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EEM Fluorescence Spectroscopy Coupled with HPLC-DAD Analysis for the Characterization of Bud Derivative Dietary Supplements: A Preliminary Introduction to GEMMAPP, the Free Data-Repository from the FINNOVER Project

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Abstract: Bud derivatives (BDs) represent a category of botanicals obtained by macerating the meristematic tissues (buds or young sprouts) of plants; widely used since ancient times in complementary medicine, they remain poorly investigated to date. In this study, a contribution is made toward the identification of the correct "a posteriori" attribution of the botanical species in commercial BDs, which is very important for ensuring the quality and safety of these natural products. Excitation–emission matrix (EEM) fluorescence spectroscopy proved to be a rapid, non-destructive and low-cost analytical instrument for providing a preliminary qualitative characterization of the BDs, allowing for the identification of specific spectral regions related to flavonol compounds and cinnamic and benzoic acids, in agreement with the targeted chromatographic analysis (HPLC-DAD), which completely defined a phytochemical profile for each BD considered. This information will be implemented in the first web application for the recognition of vegetable buds, named GEMMAPP, which was designed by the Authors and is proposed as a tool and future scientific data repository for providing information about the main, typical BDs of the France–Italy Alcotra territory.

Keywords: bud derivatives; botanicals; targeted chromatographic fingerprint; untargeted spectroscopic fingerprint; 3D fluorescence

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1. Introduction

This research takes place in the context of a European cooperative project (2017–2021) between France and Italy called FINNOVER ("Innovative strategies for the development of cross-border green supply chains") [1]. The main target of this project is the implementation of new green chain productions that exploit the biodiversity of the Alcotra territory. One of the production chains followed in the FINNOVER project concerns bud derivatives (BDs), which are commercialized in the European community as plant food supplements and represent a new category of natural herbal products [2].

BDs are produced by macerating the fresh meristematic tissues of both trees and herbaceous plants (buds or young sprouts) according to the traditional maceration procedure of the eighth edition of the *European Pharmacopeia* [3], which involves the use of a mixture of food-grade solvents, i.e., glycerol and ethanol, thus producing glyceric macerates.

Nowadays, despite the fact that these products are still poorly investigated, BDs are widely used for homeopathy and phytotherapy [4,5]. Their use has led to the birth of so-called "Gemmotherapy", an emerging branch of complementary medicine which is rapidly expanding in the market [6]. No health claims are approved to date by the European Food Safety Authority (EFSA), but a long history of their use as dietary supplements for human health has been reported in traditional medicine [2]. Moreover, pharmacognostic findings have been achieved for some of these botanicals to support their roles as adjuvants in several diseases; in vitro and in vivo biological studies are in fact available in the scientific literature [2,7–9].

As their production remains a "niche" area, there is the need to develop quality controls for BDs to ensure the correct identification of the botanical sources and their contents for the safety of both the consumers and the individuals who work in this field. Indeed, buds are often spontaneously collected in a very limited period of the year in late winter, corresponding to the germination period of the plant (balsamic period) in which the plant lacks its distinctive traits. For this reason, the attribution of the botanical species can sometimes be difficult if not carried out by expert collectors. In a previous work, Turrini et al. (2020) proposed a simple and non-destructive approach to identifying these botanicals based on tbe UV-Visible untargeted spectroscopic fingerprints of the extracts coupled with chemometrics [2].

In the present work, commercial BDs of six different plant species, i.e., *Castanea sativa* (CS), *Corylus avellana* (CA), *Ribes nigrum* (RN), *Fraxinus excelsior* (FE), *Ficus carica* (FC) and *Quercus petraea* (QP), which turned out to be among the species richest in antioxidant compounds, especially phenolic acids and flavonoids, have been investigated.

All the plant species described above are characterized by high contents of bioactive compounds with antioxidant activities that are mainly responsible for their beneficial properties. Most of these compounds, such as the many groups of polyphenols, aromatic amino acids and water- or lipid-soluble vitamins and pigments, are present in the aromatic rings of their chemical structures, π-conjugated systems and functional groups like phenols, carboxyls, hydroxyls and thiols. For this reason, they show intrinsic fluorescent properties (autofluorescence) and can be analyzed via molecular fluorescence spectroscopy [10]. In recent decades, interest in the application of non-destructive fluorescence spectroscopic techniques in the study of several foodstuffs (i.e., milk and its derivatives, wine, vegetable oils, juices, cereals and honey, etc.) and vegetable extracts has increased exponentially [11].

In the present work, this analytical method was used for the first time for the characterization of BDs, and it was chosen due to its numerous advantages, such as its high degree of sensitivity, ease of use and minimal sample preparation requirements; moreover, it represents a fast, non-destructive and low-cost analytical instrument [12]. The aim of this work was to determine a three-dimensional "fingerprint" for each of these natural products to differentiate them from each other, considering their composition in fluorophore substances. For this purpose, the multidimensional analytical method of using excitation–emission fluorescence matrices (EEMs), which provide more spectral information and a higher degree of selectivity with respect to first-order fluorescence signals [13], was applied. EEMs, which are also known as total fluorescence spectra, represent the emission intensity as a function of both excitation and emission wavelengths and provide overall comprehensive characteristics of samples that are determined via both absorption and fluorescence properties [13]. The obtained spectroscopic results were then confirmed via the application of a targeted analytical strategy (HPLC-DAD) to the same samples to fully define a phytochemical profile for each considered BD.

