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Review

Potential Applications of the *Cytisus* Shrub Species: *Cytisus multiflorus*, *Cytisus scoparius*, and *Cytisus striatus*

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Abstract: *Cytisus* spp. is present throughout the Portuguese territory. Although most of these species are considered native in Portugal, at least one species has already been reported as invasive in other parts of the world. Different measures of control have been investigated, and the application of herbicides is the most commonly used. This review gathers information about the biochemical profile and analytical methods used for the evaluation of the potential bioactivities of three species of the genus *Cytisus*, better known as brooms, which were used in traditional medicine through the production of infusions and decoctions for the treatment of several health problems, mainly due to their high value of phenolic compounds. However, little research has been conducted on its biological activities as a potential antioxidant, anti-inflammatory, and antimicrobial agent. Furthermore, one species (*Cytisus striatus*) has not been subjected to extensive research in identifying chemical compounds and evaluating their potential bioactivities. This species (known as the Portuguese broom) has a great expression in one of the forest typologies with a considerable percentage in Portugal. This research work is essential to encourage a scientific and sustainable valorisation of *Cytisus* spp. (namely *C. striatus*), which will consequently contribute to forest cleaning and management to reduce the risk of wildfires.

Keywords: *Cytisus* spp.; antioxidant activity; bioactive compounds; valorisation; analytical methods

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1. Introduction

Historically, and as their name suggests, broom species were used for broom production but also thatching, fence rows (stems), cattle fodder (green photosynthetic stems), beer production (flowers), and as a coffee substitute (seeds). For many years plant parts of diverse species have been used in traditional medicine, and today they continue to be used and are called medicinal plants [1]. From an ethnobotanical view, some parts of these plants (leaves, bark, and flowers) were used for their medicinal properties [2]. *Cytisus* species are also characterized as nitrogen-fixers. The roots form nodules from *Rhizobium*, turning the atmospheric nitrogen available in the soil by the plant's stems and leaves generates great livestock farming interest by enriching the soil and raising pasture quality. In a study based on this feature, the authors concluded that some brooms contribute substantially to the nitrogen sustainability of agricultural practices in Portugal [3,4]. This agriculture is

carried out to keep brooms on poor soils to cultivate or, in some cases, keep them close to cultivated fields during the fallow to grow faster during the following years [3,5]. These species are essential in the West Mediterranean Region ecosystems, as they are within the serial holm oak, cork oak, or deciduous oak shrublands. They are dominant in giestais (shrub type that dominates some of the regions that experience a Mediterranean climate, composed of *Cytisus* species), and other shrublands as well as in forest clearings and edges, colonising fallows lands, rocky slopes, uncultivated (agricultural) land and roadsides, in acidic and poor soils, preferably derived from sands, granites, quartzites, and schists.

On the other hand, thousands of plants are sold primarily due to their morphology, making the nursery industry a highly valued sector in some countries. Some of the plants found in this industry belong to the genus *Cytisus*. These have attracted the attention of several researchers. Brooms are native and considered "natural" in many countries (mainly Europe). However, they are starting to present a problem for vegetation in others [2,6]. Regarding the Australian nursery industry, these species trade could be negatively impacted if they are deemed to be weeds [6].

In recent years, it has been noted that these species have a great expression worldwide due to their rapid growth and can form dense stands. This evidence can lead to problems such as inaccessibility for wildlife and the risk of fires. Due to their expression, brooms were listed as Class C pest species by the California State Department of Food and Agriculture (CDFA), and some specific species (e.g., Scotch and Portuguese broom) have been placed on List A, as aggressively invasive, by the California Exotic Plant Pests Council (CalEPPC) [2].

Considering the problems exposed, this work aims to highlight the importance of creating value chains for these species so that the strategies used to control their expression are applied frequently, and their costs are minimised. The selected species will be characterised in terms of their chemical profile and biological activities so that future investigations can be carried out to present possible applications. These selections for this review work were based on the following facts: similar distribution in the world but particularly in the countries where they are considered native, species belonging to the same genus and morphology not significantly different among them.

The species selected for this review are *Cytisus multiflorus* (L'Hér.) Sweet (or (L'He'r. ex Aiton) Sweet), *Cytisus scoparius* (L.) Link and *Cytisus striatus* (Hill) Rothm., all accepted and validated names belonging to the Fabaceae Lindl. family, Faboideae Rudd (Papilionoideae de Candolle, nom. alt.) subfamily, Genisteae Bronn tribe, and *Cytisus* Desf. (or L.) genus [7]. Research platforms such as ISI Web of Science, Scopus, PubMed and Google Schooler were used for this review.

Distribution and Morphology

Brooms grow best in full sunlight, dry, and sandy soils, and different soil textures over a wide pH range. In the case of some ref, the root system is categorized by a taproot that can exceed 0.6 m in length and an extensive, branched and shallow lateral root system. Regarding the reproduction of these species, 40% of the seeds germinate soon after dispersal, and another 25% will only germinate in the second year [2]. Most of the last seeds that remain until the second year need scarification for germination since the seeds have an impermeable layer. Some factors stimulating seed germination are transportation in gravel and road materials, exposure to low-intensity grass or brush fires, and movement along watercourses. Scottish, French, Portuguese, and Spanish brooms usually take over mainly open sites such as logging roads, landings, skid trails, and harvesting areas [2].

Species belonging to *Cytisus* are shrubs, subshrubs, or small trees with alternate branches and unifoliate to trifoliate leaves, deciduous to evergreen [8]. The fruits of the Genistae tribe are mostly typical pods with more or less explosive (and valvular) dehiscence via dorsal and ventral sutures [9,10]. These three species' morphologies share specific characteristics, such as the leaves being compound with three leaflets (trifoliate), sometimes single (unifoliate) on new twigs, and deciduous. Flowers are single or paired up to four leaf axils, usually with a 2-lipped, top lip minutely tooth calyx [2,11].

C. multiflorus was first published in Hort. Brit.: 112 (1826), commonly known as White Broom, amongst other common names, and native from the West & Central Iberian Peninsula (endemism in PI), being introduced in some European countries (Belgium, France, and Italy), West USA coast (California and Oregon), India, Madagascar, New Zealand, South Australia [12]. It has 6(8)-angled, T-shape in cross-section stems, white petals and flattened, villous/villose seed pods at early stages and glabrous at dehiscence [2,8], characters that differentiate it from the other two species. Its flowers are traditionally known in Portugal to treat diabetes [13].

Concerning *C. scoparius*, this species was first published in Enum. Hort. Berol. Alt. 2: 241 (1822), and its native range is (almost all) Europe (autochthonous in Portugal), being introduced all over the world, particularly in European Turkey, Azores and Madeira, North and South America, South Africa, and Madagascar, India, West Himalaya, New Zealand, and South Australia, Tasmania, Korea, and Japan [12]. It is commonly known as Scotch broom [2], common broom, or yellow broom [12]. Distinguishing from the other brooms, this species usually has 5-angled, star-shaped cross-section stems. The flower petals are yellow or partially to entirely dark red and sometimes fragrant; the calyx is glabrous, and the seed pods are flattened, glabrous with margins densely lined with long hairs [2,11].

