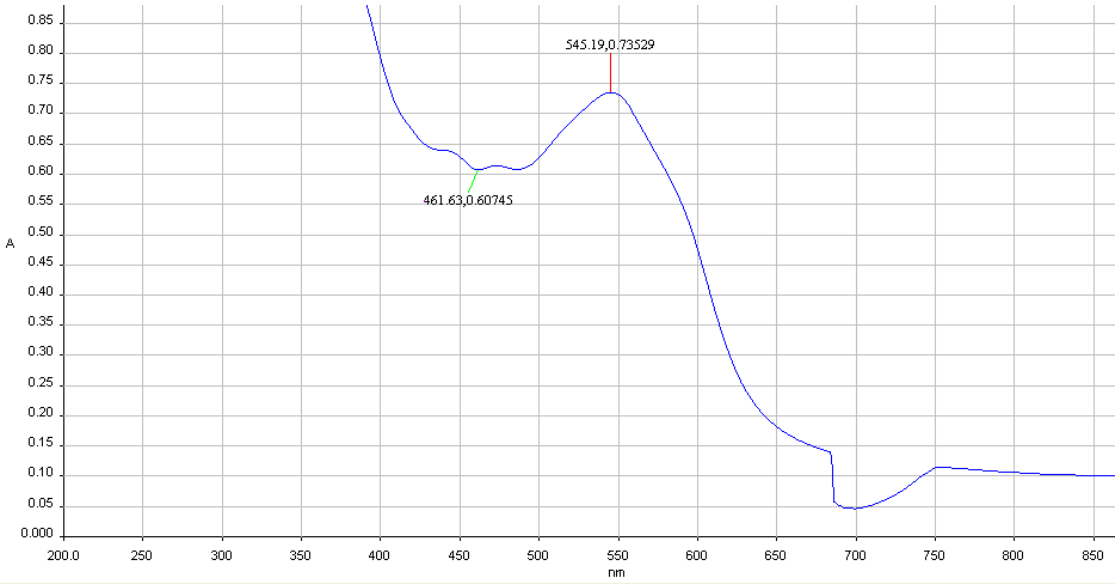
An ethanolic solution of the galvinoxyl radical (10^{-5} M) was prepared and successive titrations were carried out by adding 15 microliters of black raspberry extract. The

reduction of the galvinoxyl radical was measured at 427 nm every two minutes after adding the *Rubus niveus* ethanolic extract using a PerkinElmer Lambda 35 UV-Vis instrument. The same procedure was carried out for the emission studies using a full-scan method with a PerkinElmer FL 6500 fluorescence spectrometer.