Furthermore, a web application named GEMMAPP (http://www.gemmapp.it, accessed on 20 May 2023) was designed by the Authors and has been proposed as new data repository and tool for providing scientific knowledge about some BDs typical of the France–Italy Alcotra territory [14].

2. Materials and Methods

2.1. Plant Material

Buds were collected from plants spontaneously grown in the valleys of Chisone, Pellice, Germanasca, Bronda and Varaita (Turin, Italy) and authenticated by a professional botanist. The BDs were purchased from Geal Pharma (Bricherasio, Turin), an Italian commercial company that produces glyceric macerates and hydroalcoholic solutions from various plant materials (botanicals) located in the Piedmont region. In particular, *Castanea sativa* buds were collected from the specific sites of Bobbio Pellice, Bricherasio, Perrero, Pagno and Brondello; *Corylus avellana* buds were collected from Pagno, Bricherasio and Prarostino; *Quercus petraea* buds were collected in Bricherasio; and *Ribes nigrum* and *Ficus carica* buds were collected in Pagno and Brondello, while the sampling sites for the *Fraxinus excelsior* buds were Paesana, Pagno, Angrogna, Bricherasio, San Germano Chisone and Massello (Figure 1). The geolocalization coordinates, botanical description of each species investigated and some naturalistic botanical illustrations provided by a professional expert in representations of local flora and fauna (Figure 2) are reported in GEMMAPP, which is the first digital application for the recognition of buds and one of the products of the Finnover project [14].

Figure 1. The buds of the six BDs investigated in the present work: (**a**) *Castanea sativa*, (**b**) *Corylus avellana*, (**c**) *Ribes nigrum*, (**d**) *Fraxinus excelsior*, (**e**) *Ficus carica* and (**f**) *Quercus petraea*.

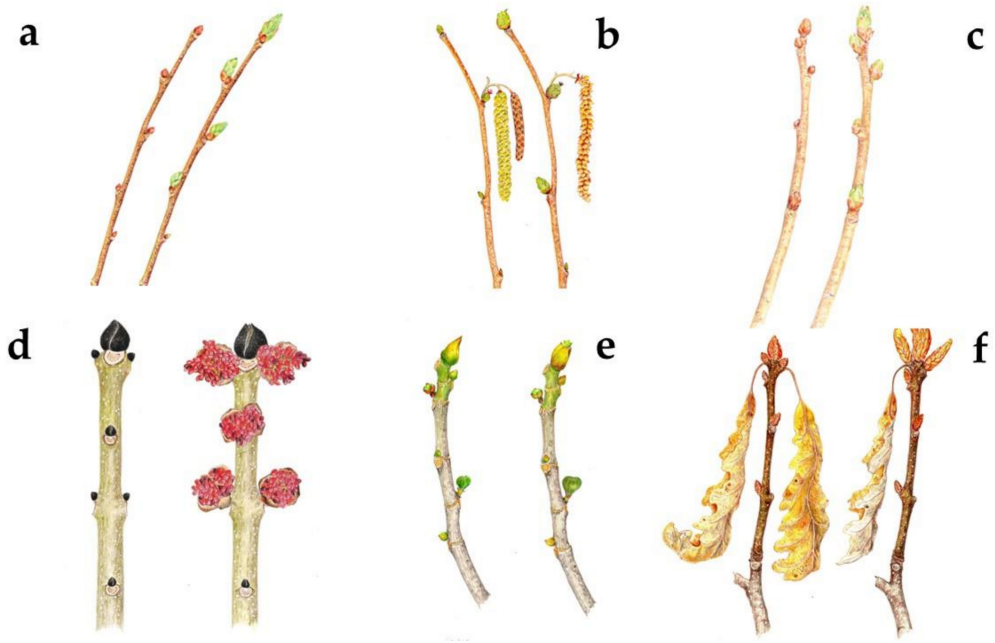

Figure 2. The naturalistic botanical illustrations of buds investigated in the present work: (**a**) *Castanea sativa*, (**b**) *Corylus avellana*, (**c**) *Ribes nigrum*, (**d**) *Fraxinus excelsior*, (**e**) *Ficus carica* and (**f**) *Quercus petraea*.

2.2. Chemicals

All chemicals employed for the preparation of the BDs and for the subsequent analysis were of HPLC grade. They were supplied by VWR International S.r.l (Milan, Italy) and Sigma-Aldrich (St. Louis, MO, USA). The purity of the standards used for the HPLC analysis of the BDs is reported in Supplementary Materials (Table S1).

2.3. Bud Derivative Manufacturing

Glyceric macerates (GCs) were prepared from the fresh buds in accordance with the detailed monograph "Homeopathic preparations" in the *French Pharmacopoeia* (8th edition, 1965) [3]. Particularly, BDs were obtained via traditional cold maceration, starting from the fresh raw material according to the official experimental conditions reported in the *French Pharmacopoeia* and reproduced in Table 1.

Table 1. Experimental conditions of BDs production.

	Experimental Conditions
Extraction solvent	Mixture of glycerol and ethanol (95%) 50:50 *w*/*w*
Bud–solvent ratio	1:20, dried weight (DW)
Time	21 days of cold maceration

After 21 days of cold maceration, the obtained extracts were filtered using filter papers (Whatman, hardened, ashless circles with diameters of 185 mm), manually pressed, and after 2 days of decanting, they were filtered again. Then BDs were stored in the dark at room temperature (25 ± 1 °C) and under a normal atmosphere (N.A.) until they were analyzed. Before the spectrofluorimetric analysis, the GCs were diluted 1:100, using pure EtOH as solvent instead of their extraction mixture (EtOH:glycerol, 50:50 *w*/*w*) to avoid the interference of glycerol with the fluorescence signal. For each of the 6 botanical species investigated in this research study, the BDs analyzed came from different batches and were obtained from different collections from spontaneous growths. The corresponding analytical data were then averaged.

