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Value-Added Products by Solvent Extraction and Steam Distillation from Elderberry (*Sambucus nigra* L.)

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Abstract: Medicinal plants are very important in sustainable economic development, in the pharmaceutical industry and in promoting the conservation of biodiversity. The main objective of this work was to obtain extracts and hydrolytes of flowers, leaves and bark of *Sambucus nigra*, and analyse their possible uses. Two sampling areas in a field were chosen, and collection was carried out at different times, according to the selected parts of the plant. Two techniques were chosen, steam distillation and ethanol and glycerol extraction. The hydrolytes of the different parts of *Sambucus nigra* can be used in therapeutic applications due to their pH. Distillations of the stems and leaves gave no indication of essential oil, at least with the quantities used. Therefore, the highest percentage of essential oil was found in the flowers. Ethanol was a better extraction solvent than glycerine, with yields of 99% and 90%, respectively. Flower extracts prepared with ethanol showed a total phenolic content of 59.3 ± 2 mg gallic acid equivalent/g and a total flavonoid content of 16.2 ± 0.2 mg quercetin equivalent/g. This research could contribute to the valorisation of this species giving a boost to its reuse from a circular economy perspective.

Keywords: *Sambucus nigra* L.; extraction; medicinal plants; glycerol; ethanol; solvents

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Citation: Sanchez, A.; Míguez, C.; Iglesias, A.; Saborido, L. Value-Added Products by Solvent Extraction and Steam Distillation from Elderberry (*Sambucus nigra* L.). *Appl. Sci.* **2022**, *12*, 2996. https://doi.org/10.3390/app12062996

Academic Editors: Monica Gallo and Roberto Romaniello

Received: 14 October 2021
Accepted: 12 March 2022
Published: 15 March 2022

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1. Introduction

Plant species were used as the first remedy for health problems. Man, intuitively, learned to differentiate those species that were harmful from the species that were beneficial, calling the latter medicinal plants. Arabic and Greek medicine used plant species to heal. Later in the Renaissance, plants were studied in depth and with the discovery of America the number of species, and therefore their uses, expanded. However, uses became more extensive with the creation of botanical gardens, which were available to be studied and catalogued in the 14th century. In the eighteenth century, there was a wide knowledge of plant species for medicinal use, which led to knowledge of the chemical composition of the substances with added value contained in them [1].

Medicinal plants have an important role in sustainable economic development, promoting the development of the pharmaceutical industry and the conservation of biodiversity [2]. For centuries uses of medicinal plants were limited to healers or some doctors, who obtained them from home crops or wild collection. In this century there has been a boom in the market, since sale and distribution takes place through herbalists and pharmacies. In Spain only 40% of traditional medicines is sold in pharmacies, compared to the rest of Europe where 80% is distributed through pharmacies [3]. The World Health Organization (WHO) strategy, 2014–2023, aims to strengthen the role of traditional medicine, emphasizing the importance of promoting and including the utilisation of medicinal plants in the health systems of member countries [4]. The use of medicinal plants in Spain is limited compared to the United States of America and European countries. Their use is also restricted to specific areas [5]. As a consequence of their increased use, legislation has been established in Europe and Spain to regulate the phytotherapeutic market—the Directiva Europea 2004/24/CE [6] and the Real Decreto 1345/2007, 11 de October [7]. Despite this legislation, the same botanical product can be classified in different categories depending on the country. For this reason, a review of the legislation is necessary, as well as adequate implementation and monitoring [8].

Galicia (Northwestern Spain), has a great plant wealth and due to its the plant richness and the edaphoclimatic conditions, the number of species that can be produced is very large. One of them is the elderberry (*Sambucus nigra*), which grows in this community naturally and spontaneously, and in relatively abundant quantities [9]. Nowadays there is a demand for fruit and flowers of *Sambucus* that have sustainability certifications, which is why cultivation of the plant is recommended. There are organic plantations in the UK and in German regions. In Galicia, crops are also starting.

