Acta Botanica Brasilica, 2024, 38: e20230185
doi: https://doi.org/10.1590/1677-941X-ABB-2023-0185

Review

Sambucus australis Cham. & Schltdl. "Sauco", a wild and native species from South America: A review for its valorization as a wild food plant with edible and medicinal properties

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ABSTRACT

Sambucus australis (Viburnaceae) is a wild and native species from South America used in traditional medicine. The objective of this review is to collect information on geographic distribution, systematics and phylogeny, morphological and anatomical characteristics, biochemical composition, and biological and ethnobotanical activity for its valorization as a wild food plant with edible and medicinal properties. *S. australis* grows naturally in Brazil, Paraguay, Bolivia, Uruguay, and Argentina. It is a shrub or small dioecious tree. Leaves are imparipinnate, generally with 11 opposite leaflets. Flowers with a 1-cm diameter are presented in inflorescences as terminal corymbs, with an inferior, pentacarpellate and pentalocular ovary. Leaves and flowers have a high content of total phenols. The following secondary metabolites were found: quercetin, isoquercetrin, quercetin, Kaempferol, hyperoside, rutin, and di-O-caffeoylquinic, chlorogenic, gallic, caffeic, and ellagic acids. Its biological activity was described as anti-inflammatory, antioxidant, allelopathic, antiproliferative, antigenotoxic, antiparasitic, hypoglycemic, lipid-lowering, antibacterial and synergizing. Ethnobotanical studies have shown that its flowers, leaves, fruits, bark and roots are used to treat asthma, colds, flu, diabetes, measles, etc. Knowledge about the characteristics, properties, and uses of *S. australis* is an important contribution for its valorization, conservation, sustainable use and domestication

Keywords: Viburnaceae, ethnobotany, biological activity, morphological, and chemical characterization.

Introduction

Latin America and the Caribbean (LAC) comprise one of the regions with the largest natural capital endowments in the world due to its great diversity and specific endemism. However, the rapid socio-economic growth of LAC has put pressure on natural resources and caused a continued loss of biodiversity. Thus, it is necessary to contribute to greater knowledge of native flora and promote its conservation and sustainable use through scientific research and technology transfer, favoring food and medicine production obtained from local biodiversity (Boeri *et al*. 2020).

Received: July 25, 2023; Accepted: November 23, 2023
Editor-in-Chief: Thaís Elias Almeida; Associate Editor: Eraldo Medeiros Costa Neto

How to cite:
Sosa AV, Arena ME, Radice S. 2024. *Sambucus australis* Cham. & Schltdl. "Sauco", a wild and native species from South America: A review for its valorization as a wild food plant with edible and medicinal properties. Acta Botanica Brasilica 38: e20230185. doi: 10.1590/1677-941X-ABB-2023-0185

Obtaining food by gathering wild plants or parts of plants (*e.g.*, stems, roots, flowers, fruits, leaves, buds, and seeds) is an ancestral practice. Neglected species, forgotten by modern civilizations, not only contribute to diet diversification but have also shown to contain equally, if not higher, amounts of nutrients than the more widely available, commercial crops; hence, if properly assessed and managed, they could be introduced into human nutrition. Furthermore, to address the growing need for food and ensure food security for an ever-growing population, plant breeders need access to new genetic resources to be used in crop breeding programs. In crop wild relatives, it is feasible to find a large reserve of genetic diversity of agronomic importance due to its high adaptability to a wide range of habitats and environmental security conditions (Borelli *et al*. 2020).

Sambucus is a cosmopolitan plant genus that has been used for centuries for the medicinal properties and nutritional value (Corrado *et al*. 2023) found in its different organs such as leaves, flowers, and fruits called "elderberries" (Hummer *et al*. 2012). European and American elderberries (*S. nigra* ssp.) are well established in cultivation (Corrado *et al*. 2023). However, in South America, several species of *Sambucus* remain wild. For example, the *S. australis* could be considered a wild food species and little information is available.

In this review, literature focusing on *S. australis* as a wild food plant for edible and medicinal uses was analyzed. The questions that frame this review are: 1. What is the state of knowledge regarding the geographical distribution, systematics and phylogeny, morphological and anatomical characterization, chemical composition, biological activity, and ethnobotany of *S. australis*? 2. What is yet to be known about its conservation and sustainable use? 3. What are the similarities and differences between *S. australis* and *S. peruviana*, a species usually cohabiting with *S. australis*? 4. Does *S. australis* present other uses rather than food? 5. Is the background information presented in this review enough for its valorization as a wild food with edible and medicinal value?