2.4. The Chromatographic Characterization of BDs

In this research, a fingerprint strategy for the phytochemical characterization of BDs was applied using HPLC–DAD protocols. Different polyphenolic groups were considered: the phenolic acids included cinnamic and benzoic acids, catechins and flavonols. The quantitative determination of phenolics was defined using the external standard method. A high-performance liquid chromatograph coupled to an UV-Vis diode array detector (Agilent Technologies, Santa Clara, CA, USA) was used for the chromatographic fingerprint. Four methods were utilized to separate the phenolic compounds on a Kinetex C18 column (4.6 × 150 mm, 5 µm, Phenomenex, Torrance, CA, USA). Several mobile phases were used for phenolic quantification, and UV spectra were recorded at different wavelengths, based on previously validated HPLC methods [4,10], with some modifications. The methods are reported in the Supplementary Materials (Table S1).

Molecules were identified in the BDs via the comparison and combination of their UV spectra and retention times with authentic standards under the same HPLC conditions. All the samples were characterized in triplicate, and the results were reported as mean values ± standard deviations.

2.5. Three-Dimensional Fluorescence Spectroscopy

The excitation–emission fluorescence matrix (EEM) measurements were obtained at room temperature on a Perkin-Elmer LS 55 Luminescence Spectrometer (Waltham, MA, USA) equipped with a Xenon discharge lamp. A 10 mm optical glass quartz SUPRASIL® cell with volume of 3.5 mL was used. EEMs were recorded, using right-angled geometry, in two different ranges:

- Range 1, which takes almost the entire emission spectrum, presents emission wavelengths from 295 to 800 nm (each 0.5 nm) and excitation wavelengths from 200 to 290 nm (each 5 nm).
- Range 2, which shows a smaller part of the emission spectrum but allows for the investigation of a different excitation range, presents emission wavelengths from 350 to 570 nm (each 0.5 nm) and excitation wavelengths from 270 to 315 nm (each 5 nm).

In both cases, the excitation monochromator slit width was set to 5 nm, while the emission monochromator slit width was set to 10 nm and the scan speed was 1000 nm/min. The spectrometer was interfaced to a computer for controlling the instrumental parameters, recording the spectra and processing the data. The fluorescent signals were registered by the BioLight (BL) Luminescence Systems Spectroscopy Software (FL Winlab, PerkinElmer). All spectra were recorded in triplicate and then averaged.

3. Results and Discussion

As shown in Figure 1, during the balsamic period of the plant, in which the spontaneous collection of fresh buds took place, the plants are often devoid of their distinctive characteristics. Therefore, having useful tools to identify the correct "a posteriori" attribution of the botanical species in commercial BDs is very important for ensuring product quality and safety.

3.1. The Chromatographic Characterization of BDs

Table 2 shows the contents of the main bioactive compounds by their chromatographic fingerprints, which were obtainable under routine conditions and at low costs.

Table 2. Chromatographic characterization of BDs obtained from the six different botanical species (*Castanea sativa*, *Corylus avellana*, *Ribes nigrum*, *Fraxinus excelsior*, *Ficus carica* and *Quercus petraea*). The mean results are expressed as mg/100 g of fresh weight (FW).

		Cinnamic Acids							
		Caffeic Acid		Chlorogenic Acid		Coumaric Acid		Ferulic Acid	
Species	ID Code	Mean Value	SD	Mean Value	SD	Mean Value	SD	Mean Value	SD
Corylus avellana	CA	2.53	0.13	n.d.	/	n.d.	/	n.d.	/
Castanea sativa	CS	1.61	0.02	14.23	0.29	n.d.	/	4.34	0.22
Ficus carica	FC	n.d.	/	n.d.	/	62.21	0.84	n.d.	/
Fraxinus excelsior	FE	43.81	0.80	489.94	0.79	n.d.	/	295.28	0.68
Quercus petraea	QP	5.08	0.65	n.d.	/	0.00	0.00	n.d.	/
Ribes nigrum	RN	22.48	0.04	n.d.	/	5.21	0.15	n.d.	/

		Flavonols									
		Hyperoside		Isoquercitrin		Quercetin		Quercitrin		Rutin	
Species	ID Code	Mean Value	SD	Mean Value	SD	Mean Value	SD	Mean Value	SD	Mean Value	SD
Corylus avellana	CA	n.d.	/	n.d.	/	171.25	14.28	83.46	4.98	79.50	2.14
Castanea sativa	CS	3.03	0.10	n.d.	/	30.64	0.23	29.01	0.82	1.54	0.12
Ficus carica	FC	63.67	0.94	n.d.	/	49.68	1.24	128.34	1.08	46.20	1.11
Fraxinus excelsior	FE	242.26	0.89	n.d.	/	176.88	0.63	79.93	1.00	0.00	0.00
Quercus petraea	QP	7.28	0.81	n.d.	/	27.91	0.38	188.45	0.79	0.00	0.00
Ribes nigrum	RN	n.d.	/	n.d.	/	49.53	0.49	30.86	0.85	17.25	0.22

		Benzoic Acids				Catechins			
		Ellagic Acid		Gallic Acid		Catechin		Epicatechin	
Species	ID code	Mean Value	SD	Mean Value	SD	Mean Value	SD	Mean Value	SD
Corylus avellana	CA	193.53	2.22	46.29	2.54	48.53	2.89	49.19	2.83
Castanea sativa	CS	48.95	0.13	94.71	0.24	1.28	0.32	31.02	0.17
Ficus carica	FC	67.29	0.89	n.d.	/	75.79	1.04	191.56	1.13
Fraxinus excelsior	FE	214.49	0.69	n.d.	/	57.74	0.79	270.51	0.89
Quercus petraea	QP	231.56	0.65	52.03	0.64	94.83	0.39	199.92	0.46
Ribes nigrum	RN	69.66	0.08	0.31	0.09	95.88	0.26	59.83	0.37

n.d. not detectable.