C. striatus was first published in Feddes Report. Spec. Nov. Regni Veg. 53: 149 (1944) and is native to the Iberian Peninsula and North Morocco (autochthonous to the Iberian Peninsula) [12]. It is commonly known as a Portuguese broom [2] and can be distinguished through its 8- to 10-ridged round in cross-section stems. The calyx is covered with short hairs (pubescent), the petals are yellow, and the seed pods are slightly inflated and densely covered with long hairs. *C. striatus* closely resembles *C. scoparius* in leaf patterns and is often mistaken for it [2]. According to Flora-On [14], *C. striatus* is the most heliophile and pioneer of the yellow broom flora indigenous to the Iberian Peninsula.

2. Bioactive Extracts, Biological Activities and Other Applications

The species *C. multiflorus*, *C. scoparius*, and *C. striatus* are found within the wild Portuguese flora and used in folk medicine for their biological properties and sometimes as a condiment for food. These species are noted for having anti-inflammatory properties. Infusions, decoctions, and tonics prepared from fresh or shade-dried flowers treat respiratory, gastrointestinal, and skin damage. They are also applied to control diabetes and cholesterol [15].

2.1. Cytisus multiflorus

C. multiflorus (Figure 1) was traditionally used for its health properties in which tea infusions were prepared from its flowers [16]. Some of these properties are based on controlling metabolism and endocrine system disorders (for example, diabetes and cholesterol), rheumatoid arthritis, headaches, and hypertension [17]. One of the main groups of compounds that can justify some of these effects caused by this species is the flavonoids, which are known to act as antioxidants. These compounds occur ubiquitously in plants with structural variations and are characterized by flavanols, flavone, isoflavones, flavanones, flavanol, and anthocyanins [18]. A study by Luis et al. [19] determined the contents of phenols, flavonoids, tannins, and alkaloids from extracts of various plants, including *C. multiflorus*. Analytical methods such as Folin-Ciocalteu's, DPPH, and β-carotene were used to correlate the chemical profile with the antioxidant activity of the aerial parts (stems, leaves, flowers, and fruits) of this species. The Soxhlet apparatus performed the extracts with methanol in which to 100 g of raw material, 1 L of solvent was added. Then the extracts underwent vacuum filtration and were distilled to a final volume of 100 mL Identification of the compounds was made using reverse-phase high-performance liquid chromatography (RP-HPLC). The results showed that *C. multiflorus* was the richest in alkaloids, mainly in the stems, and the extracts of flowers and fruits contain more flavonoids than those of stems and leaves. Gallic acid, vanillic acid, caffeic acid, chlorogenic acid, xarinic acid, p-coumaric acid, ferulic acid, quercetin, and ellagic

acid, were present in leaf extracts in the highest quantity. Stems and fruits contained all of the compounds mentioned above except gallic acid, quercetin, and ferulic acid were the major compounds. Flowers contained syringic acid, p-coumaric acid, ferulic acid (major), ellagic acid, and quercetin. Regarding the antioxidant activity by DPPH, the extract that showed an intense activity was the leaf extract, while the others were considered moderate. In addition, in the β-carotene bleaching test, the flower extract showed a higher percentage of inhibition of lipid oxidation than the other extracts.

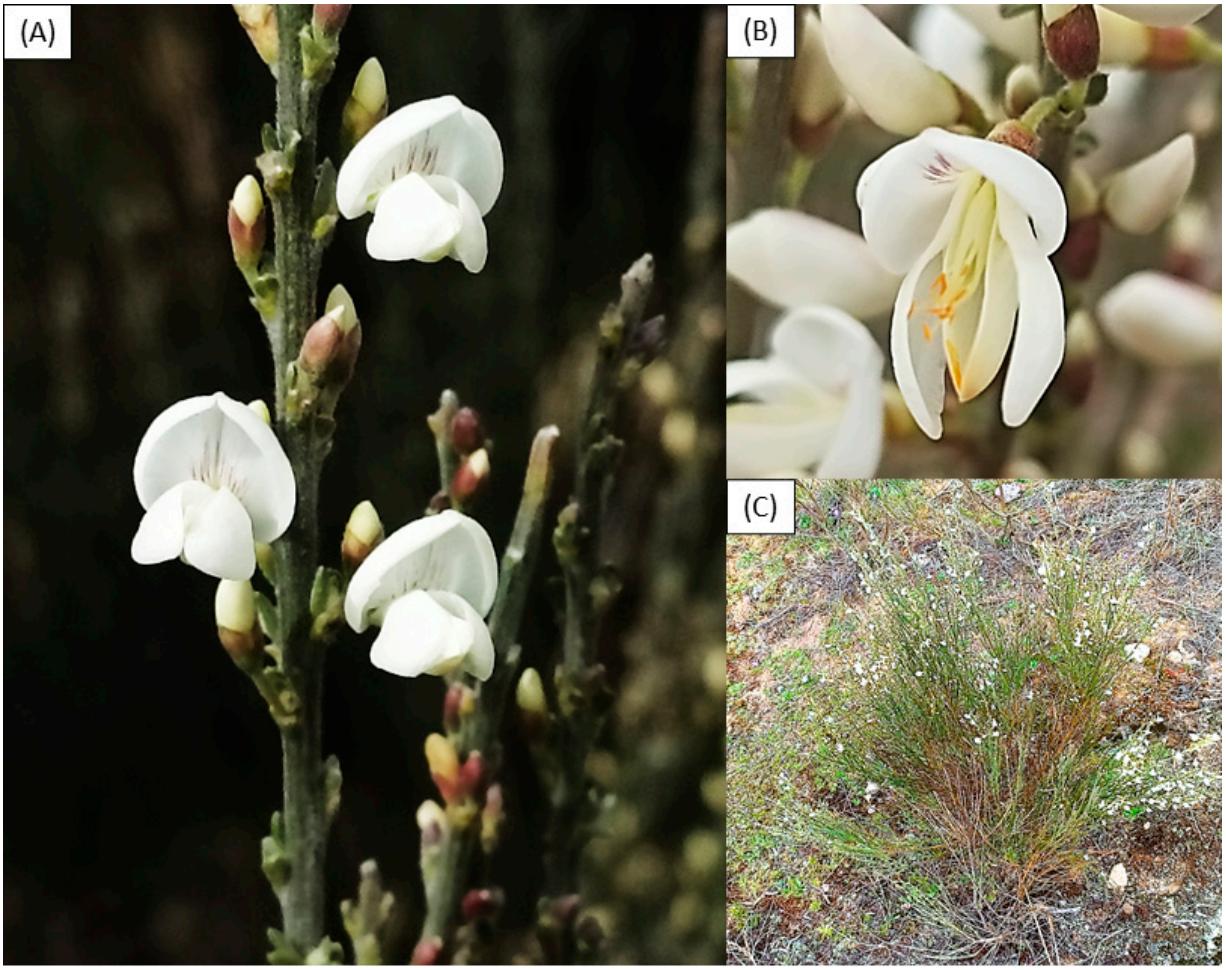

Figure 1. Some examples of *C. multiflorus* in the Beira Interior region (Portugal); (**A**) branches and flowers of the shrub; (**B**) its characteristic flower; (**C**) a complete shrub.

Another study conducted by Barros et al. [20] also evaluated the phytochemical content of *C. multiflorus* flowers, concluding that they contain a significant content of ascorbic acid and tocopherols, as well as sugars such as glucose, fructose, sucrose, and trehalose, besides their phenolic and flavonoid content. The antioxidant activity was evaluated using the following chemical and biochemical assays: DPPH, reducing power, β-carotene bleaching test and lipid peroxidation using thiobarbituric acid-reactive substances (TBARS). The samples were extracted with methanol at a concentration range of 0.03–1.00 mg/mL. The authors concluded that the results were quite satisfactory and agreed with the total phenolics, flavonoids and ascorbic acid content. They claim that these extracts may be suitable for developing products with anti-inflammatory potential or incorporating functional drinks and other health-promoting properties related to oxidative stress.