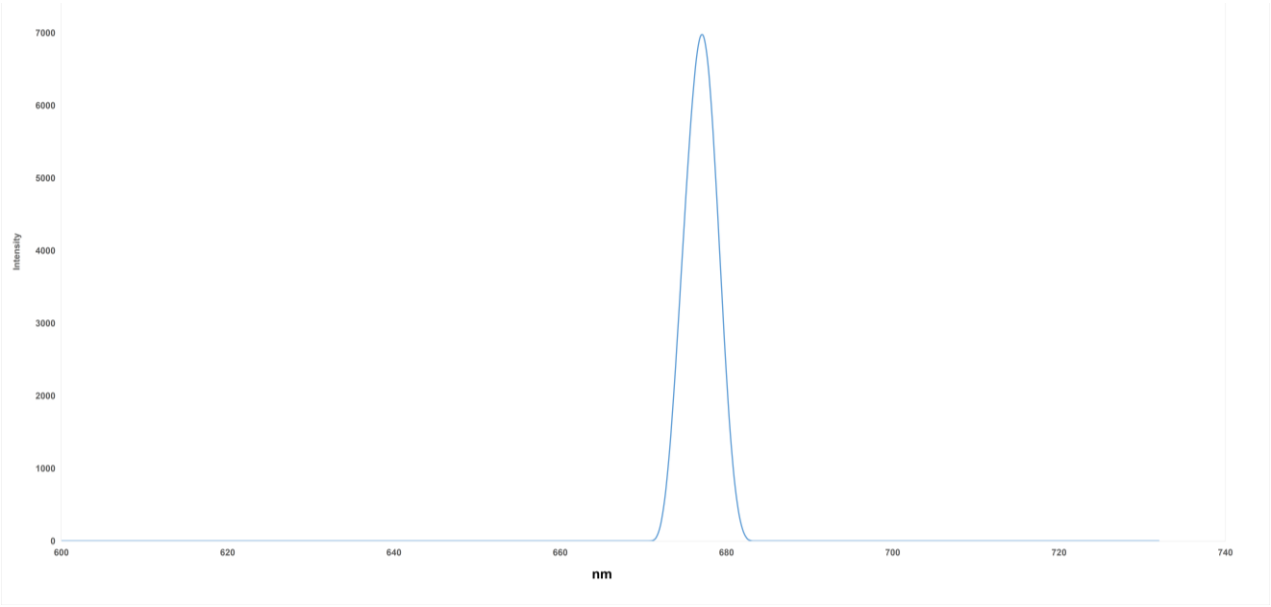
3. Results and Discussion

3.1. Black Raspberry Extract: Characterization

The UV-Vis spectrum of the ethanolic extract of black raspberry (*Rubus niveus*) is shown in Figure 1a, which shows an absorption band at 545 nm, corresponding mainly to the presence of anthocyanin compounds, which is to be expected given the dark purple colour of the extract. In addition, the emission spectra show a fluorescent emission band close to 680 nm (see Figure 1b). This demonstrates that black raspberry extract contains molecules that dissipate absorbed energy in the form of heat (non-radiative mechanisms) and also through radiative mechanisms, such as fluorescence [9,10].



(**a**)



(**b**)

Figure 1. (**a**) UV-Vis spectrum of black raspberry ethanolic extract and (**b**) fluorescence spectrum of black raspberry ethanolic extract.

On the other hand, gas chromatography and mass spectrometry (GC-MS) spectra were obtained from the ethanolic extract of *Rubus niveus* (see Figure 2a,b). With the help of the database and the fragmentation observed in the mass spectrum, it was possible to identify some compounds derived from furfural, furancarboxaldehyde, pyran and phenols, which are typical fragments of flavonoid-derived compounds (see Supplementary Materials Figures S1–S5).