The differences among the six investigated species can be summarized as follows, in accordance with previous similar studies [2,15,16]:

- FE is very rich in phenolics and cinnamic acids (more than 40% of total phenolics, as shown in Figure 2) and in chlorogenic acid (489.94 ± 0.79 mg/100 g FW), followed by ferulic acid (295.28 ± 0.68 mg/100 g FW); it is also rich in catechins, especially epicatechin (270.51 ± 0.89 mg/100 g FW), and flavonols, especially hyperoside (242.26 ± 0.89 mg/100 g FW), and benzoic acids, especially ellagic acid (214.49 ± 0.69 mg/100 g FW), as shown in Figure 2. *Fraxinus excelsior* L. (FE) has been widely used in traditional medicine due to several claimed beneficial health effects including antioxidant, anti-inflammatory, anti-rheumatic and anti-pyretic activities [17]. Several findings from in vivo studies showed that the FE extract has hypoglycemic and anti-hyperlipidemic activities, providing anti-diabetic and anti-obesity effects [18,19].
- QP is particularly rich in ellagic acid (231.56 ± 0.65 mg/100 g FW), catechins (especially epicatechin, 199.92 ± 0.46 mg/100 g FW) and quercitrin (188.45 ± 0.79 mg/100 g FW). The glyceric macerate of *Quercus petraea* L. (QP) is employed to regularize the action of the intestinal system, to counteract the state of asthenia and to fight against oxidative stress, helping to protect and preserve the well-being of the human organism [20].
- CA is rich in ellagic acid (193.53 + 2.22 mg/100 g FW), quercetin (171.25 + 14.28 mg/100 g FW) and to a lesser extent, in catechins (about 100 mg/100 g FW), while cinnamic acids (less than 2%) are only represented by caffeic acid in trace amounts (<3 mg/100 g FW), as shown in Figure 2. *Corylus avellana* L. (CA), the common hazel plant, has been known since ancient times for its astringent and antiedema properties, vasoprotective activity and mild antimicrobial effect: in fact, it was used in traditional medicine for the treatment of edema, hemorrhoids, varicose veins and phlebitis [21]. Nowadays, its characteristic compounds, cyclic diarylheptanoids, are receiving increasing interest due to

their remarkable health effects, including anti-inflammatory, anti-emetic, anti-influenza and estrogenic actions [22].

- CS is characterized by high amounts of flavonols (e.g., quercetin and quercitrin, with about 30 mg/100 g FW) and benzoic acids (e.g., ellagic and gallic acids, with about 50–90 mg/100 g FW), followed by catechins, especially epicatechin (31.02 + 0.17 mg/100 g FW), as shown in Figure 2. Cinnamic acids were quantified in small amounts (less than 15 mg/100 g FW). *Castanea sativa* Miller (CS) is one of the most widely used herbal medicines thanks to its health-promoting activities, which are due to its high contents of bioactive compounds (polyphenols, organic acids, terpenes and vitamins) [6]. In fact, CS presents antioxidant and curative properties against both cardiovascular and urinary diseases (especially recurrent cystitis), and it is well known for its positive effects on stagnant and vascular fluids [23].
- FC showed high values of catechins (75.79 + 1.04 mg/100 g FW for catechin and 191.56 + 1.13 mg/100 g FW for epicatechin), flavonols (e.g., quercitrin and hyperoside, with values of about 60–130 mg/100 g FW), and phenolic acids (e.g., coumaric and ellagic acids, with values of about 60 mg/100 g FW). *Ficus carica* L. (FC) was used in traditional medicine to cure various disorders such as gastrointestinal (dysentery, constipation, colic, ulcers and loss of appetite) and respiratory (coughs, sore throats and bronchial problems) thanks to its anti-inflammatory, antioxidant, antipyretic, antidiabetic, anthelmintic, antimicrobial and anti-carcinogenic activities [24].
- RN presented a good quercetin and quercitrin contents (about 30–50 mg/100 g FW), ellagic acid (about 70 mg/100 g FW) and catechins (about 60–100 mg/100 g FW). Cinnamic acids were identified in amounts of about 5–20 mg/100 g FW. *Ribes nigrum* L. (RN) buds are used in medicinal preparations as anti-inflammatory agents for the treatment of dermal diseases, such as eczema and psoriasis, and for the healing of wounds [25,26]. R. nigrum BDs contain high polyphenol contents, especially catechins and phenolic acids, and are endowed with antioxidant and anti-inflammatory activities which have been proven to play an important role in the human health in the prevention of several chronic diseases [16,27].