Phenolic content with ethanolic flower extracts has also been analysed by liquid chromatography with electrospray ionization mass spectrometry (LC/ESI-MS) and nuclear magnetic resonance (NMR) methods [21]. The extraction procedure was carried

out by adding n-hexane to 5 g of ground flowers three times. Then, the residue formed was extracted with a solution of ethanol 80% (*v*/*v*) and filtered. After repeating the process three more times, the solutions were combined and freeze-dried. Regarding the chemical profile, the major compounds identified were chrysin-7-*O*-β-D-glucopyranoside, 2″-*O*-pentosyl-6-C-hexosyl-luteolin, 2″-*O*-pentosyl-8-C-hexosyl-luteolin, 6″-*O*-(3-hydroxy-3-methylglutaroyl)-2″-*O*-pentosyl-C-hexosyl-apigenin and some considerable amount of rutin. The minor compounds such as 2″-*O*-pentosyl-6-C-hexosyl-apigenin, 2″-*O*-pentosyl-8-C-hexosyl-apigenin, and 6″-*O*-(3-hydroxy-3-methylglutaroyl)-2″-*O*-pentosyl-C-hexosyl-luteolin were also identified (Figure 2). The total phenolic content (140 ± 12 mg GAE/g of extract), also assessed by the Folin-Ciocalteu colorimetric method, showed to be higher than by Luis et al. [19] for methanolic flower extracts (120.4 ± 0.9 mg GAE/g of flower extract).

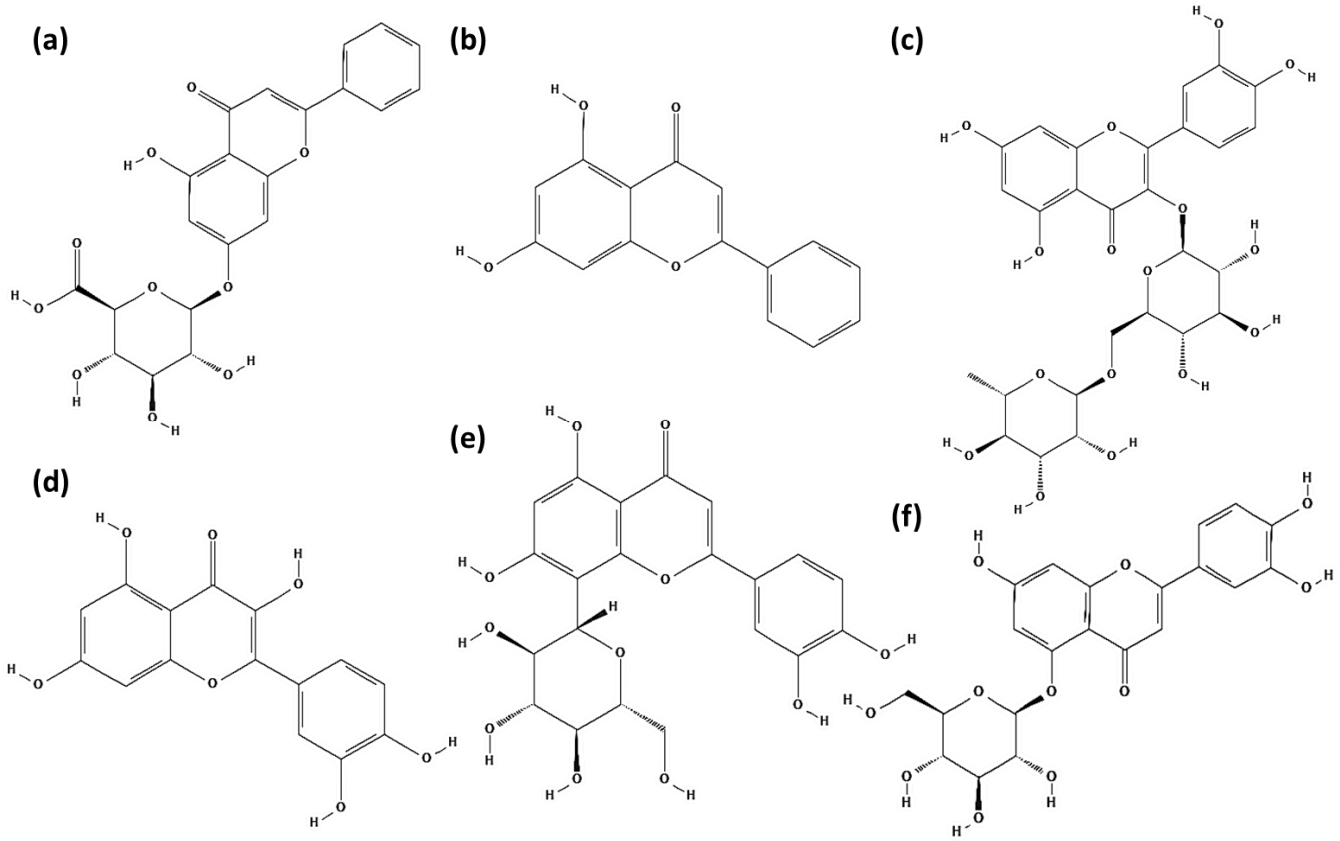

Figure 2. Some examples of flavonoids identified in *C. multiflorus*: (**a**) Chrysin 7-*O*-beta-D-glucopyranuronoside; (**b**) Chrysin; (**c**) Rutin; (**d**) Quercetin; (**e**) Orientin; and (**f**) Luteolin-5-*O*-glucoside.

In another study [16], phenolic composition and antioxidant activity were analyzed with the same type of sample (ethanolic flower extracts) of *C. multiflorus*. HPLC-DAD, ESI-MS, and NMR analysis revealed that the extract consisted of chrysin, rutin, 2″-*O*-pentosyl-8-C-hexosyl-luteolin, 2″-*O*-pentosyl-6-C-hexosyl-luteolin, and 6″-*O*-(3-hydroxy-3-methylglutaroyl)-2″-*O*-pentosyl-C-hexosyl-apigenin and the primary compound was chrysin-7-*O*-β-D-glucopyranoside. DPPH scavenging assay revealed high activity (EC_{50} of 13.4 ± 0.6) and high reducing power (95.7 ± 4.6 μg/mL). At non-toxic concentrations (50 or 200 μg/mL), the extracts were tested for their antioxidant activity in human hepatoblastoma HepG2 cells. The results demonstrated a decrease in the production rate of reactive oxygen species (ROS) in a concentration-dependent manner. A mixture of standards was also tested, simulating the phenolic composition of the plant, which provided a 50% decrease in intracellular ROS production.

These studies lead us to believe that the phenolic compounds of this species are associated with its antioxidant properties [16,22]. On the other hand, a study developed a

method to purify class III peroxidase from *C. multiflorus*. Leaves and stems were ground and incubated with agitation in a buffer (containing sodium chloride or a surfactant). The homogenate was subsequently filtered, where the resulting lower aqueous phase (containing the peroxidase) was then centrifuged, leading to the final plant extract. Considering the extraction, an interesting fact was verified: peroxidase activity of the extracts changed according to the origin and age of the plants and harvesting season. Young plants harvested during the flowering season in Almendra (Spain) area were shown to have higher peroxidase activity. After a purification process, they were subjected to photometry characterising the enzyme activity. The authors concluded that the peroxidase purified from this species might have a possible application in biotechnology, as a biocatalyst, or as a replacement for other commonly used peroxidases [23].