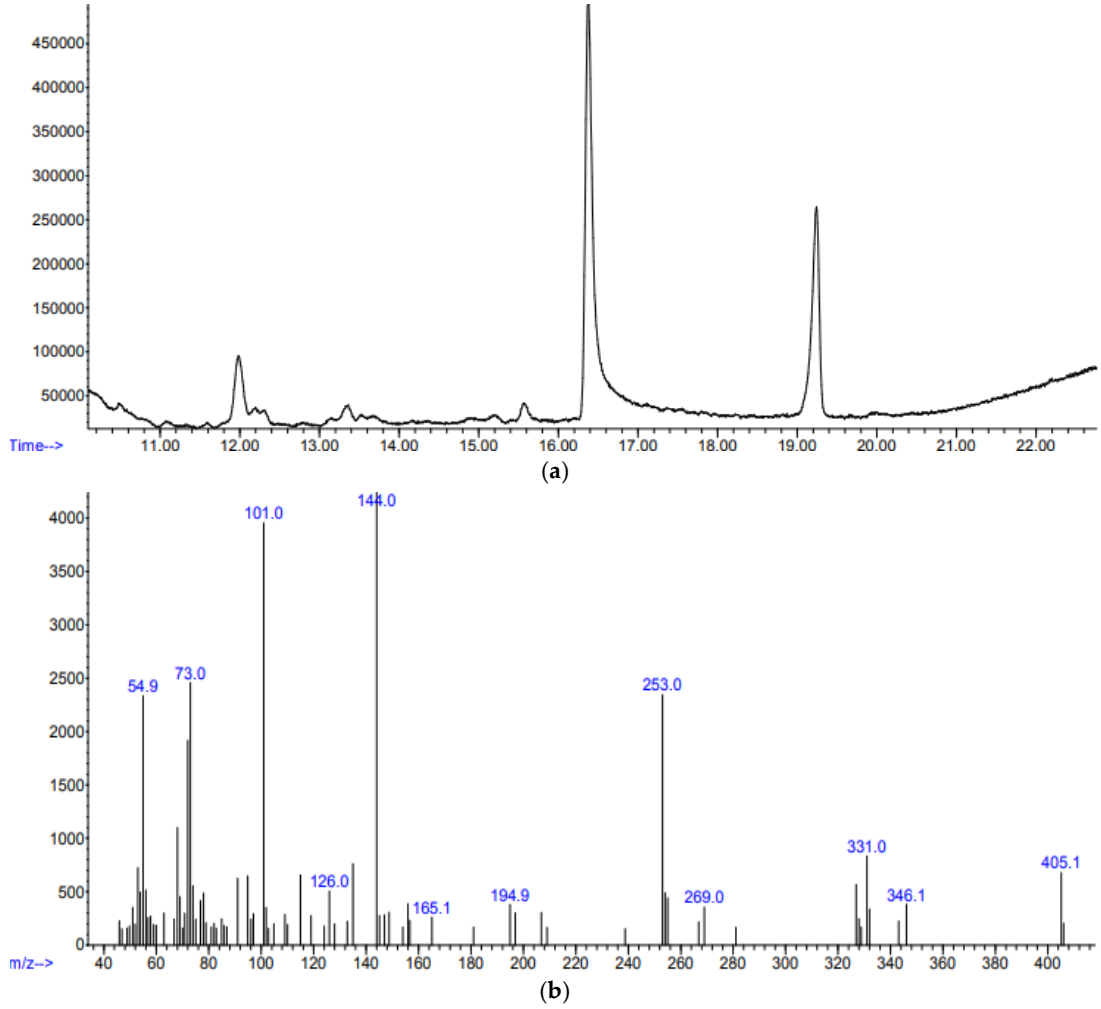


Figure 2. Spectra from (**a**) gas chromatography and (**b**) mass spectrometry of black raspberry extract.

Therefore, it can be stated that *Rubus niveus* extract contains flavonoid compounds with significant antioxidant activity, especially anthocyanins, which are the most relevant group in this plant family [11,12]. However, fluorescence and GC-MS studies suggest that there are other components that may complement the biological activity of the plant extract from a photochemical point of view, as indicated by the fluorescence band observed in Figure 1b.

3.2. Reaction with Galvanoxyl Radical: UV-VIS and Fluorescence Study

The reaction between the ethanolic extract and the galvanoxyl radical, determined by UV-VIS spectroscopy, is shown in Figure 3, where it can be observed that the maximum absorbance of the galvanoxyl radical (427 nm) decreases after the addition of 15 microlitres of black raspberry ethanolic extract every two minutes. In contrast, the absorbance at ~380 nm increases with the addition of aliquots of black raspberry extract. This behaviour indicates that the reduction of the galvanoxyl radical is effective at the concentrations of black raspberry extract.

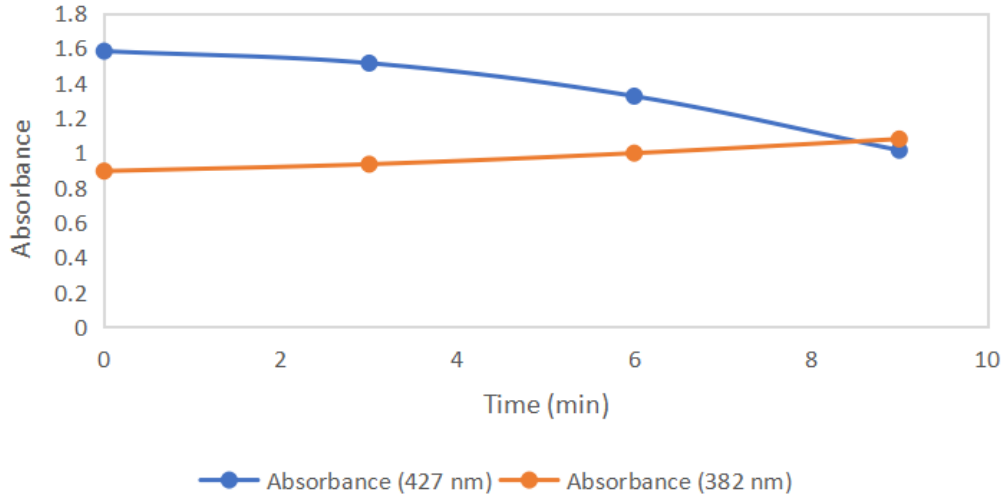


Figure 3. Reaction with Galvanoxyl Radical using UV-VIS spectroscopy.

The reaction shown in the figure above suggests a process in which a hydrogen atom is transferred from an antioxidant compound present in black raspberry extract to the galvanoxyl radical, which is converted into its non-radical form (possibly hydrogalvinoxyl). Thus, this neutralisation process leads to a decrease in the absorbance of the galvanoxyl radical at 427 nm and an increase in the absorbance of its non-radical form at 382 nm [6]. In this sense, it is confirmed that black raspberry extract has scavenging capacity.