Specific bioactive compounds can be used collectively as representative standards of an extract in quantification, as performed in this study. Bioactive substances (phenolics) were selected, comparing the BD's health-promoting properties and the most important antioxidant molecules in the literature with important roles in the positive effects on the human organism. Chromatography offers a very powerful separation ability, such that the complex chemical components in plant extracts can be separated into many relatively simple sub-fractions. The most important technique used for the characterization of plant extracts is to measure the concentration of one or very few markers or active components ("marker approach") [28]. This approach is far from satisfactory, as the biological activity is due to more than one or two single chemical compounds. The "multi-marker approach" used in this research is the natural extension of the "marker approach". It uses many, or even all, of the identified substances (the chemical profile of the considered sample) to represent the whole sample [29–31]. This approach is applied to many complex systems (e.g., herbs, herbal preparations, food supplements and foodstuffs). It is impossible to consider all the bioactive substances that may be included in the extracts due to the very high number of potential molecules; thanks to the multi-marker approach, the composition is approximated to the most important compounds with biological activities (demonstrated in the literature). The higher the number of markers, the smaller the approximation will be. In this case, four phenolic classes (13 markers) were selected for the evaluation.

Even if chromatographic fingerprints have proved to be very useful in discriminating among the BDs derived from the six considered different species [5,32], they are neither rapid nor non-destructive. However, spectroscopic techniques offer simple, rapid and non-destructive means of analyzing samples while providing quite similar information in terms of the rapid screening of different vegetable species [33].

3.2. Three-Dimensional Fluorescence Spectroscopy

Fluorescence spectroscopy has shown great potential for the characterization of food products because they contain many fluorescent molecules (fluorophores) whose emissions can be considered characteristic fingerprints of specific food samples [34,35]. In this study, fluorescence spectroscopy was applied to this category of herbal food supplements (botanicals), and the observed changes in intrinsic fluorescence were studied via plotting the fluorescence values as images (with intensity represented via color coding) of two-dimensional fluorescence excitation–emission landscapes, in which the characteristic circular patterns were used to identify regions that could be attributed to specific fluorophores. Figure 3 shows the contour plots of the six different species for each spectral range (Figure 3a,b) that were evaluated in the spectroscopic analysis. To provide a meaningful qualitative evaluation of the fluorescence data, both the ranges used for recording the fluorescence spectra are considered in the discussion.

According to the literature, it is important to consider that the emission maxima of many different phenolic compounds can be observed in approximately the same spectral region. However, thanks to the combination of the information obtained via both the spectral ranges considered, it is possible to observe two different spectral regions as a result of the fluorescent properties of BDs. Excitation in the more energetic wavelength of 250 nm results in an emission between 400 and 440 nm, while excitation at wavelengths longer than 300 nm results in an emission between 420 and 450 nm [36]. The first spectral region reported in Figure 3a is ascribable to phenolic acids and cinnamic-like and benzoic-like acids, which present maximum emissions between 400–440 nm. This peak, which was detectable in all the samples analyzed, has higher intensity values in CS and QP fluorescence emission spectra that are also rich in ellagic and gallic acids according to their chromatographic characterizations. As reported by Casale et al. [37] for green tea samples, at 270/420 nm, it is also possible to study the contents of catechins, which contribute to the generation of this broad peak. However, the second spectral range (Figure 3b) allows one to better characterize the emissions relating to other flavonol compounds, especially quercitrin and quercetin, which present excitation/emission maxima at 310/430 nm, as reported by Milosavljević et al. [38]. This characteristic peak has been observed in all six species of BDs, with higher intensities in the QP, CA, FE and FC samples, confirming the outcomes of the quantitative analysis (data are shown in the Supplementary Materials, Figure S1).

3.3. GEMMAPP

GEMMAPP, one of the products of the Finnover project, is the first digital application for the recognition of vegetable buds. The goal of this webAPP is to provide professionals with a repository of scientific information and technical data on BDs, which represent an increasingly interesting but still little-studied plant matrix.

The webAPP, which is currently presented in Italian as one of the languages of the Alcotra territory but will soon also be available in other languages, can be freely consulted (http://www.gemmapp.it, accessed on 20 May 2023) by common people and professionals, providing descriptions of the main tree species at the base of the most commercial BDs. Twenty-five botanical species are described in the webAPP, as reported in Figure 4. In particular, for each species, agronomic, environmental, biological and chemical data are reported.