Materials and methods

The literature search for this paper was conducted from 2022 to mid-2023 by using Science Direct, Google Scholar, PubMed and Google databases. For this search, the terms "*Sambucus australis*", "*S. australis*", "*Sambucus australis* + etnobotanica", "*Sambucus australis* + etnomedicina", "filogenia + Dipsacales + *Sambucus australis*" were used as selection criteria, and these combinations were also included in English. From the total number of articles found, those related to the geographical distribution, systematics and phylogeny, morphological and anatomical characterization, chemical composition, and biochemical and ethnobotanical activity of *S. australis* were taken into consideration. We found 77 articles, 5 Theses, 4 book chapters and 4 websites. Some articles on *S. australis* were excluded because they were not related to any of the topics of interest and included repeated information. In addition, 5 articles and 2 book chapters were included as secondary literature references to provide a more comprehensive context about wild foods with edible and medicinal value and about the environments where *S. australis* is found.

Results and Discussion

Geographical distribution

Sambucus australis Cham. & Schltdl. is a native species from South America. It can be found in Argentina, Uruguay, Paraguay, Bolivia, and Brazil (Hurrell *et al*. 2004).

S. australis is found in different ecoregions, such as the Atlantic forest that covers southern Brazil, northeastern Argentina, and eastern Paraguay, where the climate has a dry season in winter and a humid season in summer, with an average temperature range of 16-22 °C, and with low mountain ranges, increasing towards the east, from 1000 to 3000 m (Gallero & Miraglia 2021). The Pantanal extends across the Mato Grosso du Sur and the Mato Grosso states of Brazil, the Santa Cruz department in Bolivia and the Alto Paraguay department in Paraguay. It is a floodplain with a vegetation mosaic from the Bolivian Chaco, the Atlantic Forest, the Amazon rainforest and the Cerrado, and presents high humidity and drought during the year (Bieski *et al*. 2012). Specimens were also found in the subtropical yungas, which is the Tucuman-Bolivian forest, in two sites, one of them with mountain heights of 600 to 1800 m, warm humid climate, average temperatures of 20-22 °C and the other with mountains heights between 1500 and 3000 m of altitude, average temperatures between 16 and 18 °C, and an annual rainfall of 1000 to 1200 mm (Gallegos *et al*. 2019).

Particularly in Argentina, *S. australis* is found in the provinces of Formosa, Chaco, Córdoba, Corrientes, Santa Fe, Misiones, Entre Ríos and Buenos Aires (Hurrell *et al*. 2004; Barboza *et al*. 2009). *S. australis* grows as part of the marginal forests and "Talares" in the province of Buenos Aires, together with the *Scutia buxifolia* "Coronillo", the *Jodina rhombifolia* "Sombra de toro", the *Schinus longifolius* "Molle" and the *Erythrina crista galli* "Ceibo" among others. It is an accompanying species of *Celtis tala* "Tala", which is the main species of these forests (Rastrelli 2011). The marginal forests and "Talares" are located along the coast of the Río de la Plata and of the Atlantic from San Nicolás to Mar Chiquita. They present a maximum altitude of 50 m above sea level, average temperatures of 15 °C and annual rainfalls of 850 mm with peaks during autumn and spring (Segura 2018).

In Brazil, *S. australis* is found in the states of the southern (Rio Grande do Sul, Santa Catarina, and Paraná) and southeast (Sao Paulo, Rio de Janeiro, Espirito Santo, and

Minas Gerais) regions of the country, as it can be seen in the green area in Fig. 1 (Solbrig 1961; Hurrell *et al*. 2004; Scopel 2005; Dickel *et al*. 2011). However, numerous researchers collected samples in different sites from other states located in the central-western, northeastern, and north-central regions of the country (Table 1).

Systematics and phylogeny

Sambucus australis currently belongs to the Viburnaceae family (Wilson 2016). This family is composed by 5 genera and around 200 species, which are mainly distributed in the Northern Hemisphere, in regions where the climate is temperate. Less frequently, it can be found in subtropical climates, mostly in the forests of North America, Asia, Southeast Australia and eventually on the African continent. In Latin America, they are located in mountainous regions (Backlund & Bittrich 2016; Angiosperm Phylogeny Website 2017).

Traditionally, the genus *Sambucus* was placed within the Caprifoliaceae *sensu lato* family, which includes the genera of Caprifoliaceae *sensu stricto* and the genera *Sambucus*, *Adoxa* and *Viburnum*. Sometimes, *Sambucus* was segregated to the Sambuceae subfamily (Donoghue 1983; Thorne 1983; Applequist 2013). To clarify these relationships, different phylogenetic studies were carried out. Thorne (1983) proposed the separation of the Adoxaceae family into two subfamilies based on the morphology study of pollen grains. So, the families should be Adoxoideae, which includes the genera *Sambucus*, *Adoxa*, *Sinadoxa*, and *Tetradoxa*, and the subfamily Viburnoideae, which only includes the genus *Viburnum*. First, Reitsma and Reuvers (1975) found a great similarity between the pollen of *Adoxa moschatellina* and of *Sambucus nigra*. Later, Donoghue (1981) not only confirmed the similarity in the pollen grains of the genera *Sambucus* and *Adoxa*, but also found this similarity with the pollen of several *Viburnum* species, as for exine size, shape, and sculpture (reticulated or microreticulated). These characteristics did not coincide with the description for the Caprifoliaceae pollen, which is larger and has tected or thorned exines. Afterwards, Donoghue (1985) carried out another study on pollen diversity and evolution in *Viburnum* and Caprifoliaceae, confirming the similarities found and stating that this type of pollen is the most widespread among *Viburnum* species. Starting in the 90s, different phylogenetic trees were made based on: (I) the nucleotide sequence of the rbcL

Figure 1. Distribution of *Sambucus australis* in South America. Source: Plants of the World online. Royal Botanic Garden, Kew (POWO 2023).