Today, there is an increasing interest in forest biomass feedstock for possible applications in bioenergy. To assess this potential, it is necessary to conduct integrated characterisation and biomass ash studies of species, particularly those native to a particular region. In this context, a study by Viana et al. [24] was carried out. The aim was to characterise the fuel and ashes of some shrub species native to Portugal and Spain, including *C. multiflorus*. For the characterisation of this biomass, the following physical and thermochemical properties were analysed: essential density, moisture content (w%), fixed carbon (FC%), volatile matter (VM%), and ash yield (A%). Carbon (C), oxygen (O), hydrogen (H), sulfur (S), nitrogen (N) analysis, calorific value, energy density, and chemical composition of ash were also processed in this study. Among all of the selected species, it is important to highlight in this work that *C. multiflorus* presents high values in nitrogen content, which would be expected since it is a leguminous species. Overall, this shrub species demonstrated several advantageous properties, such as higher heating value (HHV) and quite interesting energy density values, so there might be a chance for this biomass to be used in combustion. The authors also state that further work should focus on studying the moisture content, quantifying the calorific values of other shrub species belonging to the same genus as those studied, and the variations that the energy density may undergo (depending on different harvesting seasons and logistical scenarios).

2.2. *Cytisus scoparius*

In the case of *C. scoparius* (Figure 3), it is used due to its sedative, hypnotic, antidiabetic, diuretic, and hepatoprotective properties [25,26]. Several compounds characterise this species from different classes, such as chrysanthemaxanthin, xanthophyll, and xanthophillepoxide carotenoids; quinolizidine alkaloids (sparteine, sarotamine, and lupanine) and isoquinoline alkaloids such as tyramine and hydroxytyramine. Other compounds in the benzenoid group such as phenyl ethanol and cresol are also found in this plant. The most relevant group, which has been the target of several studies, is flavonoids since they confer antioxidant properties. *C. scoparius* contains mainly rutin, quercetin, isorhamnetin, kaempferol, 6″-*O* acetyl scoparin, and isoflavones such as genistein and sarothamnoside (Figure 4) [27,28].

Marta et al. [29] investigated the potential use of ethyl lactate as a solvent to extract the bioactive compounds from *C. scoparius* for being an organic and environmentally friendly solvent. Different fractions of the plant and the whole plant were extracted with ethyl lactate and methanol: water (1:1) in a Pressurised Liquid Extraction (PLE) method to compare the extraction efficiency, considering the total content in phenols and their composition, and the antioxidant activity. Using the Folin-Ciocalteu method, the total phenols content was very similar; the hydromethanolic extract with 31.73 ± 0.016 mg GAE/g dw and the ethyl lactate extract with water was 34.27 ± 0.008 mg GAE/g dw. The antioxidant activity showed the same pattern; 3.77 ± 0.018 mM Trolox g^{-1} dw and 3.83 ± 0.004 mM Trolox g^{-1} dw for methanolic and ethyl lactate extracts. LC-MS/MS analysis revealed differences in the phenolic profile of the different plant fractions. In seed pods, the most abundant flavone was orientin; in flowers, rutin was the most abundant flavanol. The flowers also

contain quercetin and isoquercetin. Kaempferol was only found in the pods. Twigs were characterised as being a useless source of flavanols.

Figure 3. Some examples of C. scoparius in the Beira Alta region (Portugal); (**A**) stems of the shrub; (**B**) its flower; (**C**) the leaves.

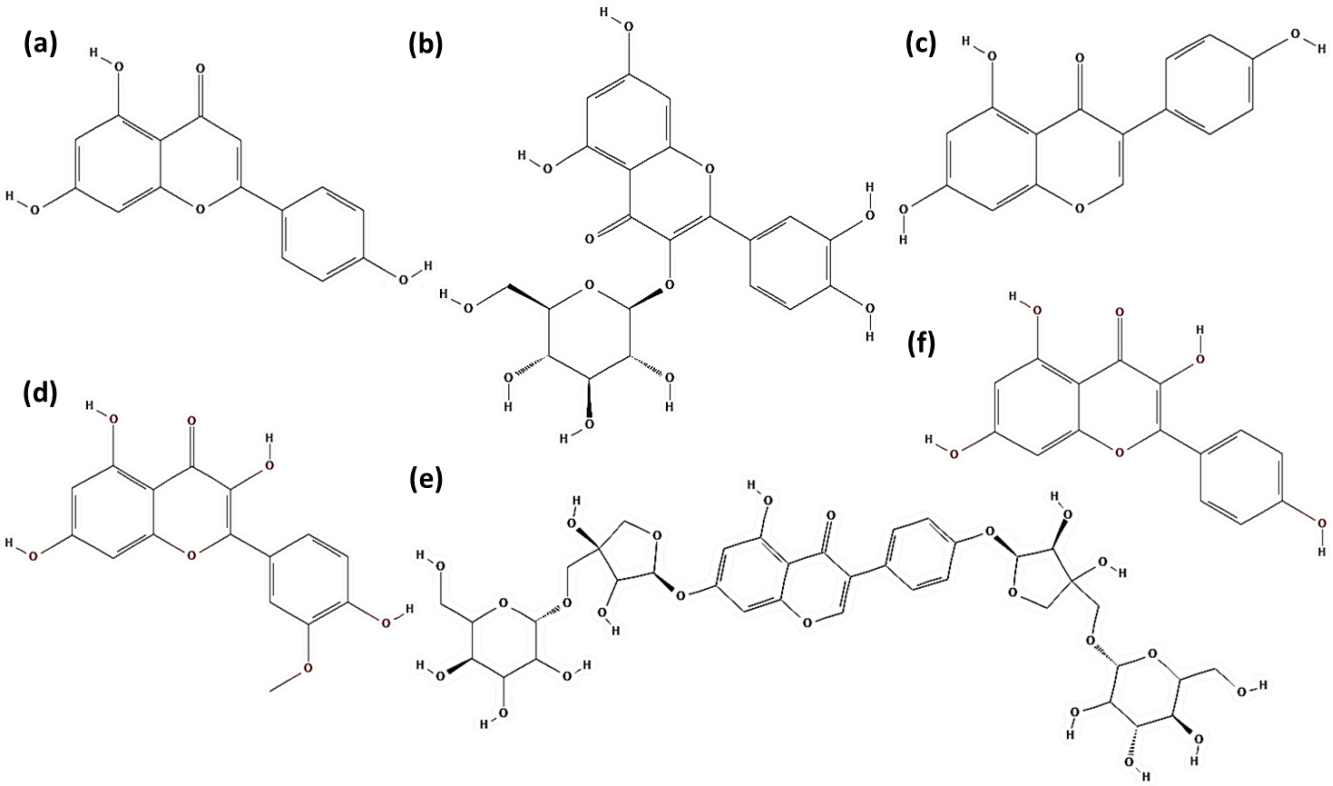

Figure 4. Some examples of flavonoids identified in *C. scoparius*: (**a**) apigenin; (**b**) isoquercetin; (**c**) genistein; (**d**) isorhamnetin; (**e**) sarothamnoside; and (**f**) kaempferol.