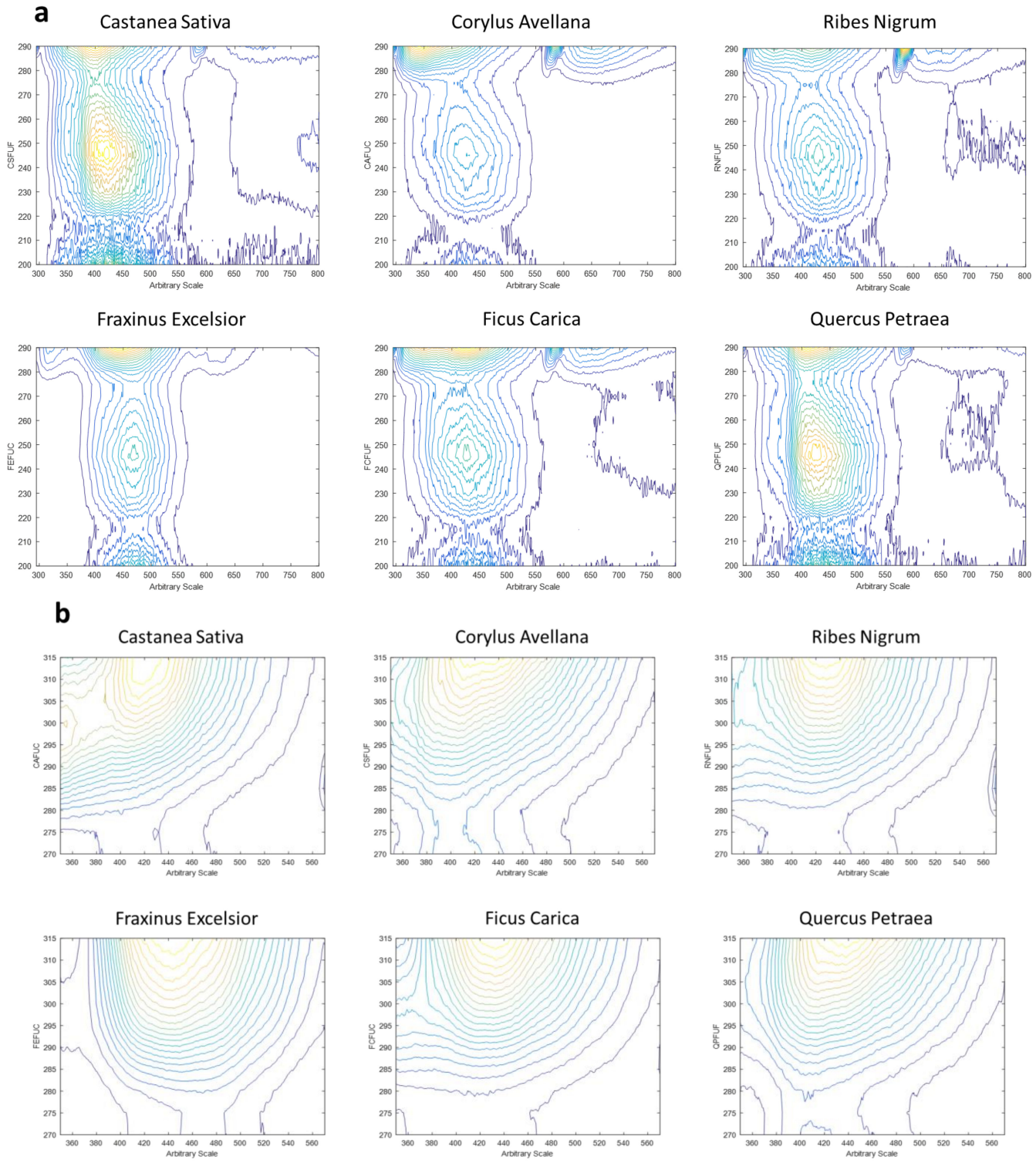

Figure 3. (**a**) BDs Excitation-Emission contour plots in Range 1 (emission wavelengths from 295 to 800 nm and excitation ones from 200. (**b**) BDs' excitation–emission contour plots in Range 2 (emission wavelengths from 350 to 570 nm and excitation wavelengths from 270 to 315 nm).