Sambucus nigra is a shrub or small deciduous tree, with cracked bark, 4–5 m high, and up to 10 m, with a very branched, short, rough trunk; opposite leaves, petiolate, odd-pinnate, elliptical, toothed, with 5 to 7 leaflets, oval or lanceolate, 3–8 cm; creamy and aromatic white flowers, small, in umbelliform cymes of 10–20 cm in diameter, which when drying turn yellow; blackish berry fruit, globular, shiny, approximately 6–8 mm, containing flat seeds [10]. Its habitat is Europe, North Africa, Asia Minor, Caucasus and Western Siberia. It is also found in Western Asia. *Sambucus nigra* (Figure 1) forms several subspecies [11].

Kingdom: Plantae
Clade: Magnoliopsida
Subclade: Asterids
Order: Dipsacales
Family: Adoxaceae
Genus: *Sambucus*
Especie: *Sambucus nigra* L.

Figure 1. Sambucus tree [11].

The wide variety of secondary metabolites produced by various species has been responsible for their use for medicinal purposes since ancient times [12]. This source of secondary metabolites or bioactive compounds plays a fundamental role in industries such as food, pharmaceuticals and cosmetics [13]. Currently, one of the strategies for reducing the environmental impact generated is the reuse of agro-industrial bio-waste to obtain new natural ingredients that could be used by these industries [14]. Here the species proposed in this study is compatible with this purpose, thus promoting the circular economy.

The bioactive compounds of this species are highly valued for their antioxidant and anti-inflammatory properties [15]. Specifically, the flowers and fruits of this species have been a traditional remedy to treat flu-like symptoms due to their antiviral properties, as well as their ability to mitigate the symptoms of respiratory disorders during infections [16,17]. Recently, and due to this antiviral capacity, studies on the inhibitory activity of *S. nigra* flower and fruit extracts against SARS-CoV2 RBD and ACE2 receptor binding have also been developed [18].

Consequently, in this study were investigated extracts and hydrolytes from flowers, leaves and bark of *Sambucus nigra* species from two extraction methods—steam distillation and extraction with ethanol and glycerol. Value-added compounds have different disadvantages associated with them and there is a need to develop strategies to improve their bioavailability, sustainable extraction technologies, and refinement and stability procedures to increase the range of applicability for industry [19]. In this context, a bioprospecting of *Sambucus nigra* species was proposed to find new compounds to be used in different sectors of human interest.

2. Materials and Methods

2.1. Sample Preparation

Two sample field-collection areas, where the trees grow naturally and are wild, were chosen. The areas were in Rianxo and Pontevedra. Collection was made at different times, according to the selected parts of the plant, and manually. The choice of tree-type was random. Thus, new leaves were collected in April, as well as green stems—to take advantage of their inner bark. In the case of flowers, several collections were made during the months of May, June, and the beginning of July. The time of harvest is very important, since the amount of essential oil and active principles of the different parts of the plant vary throughout the day. In this case, the leaves and stems were collected in the morning and the flowers at different times of the day (morning, afternoon and night), with the idea of checking if the results varied.

The collected plants were dried in a ventilated room, in contact with a stove with a continuous heat supply for 24 h. The leaves, stems and flowers were dried with the oven at a temperature of 50 °C for approximately 15 h, until the moisture content was less than 10% in both cases. The weight of the flowers, once dry, was reduced by more than 60%, reflecting the large amount moisture in them. In order for each part of the plant to be usable, it was necessary to cut and clean the plant of useless fragments. This was done by hand and fragment by fragment, for both fresh and dried plants.

2.2. Steam Distillation

The samples of green stalks, dry and green leaves, and dried flowers were subjected to steam distillation for 2.5 h, to obtain water with volatile components. Distilled water was used to generate the steam, together with an electric heating plate at 100 °C. The samples were placed in 250 mL round bottom flask. The sample quantity was not constant (Table 1).

Table 1. Mass of samples.

Type	Mass (g)
Green stalks	32 ± 2
Green leaves	40 ± 1.5
Dried leaves	44 ± 1
Dried flowers	40 ± 1.7

2.3. Extraction Procededure

The samples were subjected to an extraction process by maceration at constant temperature of 25 ± 2 °C in dim light. Alcohol and glycerine were used as solvents for the obtained alcoholate and glycerinate. Ethanol was chosen over other alcohols for its extraction capacity in these species [20]. Glycerol was used because it is a green solvent [21]. The solvent properties are shown in Table 2.