Additionally, the antimicrobial activity of the ethyl lactate extracts of *C. scoparius* was also tested, with relevant results against Gram-positive bacteria (namely *Staphylococcus aureus* and *Bacillus* spp.). However, the results against Gram-negative bacteria (*Escherichia coli*) and fungi (*Candida albicans*) were not as expected. The authors state that these results are promising, but further experiments are needed. In conclusion, ethyl lactate proved that it could be one alternative for extracting bioactive compounds with the advantage that the extracts can be used in food, cosmetic, and pharmaceutical fields.

Maria et al. [30] determined the chemical profile of volatile extracts of flowers and flowering branches of *Ulex europaeus* and *C. scoparius*. The extracts were obtained from a distillation with n-pentane for four hours with a Likens-Nickersin apparatus and analysed by gas chromatography-mass spectrometry (GC-MS). Twelve compounds present in the flowers and twigs of both species were chosen for phototoxicity dose-response assays, to be tested isolatedly on the germination and growth of two weeds (*Amaranthus retroflexus* and *Digitaria sanguinalis*). The chemical profile of the two extracts revealed that terpinen-4-ol, verbenol, α-terpineol, verbenone, and linalool were found in the twigs, the last being the most abundant compound. The flowers identified the following compounds: verbenol, *p*-menth-1,5-dien-8-ol, verbenone, and terpinen-4-ol, the latter two being the major compounds. It was clear that *C. scoparius* plant material can significantly reduce the root biomass and inhibit the germination of *D. sanguinalis* and the early growth of both weeds. This can be explained by the richness and abundance of oxygenated monoterpene content in the volatile profile of this shrub compared to *Ulex europaeus*. Although further studies are needed to evaluate the effectiveness of these species as herbicides in realistic fields, the results lead to a possible valorisation of the *C. scoparius* material for obtaining a natural product for weed control. The same author confirmed that some volatile organic compounds present in the extracts of the species *C. scoparius* show synergistic effects, increasing phytotoxicity regarding germination and/or growth inhibition of the weeds mentioned above [31].

The chemicals used as herbicides cause problems such as losing local flora and contamination in underground water supplies [32]. In recent years, the circular economy concept has gained significant relevance. One of the possible sustainable strategies, taking into account this concept, is to revalue unwanted plants (e.g., invasive alien plants) through the production of leaf protein concentrates (LPC). By being rich in essential amino acids, these protein concentrates are necessary for the human diet. The work of Ajay et al. [32] aimed to take a species considered invasive in Scotland, *C. scoparius*, and apply various methods of leaf protein extraction. Leaf samples of the species were treated with a phosphate buffer solution and subjected to multiple forms of protein estimation: Ninhydrin assay, Bradford assay, BCA (Bicinchoninic acid) assay, Pierce 660, Biuret assay, and Lowry assay. In addition, the phenol content was also tested using the Folin-Ciocâlteu, Fast Blue, and Prussian Blue methods. Estimating sugars was performed by the Lever assay, Anthrone assay, and Phenol-sulphuric acid methods. After the samples were purified, they were subjected to alkaline, autoclave, and enzyme-assisted extraction methods. The results showed that the species *C. scoparius* presents a very satisfactory protein recovery due to the enzyme action, in which 16.6 ± 2.3% (*w*/*w*) total protein was recovered. Still, glucose recovery was modest for sugars (30.8 ± 1.3%). Finally, the authors concluded that another species studied (*Ulex europaeus*) is a possible candidate to be revalued and that LPC technology can increase crop harvest rates.

Concerning antioxidant activity, several studies have been carried out with extracts of this species, which are summarised in Table 1. In a study by Raja et al. [33], the authors prepared diverse extracts from the aerial parts (chloroform, ethyl acetate, methanol, and hydroalcoholic) of *C. scoparius* and investigated their potential antioxidant in vitro activity, using the thiocyanate method. The extracts were prepared as follows: first, the aerial parts were reduced to powder, then 500 g of it was extracted with different solvents, in which hydroalcoholic extraction was carried out with ethanol-water (7:3), and finally, the solvents were entirely removed by rotary evaporator and then concentrated and dried. Hydroalcoholic extracts were submitted to other evaluation methods such as lipid peroxidation, superoxide dismutase (SOD), and catalase (CAT) activities. For these trials, albino rats were divided into two groups, which received different concentrations (250 and 500 mg/kg body weight) orally, and the treatment lasted for 14 days. In the end, the animals were sacrificed, and the heart, liver, and kidneys were removed, washed and stored. In the case of lipid peroxidation, the TABARS concentration decreased in both kidneys and liver with both doses but not in a dose-dependent manner. As for the SOD analysis, the level of this enzyme increased in both kidneys and liver but was not dose-related. CAT was significantly increased in the liver and kidneys using a 500 mg/kg dose. In a study by Nirmal et al. [34], the same methods were performed using 60% methanolic extracts of the aerial parts of *C. scoparius*. These in vivo trials involved rats induced for Chronic Unpredictable Mild Stress (CMS). The aim was to evaluate the antioxidant activity of the extracts in the brain, kidney, and adrenal tissues. The same was found in the study of Raja et al. [33]: treatment with the methanolic extracts increased SOD and CAT activity, mainly in the kidney at both doses (125 and 250 mg/kg).

Lipid peroxidation was also significantly decreased in kidney and adrenal tissues. Another study also investigated hydroalcoholic extracts for treating rats treated with tetrachloride carbon (CCl_4), in which the results demonstrated the same [35]. Another interesting work aimed to evaluate the potential topical application of an active extract of *C. scoparius*, in which aqueous and ethanolic extracts (AE and EE) of branches were characterised for their capacity to scavenge/reduce radicals, including reactive oxygen species (ROS) and reactive nitrogen species (RNS). A chemical profile analysis using HPLC revealed compounds such as flavonoids (rutin, kaempferol, and quercetin), fatty acids (gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, and benzoic acid) and some aldehydes were identified in both extracts. The extracts showed moderate lipid peroxidation and radical scavenging ability against various ROS and RNS compared to that provided by natural and synthetic compounds. DPPH and reducing power were also comparable to synthetic compounds. However, the authors state that further studies in skin models of oxidative damage will be needed since the extracts studied were not irritant in the Episkin™ test (performed within vitro reconstructed human skin tissue to analyse skin corrosivity and irritation). However, they were 9 to 10 times less effective than epicatechin [36].

Table 1. Summary of studies conducted on antioxidant activity using *C. scoparius* extracts.