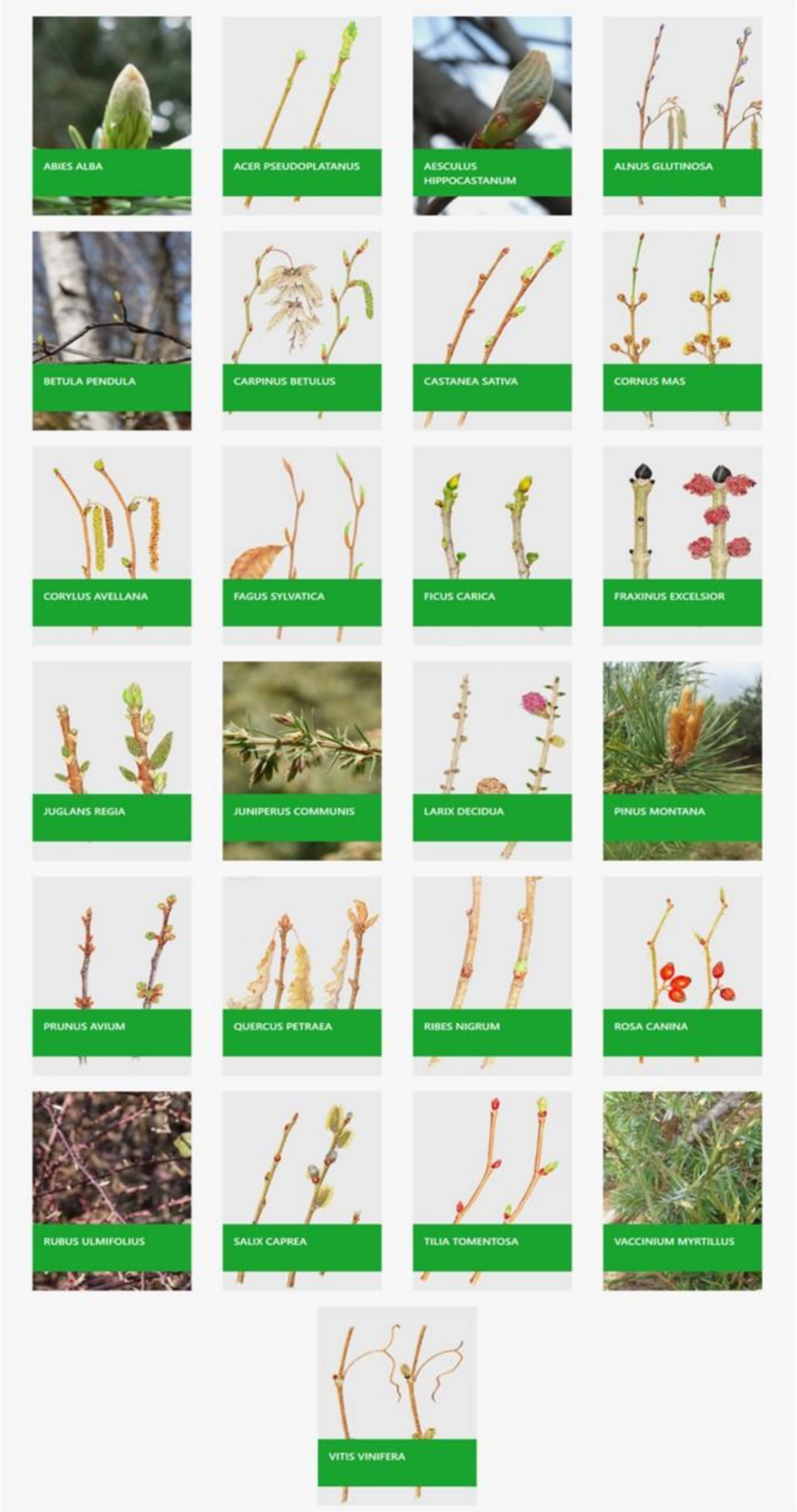

Figure 4. The 25 botanical species described in GEMMAPP.

The framework of GEMMAPP is schematized in Figure 5.

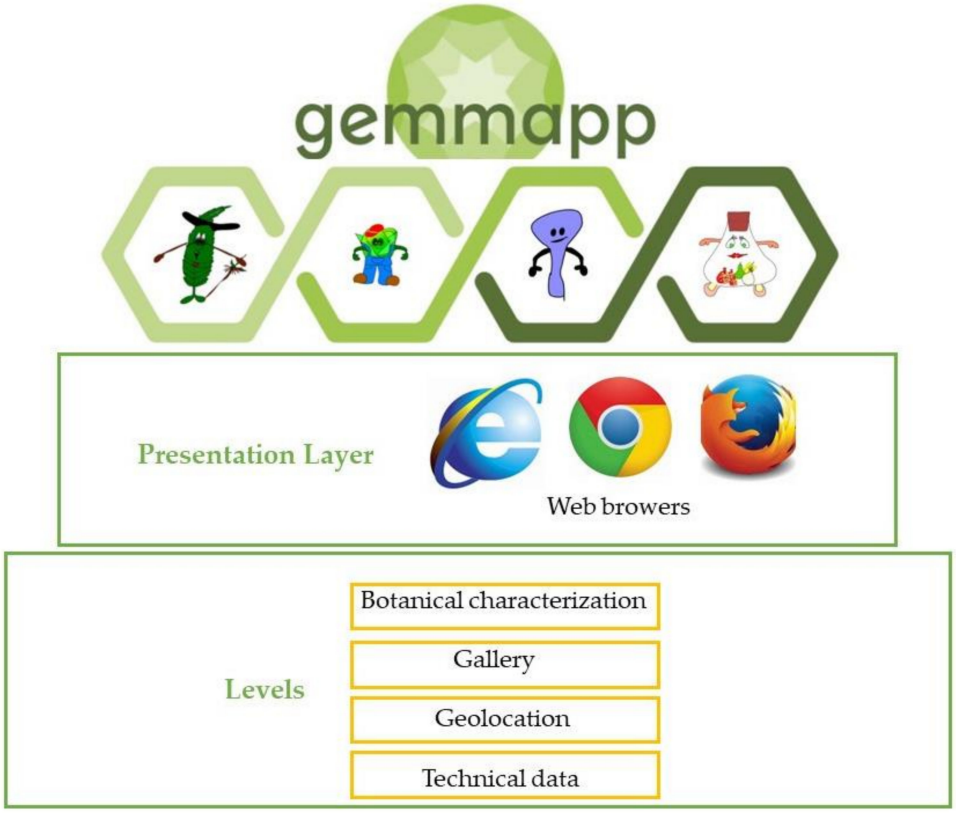

Figure 5. The framework of GEMMAPP.

Different levels can be consulted for each species: the first level presents the botanical characterization, namely, information relating to geographical distribution and habitat, size and habit, the characteristics and peculiarities of buds, elements of recognition and phenological stage at the time of collection, etc. (Figure 6a). The second level shows a gallery of pictures of buds at the correct phenological stage to help the collector properly identify them at the time of collection. In addition, in this section, there are also some naturalistic botanical illustrations which were created by a professional expert in representations of local flora and fauna (Figure 6b).

Figure 6c shows the geolocation coordinates of the main bud-collecting sites within the Alcotra territory, which includes for Italy the Valle d'Aosta Region, the Provinces of Torino and Cuneo (Piemonte Region) and the Province of Imperia (Liguria Region), while for France, the Departments of Haute-Savoie and Savoie (Auvergne-Rhone-Alpes Region) and the Departments of Hautes-Alpes, Alpes de Haute-Provence and Alpes-Maritimes (Provence-Alpes-Côte d'Azur Region) are represented.

The last level of the webApp, which is ready to be implemented, has the purpose of collecting all the scientific data obtained not from the starting vegetable matrix (buds) but from the BDs, namely, the finished products. Untargeted spectroscopic analyses (UV-Visible and 2D/3D Fluorescence, FT-IR) targeted chromatographic data and biological test results will be inserted to provide an overview of the Finnover project results.