Table 2. Solvent properties.

	Glycerol	Ethanol
NBP (°C)	290	78.37
Vapor pressure at 50 °C (mmHg)	<1	349
Dielectric constant (25 °C)	42.5	24.55
Viscosity (cP) (30 °C)	629	1.040
Density (g/mL)	1.29	0.789

Twenty grams of dry flower was placed in a 500 mL Erlenmeyer flasks, mixed with solvent (solid/liquid ratio 1:10 g/mL) and shacken once a day. The extraction process was controlled by a SPECTRO 22 digital spectrophotometer at 550 nm.

Once the extraction process was finished, the extraction yield was calculated. The extraction results were obtained by absorbance measurements. These values were measured once a day until constant. All experiments were conducted in triplicate.

If the yield was calculated as follows:

$$\text{Yield}(\%) = |\text{Absf}| - (\text{Absi}/\text{Absf} \times 100) \quad (1)$$

where Absi is absorbance before each method is applied and Absf is absorbance after each method is applied.

2.4. Analytical Method

The total phenolic content of the extract was determined by the Folin–Ciocalteu method, carrying out the analysis of the samples at the Scientific and Technological Support Center for Research (CACTI) of the Ourense campus. Polyphenols in plant extracts react with specific redox reagents (Folin–Ciocalteu reagent) to form a blue complex that can be quantified by visible-light spectrophotometry [22]. The intensity of the blue colour is proportional to the amount of reactive phenolic compounds in the sample. The concentrations of polyphenols of the plant sample were determined using a calibration curve of standard reference gallic acid [23]. An Agilent spectrophotometer, Cary-60 VIS-UV, as double internal beam with pulsing xenon lamp was used and absorbance was read at 760 nm. A calibration curve was built at five gallic acid concentrations (50, 25, 10, 5, 0) mg/L, ($y = 0.0109x - 0.001$; $R^2 = 0.9998$). The total phenolic content was expressed as mg of gallic acid equivalent (GAE) per g dry part plant.

For the flavonoid content, quercetin levels were measured after acid hydrolysis [24]. The absorbance was recorded at 530 nm and the results were expressed as quercetin equivalent based on dry extract. We worked with a calibration curve at five concentrations (30, 15, 10, 5, 0) mg/L, ($y = 0.0055x + 0.0082$; $R^2 = 0.9767$).

3. Results and Discussion

The steam extraction experiments gave a final water product (55–75 mL) and a small quantity of essential oil. This increased when leaves were used. The pH range of the hydrolates obtained was 4.5–5.2. For this reason the hydrolates could be applied to human skin (pH = 5). Hydrolates are lighter therapeutic agents than essential oils. They are mild, non-toxic and therefore ideal for use as a skin tonic or for therapeutic baths [25].

The initial absorbance was measure from each experiment, to determine the extraction efficiency and thus to compare the extractions of ethanol and glycerine. The absorbance values are presented in Figures 2 and 3. The results showed that maceration with ethanol is slower that glycerine maceration. Recent research demonstrated the high capacity of glycerol as an extracting solvent, alone or combined eutectic solvents [26–28].

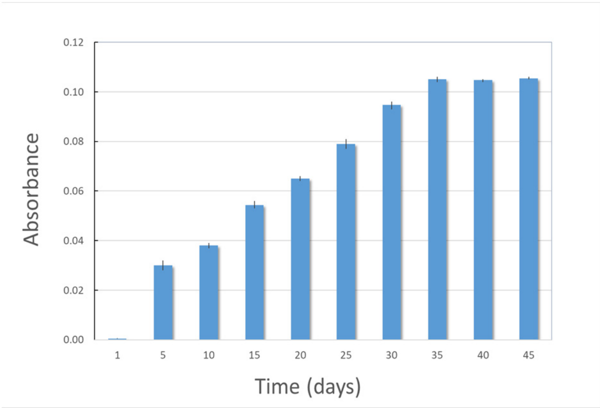

Figure 2. Time variation of absorbance in ethanol maceration.