Part(s) of Plant	Extraction Method	Methods	Results	Units	Reference
Aerial parts	It was dried at room temperature and reduced to a coarse powder. Then extracted with ethanol: water (7:3 ratio) for 48 h. After evaporation (rotary evaporator), the extract was dried and stored in a vacuum desiccator.	**DPPH:** vitamin C as standard **NOR:** Rutin as standard **SAS:** Curcumin as standard **LPA:** Vitamin E as standard **HRS:** Vitamin E as standard **RP:** Butylated hydroxytoluene as standard **TPC:** Pyrocatechol as standard	**DPPH:** IC_{50} = 1.5 µg/mL (lesser than the standard). **NOR:** IC_{50} = 116.0 (lesser than the standard). **SAS:** IC_{50} = 4.7 (lesser than the standard). **LPA:** IC_{50} = 104.0 (lesser than the standard). **HRS:** IC_{50} = 27.0 (lesser than the standard). **RP:** Lesser reductive activity than the standard **TPC:** 0.0589	**DPPH:** µg/mL **NOR:** µg/mL **SAS:** µg/mL **LPA:** µg/mL **HRS:** µg/mL **RP:** µg/mL **TPC:** µg pyrocatechol equivalent/mg	[37]
Aerial parts	Dried at room temperature and reduced to powder. After the extraction with ethanol: water (7:3 ratio) at 60 °C, the solvent was evaporated, and the extract was freeze-dried and stored in a vacuum desiccator.	**LPA, SOD, CAT, GSH, glutathione peroxidase, glutathione-s-transferase activity** and **glutathione reductase activity** The standard used for all methods was silymarin.	**LPA:** Plant extract of 500 mg/kg dose—decrease in LPO. **SOD:** 500 mg/kg of plant extract—increase **CAT:** At 500 mg/kg dose—increase **GSH:** At the dose of 500 mg/kg—increase **Glutathione peroxidase:** Treatment with 500 mg/kg—increase **Glutathione-s-transferase activity:** At 500 mg/kg dose—increase **Glutathione reductase activity:** Treatment with 500 mg/kg—increase	**LPA:** nM/mg of protein **SOD:** Units/mg of protein **CAT:** Units/mg of protein **GSH:** µg/g of protein **Glutathione peroxidase:** mUnits/mg of Protein **Glutathione-s-transferase activity:** nM CDNB formed/min/mg of protein **Glutathione reductase activity:** nM NADPH oxidized/min/mg of protein	[35]
Aerial parts	Dried at room temperature and reduced to powder. 500 g of powder was extracted with chloroform, ethyl acetate, and methanol for 48 h. Then the solvent was evaporated and stored in vacuum desiccators. Another 500 g of powder was extracted with a hydroalcoholic mixture (7:3).	For hydroalcoholic extracts: **FRAP:** standard: ferrous sulphate **LPA (TBARS):** standard: alpha-tocopherol **SOD**; **CAT**; **GSH**.	**FRAP:** increase on groups 2 and 3 (1224 and 1416) on days 7 **LPA:** decrease in TBARS concentration in liver and kidney **SOD:** increase in kidney and liver of the rats at dose 250 mg/kg (32.3 and 41.4) **CAT:** 31.4 for liver and 44.2 for kidney **GSH:** no significant change of reduced glutathione	**FRAP:** nM Fe^{2+}/Liter **LPA:** nM/g tissue. **SOD:** Units/mg of protein **CAT:** Units/mg of protein **GSH:** µg/g tissue	[33]
Aerial parts	Dried in the shade and then reduced to powder, it was then macerated with 60% methanol at room temperature for 72 h. The filtrate was evaporated in a rotary vacuum evaporator. Then the extract was stored in a desiccator.	**TPC:** standard: pyrocatechol **SOD**; **CAT**; **Ascorbic acid**; **LPA (TBARS):** standard: 1, 1, 3, 3-tetra ethoxypropane	**TPC:** 8.54 ± 0.16 **SOD:** improved in all of the tissues (at 125 and 250 mg/kg) **CAT:** increase in kidney and adrenals. No significant increase in brain **Ascorbic acid:** decrease in adrenals and any significant observation in kidney and brain. **TBARS:** decrease in kidneys and adrenal tissue.	**TPC:** % *w*/*w* **SOD:** Units/mg protein **CAT:** Units/mg protein **Ascorbic acid:** µg/g of adrenals **LPA:** nmol of MDA/mg tissue	[34]

Table 1. *Cont.*

Part(s) of Plant	Extraction Method	Methods	Results	Units	Reference
Aerial parts	Dried at room temperature (3 months) and reduced to powder. The ethanolic extracts were performed with Shoxlet apparatus using 100 g of plant and 100 mL of solvent. Aqueous extractions were carried out by refluxing the plant with 100 mL of water. After being filtered under vacuum solvents, each extract was diluted in 45 mL of methanol. Finally, 50 mL of extracts were evaporated and dryness for calculation of extraction yield.	**TPC:** standard: gallic acid in methanol **TFD:** standard: quercetin **DPPH:** standards: gallic acid and Trolox ***β*-carotene test:** standard: butylated hydroxytoluene in methanol	**TPC:** Ethanolic extract (225.32 ± 4.08) and aqueous extract (134.67 ± 0.14) **TFD:** Ethanolic extract was approx. 40 and aqueous extract was 15 **DPPH:** Ethanolic extract (65.43 ± 2.47) and aqueous extract (120.42 ± 5.33). The standard Trolox (7.10 ± 0.08) and gallic acid (1.81 ± 0.02) ***β*-carotene test:** At 500 µg/mL, the ethanolic and aqueous extract showed 60 and 90, respectively	**TPC:** mg/g of dry mass **TFD:** mg/g of dry mass **DPPH:** mg/L ***β*-carotene test:** % of inhibition	[38]
Branches	Dried at room temperature and grounded. Selection of two-particle sizes, between 0.25 and 2 mm (S1) and smaller than 0.25 mm (S2). Extraction in soxhlet with hexane during 8 h. Extracted solids of the particles were processed with acetone: water, and the raffinate was processed with 70% ethanol: 1% acetic acid. After filtration, the extracts were subjected to evaporation and freeze-dried.	**TPC:** Standard was gallic acid **DPPH**; **ABTS:** standard: Trolox **FRAP:** standard: solution of Fe (II) **RP:** standard: ascorbic acid (AA) ***β*-carotene:** standards: Butylhydroxytoluene (BHT) and butylhydroxyanisol (BHA); **O_2^-**; **H_2O_2 assay**; **HOCl assay**; **$^1O_2$ assay**; **ROO. assay**; **$ONOO^-$ assay**; **·NO assay**.	**TPC:** AE 1.8 (S1) and 19.0 (S2); EE extracts 7.6 (S1) and 11.6 (S2) **DPPH:** IC_{50} of AE was 0.12 (S1) and 1.06 (S2); For EE was 1.32 (S1) and 1.29 (S2) **ABTS:** IC_{50} of AE was 1.66 (S1) and 1.70 (S2); For EE was 1.69 (S1) and 1.70 (S2) **FRAP:** AE was 3.71 (S1) and 1.20 (S2); EE was 0.76 (S1) and 1.37 (S2) **RP:** AE was 0.546 (S1) and 0.551 (S2); EE was 0.26 (S1) and 0.47 (S2) ***β*-carotene:** AE was 749 (S1) and 662 (S2); EE was 875 (S1) and 998 (S2) **O_2^- assay:** IC_{50} of AE was 48.8 ± 19.9 (S2); For EE was 48.8 ± 19.9 (S2) **H_2O_2 assay:** no activity was found **HOCl assay:** IC_{50} of AE was 56.0 ± 5.0 (S2); For EE was 60.0 ± 4.4 (S2) **$^1O_2$ assay:** IC_{50} of AE was 15.3 ± 0.9 (S2); For EE was 48.8 ± 2.8 (S2) **ROO. assay:** AE was 0.97 ± 0.09 (S2); EE was 0.37 ± 0.10 (S2) **$ONOO^-$ assay:** IC_{50} of AE was 1.21 ± 0.07 (S2); For EE was 5.39 ± 1.19 (S2) **·NO assay:** IC_{50} of AE was 8.36 ± 0.73 (S2); EE was 13.6 ± 1.6 (S2)	**TPC:** g GAE/100 g **DPPH:** mg/mL **ABTS:** mM Trolox **FRAP:** Mm $FeSO_4$ + $7H_2O$/g E **RP:** mM AA/g E ***β*-carotene:** Antioxidant activity coefficient **O_2^- assay:** µg/mL **HOCl assay:** µg/mL **$^1O_2$ assay:** µg/mL **ROO. Assay:** µmol Trolox equiv./mg extract **$ONOO^-$ assay:** µg/mL **·NO assay:** µg/mL	[36]