On the other hand, the reaction between black raspberry extract and the galvanoxyl radical, analysed by fluorescence, is shown in Figure 4, where three measurements with different fluorescent emission wavelengths can be observed. Thus, it can be observed that, after adding aliquots of black raspberry, the main fluorescent emission band of the galvanoxyl radical (at 660 nm) decreases in intensity. This behaviour suggests a photophysical interaction occurring in the excited state of the radical.

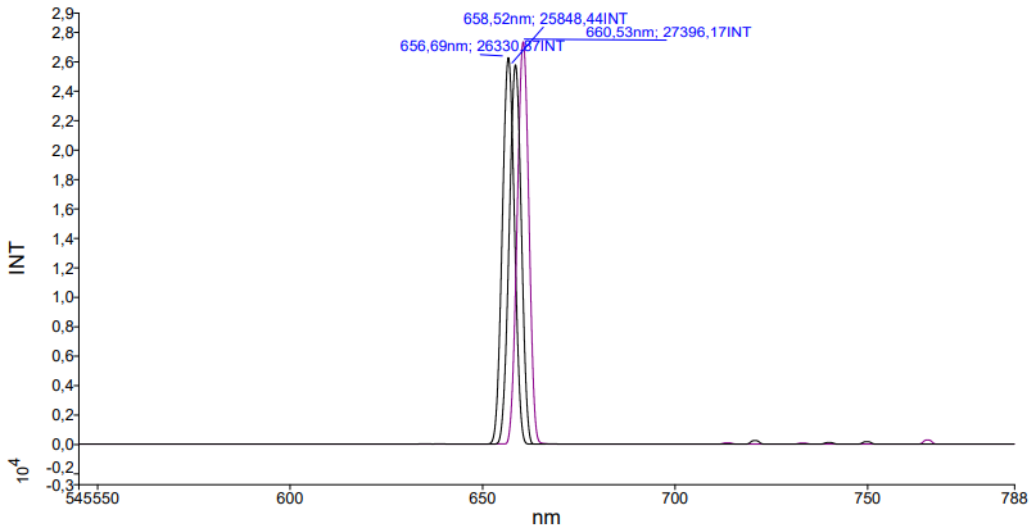


Figure 4. Reaction with Galvanoxyl Radical using UV-VIS spectroscopy.

The deactivation of the excited state of the galvanoxyl radical in the presence of black raspberry extract may suggest the effect of mechanisms other than scavenging. In this regard, it should be noted that the scavenging process promotes the generation of less reactive free radicals, in this case forming radicals from the bioactive compounds in the natural extract. However, fluorescence quenching reflects a more direct neutralization of the

excited radical species, likely through energy transfer or electron exchange [6,13]. Thus, the evidence supports that the bioactive compounds of *Rubus niveus* have a scavenging and quenching effect on the galvoxyl radical, as suggested by the UV-Vis and fluorescence experiments shown in Figures 3 and 4, respectively.

4. Conclusions

In summary, *Rubus niveus* fruit extract has scavenging and quenching activity against the highly reactive galvanoxyl radical, as shown by UV-Vis and fluorescence assays, respectively. This behaviour is mainly due to the presence of bioactive compounds, such as anthocyanins and other flavonoid derivatives, which contribute to the deactivation of reactive radicals and suggest antioxidant activity of *Rubus niveus* extract in vitro, which may be an aspect to consider in future research linking the properties of these extracts to health promotion and disease prevention.

Furthermore, the integration of both absorbance and fluorescence measurements affords a more comprehensive perspective on both the scavenging (via the process of radical reduction) and the quenching (via excited-state interactions) mechanisms. This dual approach, in conjunction with the specific evaluation of the galvinoxyl radical in vitro, provides novel experimental data. These results enhance the understanding of the functional antioxidant mechanisms of *Rubus niveus* fruit, which had not previously been characterized in this manner.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/doi/s1, Figure S1. 2-Furancarboxaldehy,5-methyl in Black raspberry extract; Figure S2. 2-Furancarboxaldehyde, 5-(hydroxymethyl)- in Black raspberry extract; Figure S3. Furfural in Black raspberry extract; Figure S4. 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- in Black raspberry extract; Figure S5. Phenol, 2,4-bis(1,1-dimethylethyl)- in Black raspberry extract.

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Abbreviations

The following abbreviations are used in this manuscript:

ROS	Reactive Oxygen Species
GC-MS	Gas chromatography–mass spectrometry
UV-Vis	Ultraviolet-Visible spectroscopy

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