Table 1. *Sambucus australis* specimens collected by different authors in the central-western, northeastern and north regions of Brazil (collections in nurseries, orchards and herbariums are excluded).

Reference	City	State	Region
Almeida *et al.* 2014	San Franscisco de Conde	Bahía	Northeastern
Medeiros & Albuquerque 2012	Olinda	Pernambuco	
Silva *et al.* 2008	San Antonio, Pedrafogo	Paraíba	
Maia *et al.* 2021	Timbó	Paraíba	
Junior *et al.* 2016	Crato	Ceará	
Rao *et al.* 2011	Guaramiranga	Ceará	
do Nacimento *et al.* 2014	Guaramiranga	Ceará	
dos Santos Silva *et al.* 2020	Bragança	Pará	North
	Vigía	Pará	
Bieski *et al.* 2012	Región del Pantanal	Mato Grosso	Central-western
Maia 2018	Ponta Porá	Mato Grosso do Sul	

chloroplast gene (Donoghue *et al*. 1992; Chase *et al*. 1993; Olmstead *et al*. 1993; 2000; Fan *et al*. 2018; Wang *et al*. 2019; Ran *et al*. 2020); (II) morphological characteristics (Judd *et al*. 1994; Donoghue *et al*. 2003; Moore & Donoghue 2007; Jacobs *et al*. 2010); (III) morphological characteristics and internal transcribed spacer (ITS) of nuclear ribosomal DNA (Eriksson & Donoghue 1997) or DNA sequences (ITS, trnK, matK) (Jacobs *et al*. 2010); (IV) nucleotide sequences of the chloroplast rbcL gene with three mitochondrial loci sequences (Winkworth *et al*. 2008). Results of these studies allowed to conclude that *Sambucus*, *Adoxa* and *Viburnum* should be separated from the Caprifoliaceae family and included in the Adoxaceae family (The Angiosperm Phylogeny Group 2003), although Reveal (2008) objected the family name change to Viburnaceae (Applequist 2013). Finally, in 2016, the Vascular Plant Nomenclature Committee (NCVP) approved the family name change from Adoxaceae to Viburnaceae (Wilson 2016), and rejected Sambucaceae and Adoxaceae as family names, taking Viburnaceae as the accepted family name which includes the genera *Sambucus*, *Adoxa* and *Viburnum* just as Reveal (2008) had suggested.

Morphological and anatomical characteristics

Sambucus australis is a semi-evergreen and dioecious species. It can be a highly branched, woody shrub or a small tree (Fig. 2A) reaching up to 4 meters according to Scopel (2005), or up to 6 meters (Solbrig 1961; Hurrell *et al*. 2004). It has a grayish brown bark with very marked longitudinal cracks (Fig. 2B) (Hurrell *et al*. 2004).