Table 1. *Cont.*

Part(s) of Plant	Extraction Method	Methods	Results	Units	Reference
Seeds	Dried seeds were reduced to powder, and 50 g were mixed with 180 mL of different solvents using a Soxhlet apparatus. Extracts with petroleum ether (CPE), chloroform (CCF), ethyl acetate (CEA), acetone (CA), and methanol (CMT) were extracted for 2–3 h and then were kept at 4 °C.	**DPPH;** **Nitroblue tetrazolium assay;** **H_2O_2 scavenging assay;** **ABTS scavenging assay;** The standard used for all of the methods was BHT.	**DPPH:** at 200 µg/mL: the order of increasing radical scavenging of extracts was CA > CMT > CCF > CEA **Nitroblue assay:** at 200 µg/mL, the order of increasing radical scavenging of extracts was CEA > CCF > CMT > CA **H_2O_2 assay:** at 200 µg/mL, the order of increasing radical scavenging of extracts was CEA > CA > CMT > CCF **ABTS:** at 200 µg/mL, the order of increasing radical scavenging of extracts was CCF > CMT > CEA > CA	**DPPH:** % of inhibition **Nitroblue assay:** % of inhibition **H_2O_2 assay:** % **ABTS:** %	[39]

NOR, nitric oxide radical; SAS, superoxide anion scavenging; LPA, lipid peroxidation assay; HRS, hydroxyl radical scavenging assay; RP, reducing power; TPC, total phenolic compounds; SOD, superoxide dismutase; CAT, catalase; GSH, reduced glutathione; TFD, total flavonoids determination; O_2^-, superoxide radical; H_2O_2, hydrogen peroxide; HOCl, hypochlorous acid; $^1O_2$, singlet oxygen; ROO., peroxyl radical; $ONOO^-$, peroxynitrite; NO, nitric oxide; AE, acetone: water extracts; EE, ethanolic extracts.

2.3. *Cytisus striatus*

The above leguminous species can potentially use atmospheric nitrogen to fertilise the soils on which they are found. For this reason, many researchers have been working to prove its power as a nitrogen fixer in soils. A study carried out by Rodríguez-Echeverría and Pérez-Fernández [40], using Portuguese broom, stated that plant growth is limited by soil acidity in which the leading players are phosphorus (P), calcium (Ca), and magnesium (Mg), and the phytotoxic substances soluble aluminium (Al), and manganese (Mn). Plots with shrubs showed that the phosphorus and nitrogen content in the soil was higher, suggesting that *C. striatus* (Figure 5) can accumulate nutrients under the canopy. It was also concluded that the nitrogen content of other plants was higher in the plots where there was a dense canopy of Portuguese broom. This was explained by observing a higher nitrogen content in the shrub litter exploited by the herbs. Although this high nitrogen content was verified, the shrubs in the ecosystem do not exert an effect that facilitates plant growth in their understory.

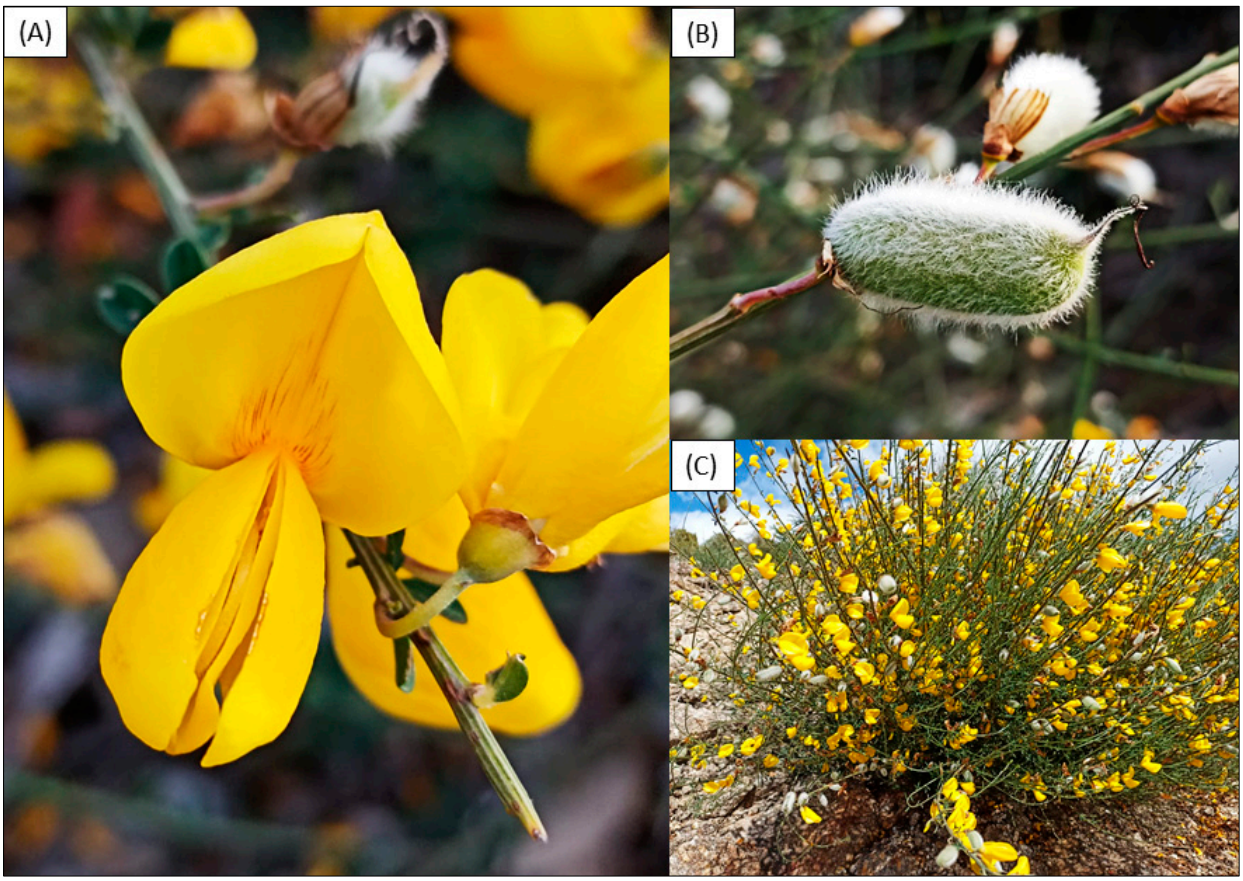

Figure 5. Some examples of *C. striatus* in the Beira Interior region (Portugal); (**A**) flowers of the shrub; (**B**) its characteristic fruit; (**C**) a complete shrub.