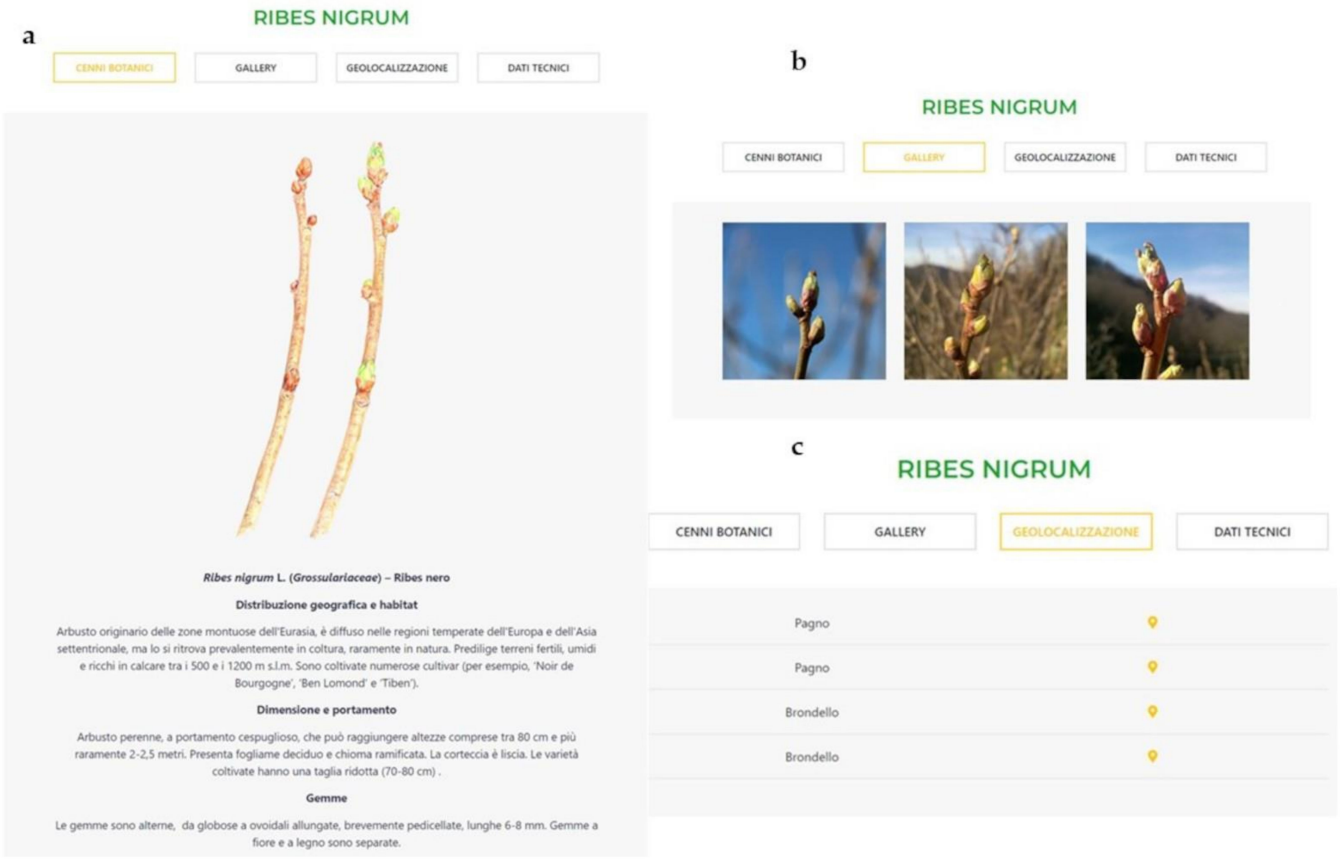

Figure 6. Botanical characterization, gallery and geolocation of the main collecting sites within the Alcotra territory of *Ribes nigrum*.

4. Conclusions

The aim of this study was to propose a method for accurately identifying the botanical species in commercial bud derivatives (BDs) in order to ensure their quality and safety. It is important to highlight that many commercial products derived from vegetable buds use plant material obtained from spontaneous harvests. This can lead to the false identification of the botanical species to which the material belongs and the consequent placement = of non-conforming products on the market, which may sometimes even potentially dangerous for the consumer. The development of rapid and easy-to-use methods for self-monitoring for the producing companies constitutes an important step forward to ensure the authenticity of these supplements. However, the applicability of rapid, non-destructive analytical methods (i.e., spectroscopic methods) requires a preliminary study to ensure their efficiency and validity in providing data that are as useful the data obtained via official methods (i.e., chromatographic methods).

The researchers applied a fingerprint strategy, using high-performance liquid chromatography with diode array detection (HPLC-DAD) protocols for the phytochemical characterization of the BDs. Additionally, they used emission fluorescence spectroscopy to provide a preliminary qualitative characterization of the samples.

The results showed that the proposed fluorimetric method was a rapid, non-destructive and cost-effective tool for identifying specific spectral regions related to the bioactive compounds (i.e., flavonols and cinnamic and benzoic acids). These findings were consistent with the outcomes of the targeted chromatographic analysis (HPLC-DAD), which provided a comprehensive phytochemical profile for each BD.

In the future, further research will be needed to validate these preliminary results obtained with six botanical species. Additionally, expanding the dataset will allow for the

application of more advanced multivariate approaches for both qualitative and quantitative characterizations of the phenolic profiles of different botanical species.

The data obtained from this research can be included in the GEMMAPP database, along with other published data, to provide a comprehensive overview of BDs and buds. These natural products are increasingly used, but the research in this field is still limited. By adding more data to the database, researchers can gain a better understanding of these products and ensure their quality and safety in the market.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app13158679/s1, Table S1: Conditions of the HPLC methods used for the chromatographic analysis; Figure S1: 3D EEM excitation–emission spectra.

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