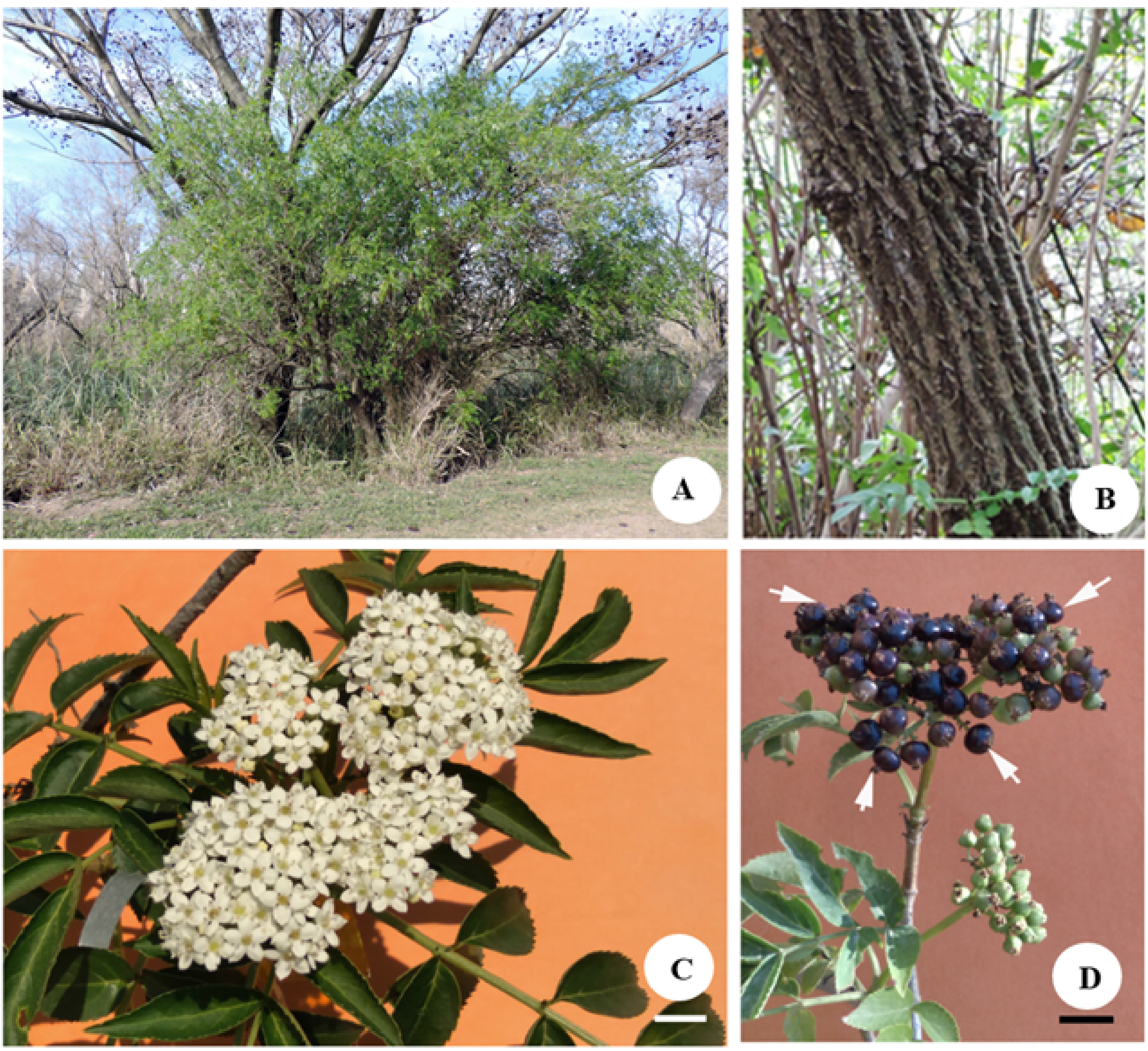

Figure 2. *Sambucus australis* grown in Argentina. (A) tree grown in Reserva Natural Costanera Sur, CABA; (B) detail of bark; (C) inflorescence of staminate flowers; (D) Mature fruits (arrows) and immature fruits. Bars = 1 cm.

Leaves of *S. australis* are imparipinnate, opposite, and petiolate, 15 to 20-cm long and are generally composed by 11 opposite leaflets, with a variation from 5 to 19 (Solbrig 1961; Hurrell *et al*. 2004; Scopel 2005; Nunes *et al*. 2007). According to Hurrell *et al*. (2004), leaflets are ovate and can be 3.5 to 7.5 cm long and 1.5 to 3 cm wide; however, Solbrig (1961), Scopel (2005) and Nunes *et al*. (2007) described them as oval-lanceolate, with variable sizes. They also agreed that the leaflets are glabrous, with serrated edges, an acute apex, and a short petiole, with a grooved upper part that is continuous along the pinna axis. According to the study by Arambarri *et al*. (2008), leaflets have a striated cuticle, are hypostomatic, and have a domed (convex) central vein on their adaxial side. The sclerenchyma tissue is also poorly developed or absent, and the blade and petiole are characterized by having collateral vascular bundles.