Within the initial theme, plants considered good at nitrogen fixation can draw attention to applications such as phytoremediation processes. It is prevalent to carry out studies on applying inoculants, from plant growth-promoting (PGP) bacterial strains to developing species under everyday environmental stresses. Maria Romero et al. [41] investigated the effects of a collection of PGP bacterial inoculants on two species (*C. striatus* and *Lupinus luteus*) planted under two different conditions: unstressed growth on perlite and diesel-contaminated soil. The results obtained in the study showed, under non-stressed conditions in the perlite experiments, that PGP strains improve (overall) plant growth. In

the case of *C. striatus*, individual inoculation was more effective in terms of length and weight than combinations.

Furthermore, no significant changes in enzyme activities occurred with these inoculants and under these conditions. On the other hand, under contaminated soil conditions, they only concluded against the *L. luteus*, where a reduction in oxidative stress was observed. The main conclusion of this work was that the PGP strains used have a potential application in phytoremediation to improve plant development but under contaminant soil conditions. Therefore, significant results were only presented for the other species and not for *C. striatus*, which leads us to believe that more studies must be carried out on this subject. Considering the density and amount of biomass that characterises this particular species, a problem has gained prominence in recent years: the increased risk of rural fires. Raposo et al. [42] state that it is necessary to adopt fire prevention strategies and that vegetation control should be selected. These authors also state that shrubs, particularly species in the *Cytisus genus*, should prioritise fuel control and management in Mediterranean forests. In Portugal, where the summer is usually arid and hot, there is a necessity to control the density of *C. striatus* by carrying out regular cleaning operations.

For this reason, a study by Leonel Nunes [43] aimed to evaluate the biomass properties of this species to assess its potential use in the production of biomass pellets. The samples of *C. striatus* were collected in the region of Guarda (Portugal), cut, and moved to an industrial pellet production unit. The shredding process allowed the formation of *C. striatus* chips with a dimension of 30 mm × 10 mm × 5 mm. In this process, it was soon found that the density of the chips was lower than wood chips of other plant species. The laboratory characterisation of the wood chips and pellets made it possible to ascertain some differences, namely in ash content and fixed carbon content.

On the other hand, the hydrogen content was relatively high (above the standard values admitted by ENPlus®) both in the pellets and the wood chips. Regarding the physical characterisation, the pellets presented values below the standard values considered by the ENPlus® standard. The results obtained to the density and shape of the chips and the physicochemical characterisation tests of the *C. striatus* biomass indicate that there may be difficulties in handling the material, and possibly this biomass is not energetically valuable. However, the author stated that the material of this species could be added to other mixtures, diluting some negative characteristics so that this species is valued and control and cleaning operations become more regular.

One of the few studies carried out with this broom species had its main objective to evaluate the antibiotic-potentiating activity of different extracts proving their antimicrobial activity [44]. Subsequently, the work aimed to isolate compounds that may be involved in the potentiation of signals for this activity through NMR-based metabolomics. Identifying the compounds and testing various combinations for methicillin-resistant *S. aureus* (MRSA) bacterial strains were also evaluated. To show the mode of action of the extract and its interaction with the antibiotics, seven strains of *S. aureus* were chosen for this experiment: RWW337, RWW50, M166, M82, RN6390, CECT 976, and SA1199B as control strains. The ethyl acetate fractions (0.5 mg/mL) used for these trials were obtained from methanolic extracts of leaves, flowers, and twigs of *C. striatus* (50 mL of solvent for 5 g of plant material) in which they were filtered, evaporated and redissolved in 10 mL of methanol. The extract was partitioned with n-hexane, and the remaining methanolic phase was evaporated and redissolved with water/methanol (95:5), which was finally extracted with 3 × 10 mL portions of ethyl acetate. These extracts were combined and evaporated. In addition to ethyl acetate fractions, hexane and remaining water/methanol fractions were also tested to ensure the absence of compounds of interest. The potentiating effect on ciprofloxacin and erythromycin activity was evaluated for all *S. aureus* strains. The extract that showed the best potentiating effect on both ciprofloxacin and erythromycin against several strains was the leaf extract, followed by the flower extract (with some additive and potentiating effects) and the twig extract (which showed no antibiotic potentiation effect). These results were corroborated by visual inspection results

of the ^{1}H-NMR spectrum of the ethyl acetate fractions of the different extracts, which reveal differences at the chemical profile level. The methanolic extracts of the leaves were analysed at the level of their chemical structure, separated by a silica gel chromatographic column, obtaining two fractions (B5 and B6). The B6 fraction was identified as luteolin, and in the B5 fraction, the following compounds were identified: apigenin, chrysin, daidzein, genistein, 2′-hydroxygenistein, and 3′-hydroxydaidzein. Identification of these compounds was carried out by using semi-preparative RP-HPLC and by ^{1}H-NMR. After testing the antibacterial activity of the components separately, isolated from *C. striatus* leaves, the authors concluded that luteolin was the only compound that showed an antibacterial effect against various *S. aureus* strains (MIC between 30 and 120 μg/mL). Furthermore, other isolated compounds showed potentiation of the antibiotics against RWW337, M116, and RWW50 strains- apigenin and genistein- and ciprofloxacin potentiating activity against the resistant line SA1199B, which was the case of genistein and daidzein. Overall, all of these results obtained point towards other questions at a scientific level, such as the structure-activity relationship of isoflavonoids, which could be interesting to evaluate for other potentiating effects on other pharmacological activities.

3. Conclusions and Future Perspectives

The main objective of this work was to gather as much information as possible about three species of the genus *Cytisus* native to the Iberian Peninsula (*C. multiflorus*, *C. scoparius* and *C. striatus*). Taking into account the use of brooms in traditional medicine, some studies on their bioactivities and possible applications have been conducted. However, few studies corroborate their results with the traditional use of these species. The chemical profile is not much explored either. Regarding this, it is possible to conclude that some flavonoids have been identified in all three species, not significantly different among them. In addition, most of the described works (mainly about *C. scoparius*) are directed at the antioxidant activity. Although there are some interesting studies that approach other kinds of applications for the extracts of the different brooms, we believe that more studies could be carried out. In terms of extraction methodology of the compounds, it is mostly very similar among the species, using almost the same method but in some cases with different solvents. It could be interesting to conduct studies comparing two different extraction methods and observe which one will be more appropriate or even discover other classes of compounds. Moreover, it is essential to emphasise that *C. striatus* is used as infusions and decoctions of the flowers to treat rheumatic diseases, hypotension, heart failure, muscle pain, and liver failure [15]. However, very few studies have been conducted to characterise its chemical profile and antioxidant, anti-inflammatory, and diuretic activities. Furthermore, by presenting similarities with *C. multiflorus* and *C. scoparius*, it is possible to assert that this species may offer an acceptable content of flavonoids and promising results in pharmacological activities, which should be the target of future investigations. *C. scoparius*, on the other hand, is considered an invasive alien species in some countries. Besides *C. scoparius*, other *Cytisus* species may also be classified as such, emphasising the importance of investigating the potential applications of its extracts or bioactive compounds in various areas such as biotechnology, phytopharmaceutical, and bioherbicide production.

These species have been spreading worldwide due to their rapid growth. This fact and the formation of dense stands can present some ecological problems. A strategy to confront some of these problems is based on controlling the expression of these shrubs. For that, it is important to find methods so they can be valorised. The valorisation is based on the discovery of possible applications for plant extracts. In order to perform this, it is necessary to analyse bioactivities or the effects these extracts can exert.

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