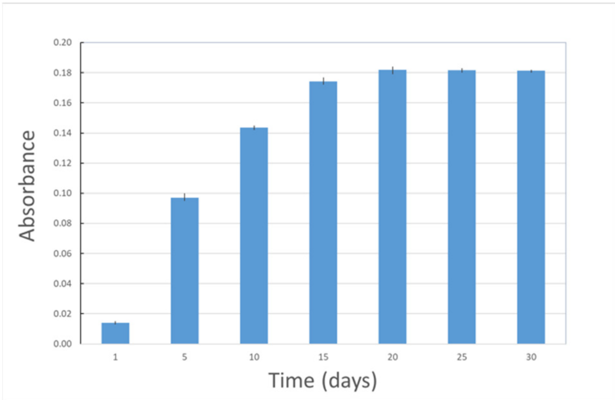

Figure 3. Time variation of absorbance in glycerine maceration.

The yield was higher with ethanol than with glycerine—99% for ethanol and 90% for glycerine. The product obtained from ethanol maceration is a fluid liquid with a sweet and vegetal smell, soluble in water and alcohol. The product obtained from glycerine maceration is a viscous liquid with a sweet and vegetal smell, soluble in water and alcohol. The glycerine extract can be used as a dietary supplement because it contains appreciable amounts of anthocyanins and other nutrients [29].

The total phenolic content of the flower extract was 59.3 ± 2 mg gallic acid equivalent/g extract. The total flavonoid content of the extract was 16.2 ± 0.2 mg quercetin equivalent/g extract. The alcohol extract of the flower has a good antioxidant activity similar to other extracts [30]. This antioxidant activity can be supported by previous literature, where the antioxidant effects of the flowers of *Sambucus nigra* species have been demonstrated. Ferreira-Santos et al. (2021) carried out aqueous extractions of elderflower, in which it was found to be a good source of natural antioxidants. Antimicrobial activity was also evaluated. The results were promising, showing the great potential of its bioactive compounds as nutraceuticals, pharmaceuticals and cosmetics for therapeutic purposes [31]. Other studies, such as those by Domínguez et al. (2020), highlighted the antioxidant activity of the fruits of this species due to their phenolic-compound content. Both studies found that the flavonoid with the greatest presence was quercetin, which would explain part of the high antioxidant activity of *Sambucus nigra* [20,32].

In conclusion, phenolic compounds have different disadvantages associated with them and it is necessary to develop strategies to improve their bioavailability, sustainable extraction technologies, and refinement and stability procedures to increase the range of applicability for industry [19].

Sambucus nigra species could be an important source of new compounds to be used in different industrial sectors, not only for the widely known pharmaceutical use but also for other sectors. The antimicrobial activity of various species is generally attributed to phenolic compounds present in the essential oils [33]. This makes it interesting to apply essential oils to the food industry as a preservative, thus avoiding the synthetic additives widely used today in many foodstuffs [34].

4. Conclusions

The hydrolates of the different parts of *Sambucus nigra* can be used in therapeutic applications due to their pH and minimal amounts of oils. The hydrolates obtained have the properties and active principles of elderberry bark and stems, with a pH range of 4.5–5.2. Ethanol was better solvent than glycerine, with a yield of 99% for ethanol and 90% for glycerine. The flower extract had total phenolics and flavonoids. The alcohol extract of the flower had good antioxidant activity and glycerine extract contained appreciable amounts of anthocyanins and other nutrients.

The recovery of functional phenolic compounds and essential oils, from a strategic point of view, promotes the efficient use of agricultural waste that can be reintroduced into the economy. Therefore, it is necessary to advance this research further to propose simple and efficient extraction methods that can be adapted to industrial scale.

Author Contributions: Conceptualization, A.S. and C.M.; methodology, A.S.; software, A.I.; validation, C.M. and A.I.; formal analysis, C.M.; investigation, A.S. and L.S.; resources, A.S.; data curation, A.S.; writing—original draft preparation, A.S.; writing—review and editing, C.M.; visualization, A.S.; supervision, A.I.; project administration, A.S.; funding acquisition, A.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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