Flowers are presented in inflorescences as terminal corymb (Fig. 2C) forms measuring 9 to 15 × 10 to 13 cm (Pozner 2023). According to Hurrell *et al*. (2004), flowers located on the periphery are zygomorphic, while those in the rest of the inflorescence are actinomorphic; however, Scopel (2005) and Nunes *et al*. (2007) who have particularly studied the flower's botany, considered them to be actinomorphic. Morphologically, flowers are monoclines. They are white to yellowish-white, dichlamydeous, gamopetalous, and pentamerous (rarely tetrameric). The flower diameter usually ranges from 7.0 to 10.1 mm and has three very small green bracts with a triangular, oblong or elliptical shape and these are distributed at different heights of the pedicel, calyx or receptacle. They measure between 0.5 to 0.6 mm wide and 0.9 to 2.8 mm long. Tector or glandular trichomes are present in the basal portion of the adaxial sepal surface. The calyx is formed by 4-5 sepals fused at the base, with a triangular to oval shape. They are yellowish-green in color and have glandular and tector trichomes. The corolla consists of 5-pointed, star-shaped petals, which are yellowish-white in color, and very labile. Petals are fused together to form a short, oval to elliptical tube with its apex retracted towards the petiole base. Petals measure 2.5–5.0 mm long and 1.5–3.0 mm wide. Flower buds are 1.0–3.0 mm in diameter and are similar in color to the corolla throughout their development, as described by Scopel (2005). The androecium is composed of five, sometimes four, epipetalous stamens; the filament length is variable, and pistillate flowers are 1.0-2.0 mm long while staminate flowers are 3.0-4.0 mm long. The stamen is cylindrical and glabrous with 1 mm-long, , dithecal, extrorse, dorsifixed and oblong anthers. Pistillate flowers have indehiscent anthers while staminate flowers are dehiscent (Scopel 2005; Nunes *et al*. 2007). According to Bauermann *et al*. (2009), pollen grains are small monads with a subtriangular scope, and are subprolate, tricolpade in shape, with lalongate endo-aperture. The exine presents reticulated ornamentation with spaced lumens. Pollen is 20 - 25 μm, and 13 - 20 μm for polar and equatorial diameters, respectively. On the contrary, Scopel (2005) and Nunes *et al*. (2007) described the pollen grain as elongated, ellipsoidal in polar view and rounded in equatorial view with a variable diameter of 18 to 34 μm. However, they agreed that the pollen grain is tricolpate and has a reticulated surface. *Sambucus* has an inferior ovary soldered to the coronary tube, which is generally pentacarpellate and pentalocular, rarely tetracarpellate or tricarpellate and tetralocular or trilocular. It also has well-defined carpels, and one seminal rudiment per loculus with axial placentation. The gynoecium is globose-papilose, and has a short style. The stigma is five-lobed, greenish in color, covered by a nectar drop (Hurrell *et al*. 2004; Scopel 2005; Nunes *et al*. 2007; Pozner 2023). In addition, Scopel (2005) and Nunes *et al*. (2007) described three prominences emerging from each lobe. Fruits are globose or oval drupes of shiny black color (Fig. 2D) (Hurrell *et al*. 2004; Solbrig 1961; Scopel 2005; Nunes *et al*. 2007; Pozner 2023), with a membranous pericarp (Solbrig 1961), and five seeds (Solbrig 1961; Pozner 2023). Jacobs *et al*. (2010) concluded that the seeds have the size of the pyrene, measuring 2.6 mm × 1.4 mm and 3.1 mm x 1.8 mm, respectively. The embryo is cylindrical, long, and thin, and occupies the entire seed.

Among the characteristics described above, the number of leaflets, locules and carpels, the corolla diameter and its dioecious condition are the most distinctive characteristics that the S. *australis* possesses compared to other species of same genus. Morphological differences with *S. peruviana* Kunth, the other species native from South America cohabitating with *S. australis* in the Andean region of South America and northeastern Argentina (Scopel 2005) are that its leaves are bigger than the *S. australis'* (25-30 cm long), with 7-10 pubescent leaflets and veins (Solbrig 1961). Its flowers are hermaphrodite with 3.1-4.5 mm-long petals, and fruits up to 4 mm in diameter (Pozner 2023).

Chemical composition

There are few studies on the chemical composition of *S. australis* (Table 2). Almeida *et al*. (2003) reported that the *S. australis* leaf is 9.75% total ash, 0.41% silica, 72% moisture, 8.08% crude fiber and 6.26% fat, highlighting that it is the second species with the highest fat content among ten medicinal species studied. However, a high ash level and a low silica content indicate high mineral richness in *S. australis*. On the other hand, Clemes *et al*. (2015) evaluated the differences in the total phenol concentration and the antioxidant capacity in the aqueous and ethanolic extracts of leaves and petioles using the Folin-Ciocalteu and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) methods, respectively. Phenol concentration in the aqueous extract was 0.26 mg GAE (gallic acid equivalents)/g, while this content in the ethanolic extract was 12.84 mg GAE/g, with significant differences between them. No significant differences were observed for the antioxidant activity between both extracts (0.87% for the aqueous extract and 1.10% for the ethanolic

Table 2. Literature regarding composition and antioxidant activity of *S. australis*. Author, organ used, methodology, composition and antioxidant activity are included.

Reference	Organ	Methodology	Composition	Antioxidant activity
Almeida *et al.* 2003	Leaf	AOAC (1992)	9.75% total ash, 0.41% silica, 72% humidity, 8.08% crude fiber, 6.26% fat	
Clemes *et al.* 2015	AqLe-Pe	Folin-Ciocalteu DPPH	Ph (0,26 mg GAE/g)	0,87 %
	EtLe-Pe:	Folin-Ciocalteu DPPH	Ph (12,84 mg GAE/g)	1,10%
Tedesco *et al.* 2017	AqLe	HPLC	Ga, Ca, El, Ru, Qtin, Qtrin, Is, Ka, Ch	
	AqFl	HPLC	Qtrin, Is, Qtin, Ka, Di, Hy, Ru, Ch	
Benevides *et al.* 2017	EtLe	Folin-Ciocalteu PVPP LC/ESI-MS/MS	Ph (395,24 mg/g of pyrogallol) Ta (77,38 mg/g) Ca, Ch, Ru, Qtin	
	EtBa	Folin-Ciocalteu PVPP LC/ESI-MS/MS	Ph (381,35 mg/g of pyrogallol) Ta (47,62 mg/g) Ca, Ch, Ru, Qtin	
dos Santos *et al.* 2020	Le	Spectrometer	Al (21.41 μg/g), Ba (2.39 μg/g), Ca (1480.20 μg/g), Cr (0.08 μg/g), Cu (1.97 μg/g), Fe (2.86 μg/g), K (9868.00 μg/g), Mg(1091.40 μg/g), Mn (26.18 μg/g), Na (378.50 μg/g), Ni (0.35 μg/g), Zn (6.14 μg/g)	
Alerico *et al.* 2016	Sb	Lugol reactive Dittmar reagent Sudan IV Sulphuric acid 50% Folin-Ciocalteau UV-VIS spectrophotometry	Starch Alkaloids Lipids Calcium oxalate crystals Ph (48,32 mg GAE/g) Ru (0,62 mg/g)	
Alice *et al.* 1991	EtFl	Rizk (1982), Hussein Ayoub and Kingston (1982) and Krebs *et al.* (1969)	Fl, St, Tr	
Maciel *et al.* 1998	Fl		9.59% total ash, 11.3% humidity, Fl, Po, Ta	
Scopel 2005, Scopel *et al.* 2010	EtFl	Farmacopéia Brasileira Infrared Scale Drying HPLC	Total ash (9.59%) Humidity 10.90% Qtrin, Is, Qtin, Ka, Di, Hy, Ru, Ch	

Organ: Le: Leaf; Fl: flower; Sb: Stem bark; AqFl: aqueous flower extract; AqLe: aqueous leaf extrac; AqLe-Pe: aqueous lear and petiol extract; EtBa: ethanolic bark extract; EtFl: ethanolic flower extract; EtLe: ethanolic leaf extract; EtLe-Pe: ethanolic leaf and petiol extract. **Composition**: Ca: caffeic acids; Ch: chlorogenic acid; Di: di-O-caffeoylquine acid; El: ellagic acid; Fl: flavonoids; Ga: Gallic acid; Hy: hyperoside; Is: isoquercetrin; Ka: Kaempferol; Ph: phenols; Po: polyphenols; Qtin: quercetin; Qtrin: quercetrin; Ru: rutin; St: sterols; Ta: tannins; Tr: triterpenes.

extract). Tedesco *et al*. (2017) identified gallic acid, caffeic acid, ellagic acid, rutin, quercetin, quercitrin, isoquercitrin, Kaempferol and chlorogenic acid, particularly the latter in greater proportion, by using aqueous leaf extracts in high-performance liquid chromatography. These results were consistent to those of Benevides Bahiense *et al*. (2017), who used Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometric (LC/ESI-MS/MS), and obtained caffeic and chlorogenic acids, rutin and quercetin as the main compounds of the ethanolic leaf extract. The cited authors also found differences between the total phenol and tannin amounts present in the ethanolic extract of leaves and bark, using Folin-Ciocalteu and Insoluble polyvinyl polypirrolidone (PVPP) methods, respectively. In both cases, the values from the leaf extract were higher than those from the bark, without significant differences for total phenols (395.24 and 381.35 mg/g of pyrogallol, respectively). However, there were statistical differences for tannins (77.38

and 47.62 mg/g, respectively). Overripe fruits presented 338 mg of tannic acid/100 g fresh weight and 87.8 mg of anthocyanin/100 g fresh weight, while higher phenol contents were also found in flowers compared to the fruits, being all these highlighted values (Sosa *et al*., unpublished results). Finally, dos Santos Silva *et al*. (2020) determined the mineral elements present in the leaves of *S. australis* with a spectrophotometry. They did not find metals such as lead, molybdenum, lithium, cobalt, cadmium, and beryllium. On the contrary, they found aluminum (21.41 μg/g), barium (2.39 μg/g), calcium (1480.20 μg/g), chromium (0.08 μg/g), copper (1.97 μg/g), iron (2.86 μg/g), potassium (9868.00 μg/g), magnesium (1091.40 μg/g), manganese (26.18 μg/g), sodium (378.50 μg/g), nickel (0.35 μg/g), and zinc (6.14 μg/g). Copper, magnesium, manganese, potassium, sodium, and zinc levels were higher in *S. australis* compared to the other species grown in similar conditions (dos Santos *et al*., 2020), indicating a possible accumulation of these elements in the leaves. They also studied if the amount of these mineral elements in the *S. australis* leaf infusion were within the allowed ranges for human consumption set by the World Health Organization (WHO). Most of the elements resulted in a value lower than the maximum suggested by the WHO. In the case of aluminum, values between 0.6 and 0.18 mg were obtained, and although these values are lower than the 0.20 mg suggested by the WHO, the consumption of more than one cup of tea could be harmful to health. Manganese is the only element whose average value (0.16 mg) exceeded that recommended by the WHO (0.10 mg). It can be then concluded that a 250-mL cup of tea per day is the maximum dose recommended for an adult.

Alerico *et al*. (2016) studied the chemical compounds present in the stem bark of *S. australis*, since an ointment with apparent healing properties can be prepared with it. They detected starch grains, in a greater proportion, and alkaloids, lipids and calcium oxalate crystals, in a lesser proportion, and absence of saponins and tannins in the parenchyma cells. In addition, they compared results between different extraction methods for rutin and total phenolic compounds. Rutin quantification was done by UV-VIS spectrophotometry and total phenolic compounds by the Folin-Ciocalteau method, resulting in an average of 0.62 mg/g for rutin and 48.32 mg for GAE/g, respectively, recommending 80% ethanol as the most efficient solvent and the method "Four-hour Soxhlet" as the best extraction method. These chemical compounds in the stem bark would explain the ointment effectiveness as a healing agent since they promote skin cell growth.

Regarding the chemical study of the flower, Alice *et al*. (1991) in their work on medicinal plants of southern Brazil detected the presence of high amounts of flavonoids and small amounts of sterols and triterpenes, and the absence of alkaloids, saponins, coumarins, anthraquinones and tannins, using ethanolic extracts determined with the methods of Krebs *et al*. (1969), Rizk (1982) and Hussein Ayoub and Kingston (1982). These results are consistent with those found by Maciel and Brandão (1998), who observed the presence of flavonoids, and polyphenols and only small traces of tannins, 9.59% total ash and 11.3% humidity of. These outcomes are also consistent with those found by Scopel (2005), who reported 9.59% total ash and 10.90% humidity. Scopel (2005) and Scopel *et al*. (2010) used HPLC to obtain the chromatographic profile with the ethanolic flower extract. They observed the presence of quercetrin, isoquercetrin, quercetin, kaempferol, di-O-caffeoylquine acid, hyperoside, rutin and chlorogenic acid, which many were also reported by Tedesco *et al*. (2017) in aqueous flower extracts. On the other hand, Scopel (2005) and Scopel *et al*. (2010) verified that rutin is a good chemical marker in samples of *S. australis* and *S. nigra*, since it was the main component found in both species and decreases with time.

Biological activity

Anti-inflammatory activity of the aqueous and hydroethanolic flower extracts was verified through in vivo tests on edema induced on rat paws (Scopel 2005). The aqueous extract showed 81.70% maximum inhibition and the hydroethanolic extract showed 86.24%. In addition, the antioxidant activity was analyzed using the DPPH method, where the antioxidant activity percentage of the hydroethanolic extract ranged between 16.32 and 89.75%, while the oscillation was between 9.03 and 89.35% with the aqueous extract at the same concentration. These results allowed to conclude that both the anti-inflammatory and the antioxidant activity are related to the flavonoid content found in the same work.

On the other hand, Ribeiro *et al*. (2007) used a commercial extract of *S. australis* to verify if its consumption interfered with the labelling of blood constituents with Technetium-99m, altering the diagnostic images performed in nuclear imaging tests. Authors demonstrated that there was a significant decrease in the percentage of rat blood cells labeled with Technetium-99m, possibly due to the presence of some chelating agents or chemical compounds with oxidation-reduction properties.

To verify if the aqueous leaf extract from the *S. australis* had an allelopathic effect and could be used as a natural herbicide, Piccolo *et al*. (2007), Lima *et al*. (2009) and Fortes *et al*. (2009) carried out tests with different species. *S. australis* significantly affected the *Sida rhombifolia* germination (Piccolo *et al*. 2007) and the root growth of *Ipomoea grandifolia*, although it did not inhibit its germination (Lima *et al*. 2009). Fortes *et al*. (2009) showed that the hot aqueous extract of *S. australis* significantly decreased the seed germination of *Bidens pilosa* and *Glycine max* (soybean), confirming its allelopathic effect. The authors concluded that the hot aqueous leaf extract of *S. australis* could not be used as an herbicide in soybean plantations to fight *Bidens pilosa* because it would affect the normal growth of soybean plants.

On the other hand, Maria *et al*. (2010) evaluated the antiulcerogenic activity of *S. australis* in ulcers induced in rats with ethanol using the aqueous extract from the aerial part of the plant, except the flowers; however, its effect could not be verified. Tedesco *et al*. (2017) used the aqueous extract from the leaf and flower of *S. australis* to test the possible antiproliferative and antigenotoxic activity in *Allium cepa* roots. These effects were verified with both aqueous extracts since they significantly inhibited cell division and did not present genotoxic potential. In addition, at a concentration of 0.012 g/mL of the aqueous leaf extract, significant genotoxic activity was observed, showing a decrease in the damage caused by glyphosate.

The antiparasitic effect was also attributed to the *S. australis* leaf. Jorge *et al*. (2009) used a hydroalcoholic extract to study its pediculicidal activity. Initially, the lice mortality rate was low but it considerably increased after a 5-minute exposure, to finally obtain an 80% mortality. On the other hand, Krawczak *et al*. (2011) used an ethanolic leaf extract to test the possible acaricide activity on *Rhipicephalus microplus*. Future studies using different concentrations of hydroalcoholic extracts or different extraction methods are necessary to verify the acaricide activity.

Since the *S. australis* leaf is rich in ursolic acid, which has a wide range of biological activities, Rao *et al*. (2011) studied if ursolic acid prevents adiposity using mice fed on a high-fat diet. Abdominal adiposity was prevented with the *S. australis* leaf since it causes hypoglycemia and dyslipidemia without modifying plasma insulin values. On the other hand, Do Nascimento *et al*. (2014), in search of possible antibacterial and antioxidant activity, used the aerial parts of the plants, including the flowers, to extract ursolic acid and then, synthesize two derivatives. The three compounds presented antibacterial activity in many of the tested strains, synergy with antibiotics and antioxidant activity. Carneiro *et al*. (2019) also used ursolic acid extracted from the leaves to test the anti-inflammatory potential through nuclear factor kappa B (NF-kB) inhibition. These authors concluded that the *S. australis* has anti-inflammatory potential because it inhibits the transcription factor NF-kB activation, increases the IL-10 production, and reduces the inflammatory cytokines and nitric oxide levels. Another study on anti-inflammatory potential was that of Benevides Bahiense *et al*. (2017) who use the ethanolic extracts from the leaf and bark. In addition, they analyzed the possible antioxidant, cytotoxic and antimicrobial activities. These results are consistent with those of Carneiro *et al*. (2019), since both ethanolic extracts have anti-inflammatory potential as they reduce nitric oxide, transcription factor NF-kB, TNF-α and cytokine concentrations. On the other hand, both extracts showed a moderate DPPH sequestering activity, and a weak iron-reducing antioxidant power and did not present cytotoxic effects on any of the tested cell lines. Both ethanolic extracts also presented antimicrobial activity against Gram-negative bacteria and only the ethanolic leaf extract presented weak activity against Gram-positive bacteria.

Finally, Bertolotto and Degen (2011) studied the inhibitory activity on the aldose reductase enzyme by applying solutions prepared with *S. australis* leaves, which were previously lyophilized, and with dimethyl sulfoxide to rat lenses *in vitro*. The results showed a low inhibition percentage (28%) compared to the solutions with other species studied.

Ethnobotany in South America

The largest number of works related to ethnobotany, and particularly referred to the uses of *S. australis*, is recorded in Brazil. Several authors (Azevedo & Silva 2006; Almeida *et al*. 2014; Ferreira Júnior *et al*. 2016; Fernandes & Boff 2017; Maia 2018) have provided little information, because they only refer to the medicinal use of the species which is kept in backyards or collected in areas surrounding homes due to its large size. Furthermore, Almeida *et al*. (2014) mentioned that the species is recorded in the 1st (1929) and 5th (2010) edition of the Brazilian Pharmacopoeia. On the other hand, some authors (Stalcup & Stalcup 2000; Vendruscolo & Mentz 2006; Silva *et al*. 2008; Bieski *et al*. 2012; Medeiros & de Albuquerque 2012; De Luca *et al*. 2014; Bolson *et al*. 2015; Maia *et al*. 2021) presented more detailed works, mentioning which parts of the plant are used, how they are consumed and what diseases or ailments treat (Table 3). Medeiros and de Albuquerque's (2012) contribution is highlighted because they presented a compendium of Dr. Serpa's (1823-1829) handwritten recipe book, where the use of the flowers, fruits, bark, and root from *S. nigra* and *S. australis* as purgative was cited. On the other hand, De Luca *et al*. (2014) mentioned the consumption of *S. australis* leaves mixed with lemongrass (*Cymbopogon citratus*) leaves as a decoction against colds.

In Paraguay, Basualdo *et al*. (2004) conducted a survey in markets of Asunción and Gran Asunción, where they found that the *S. australis* leaves are marketed as antispasmodic and antigastritis medicine. The same result was obtained by Soria Rey (2021) in the interviews carried out with consumers and sellers from various cities in Paraguay. Finally, Goyke (2017) carried out his study in the areas of Chamorro Cué, Gral. E. Aquino, and San Pedro, where *S. australis* is among species rarely used by the interviewees.

On the other hand, Bussmann *et al*. (2016) showed a list of species commercialized in the markets of La Paz and El Alto, Bolivia. When comparing this list to similar works from previous years, they noted that the *S. australis* was only mentioned one year in the Rodríguez de La Paz market, possibly because the surveys were carried out at different times of the year in which the sellers did not have stock.

Table 3. Literature on ethnobotany that contains *S. australis*. Author, state, type of interviewee, organ used, therapeutic action/disease that cures, and method of preparation are included.

Reference	State/province	Interviewed	Organ	Therapeutic action/ disease	Preparation method
Fernandes & Boff 2017	Rio de Janeiro	Herbalists	Le, Fl	I) Ast, Am	In
Stalcup & Stalcup 2000	Río Grande do sul	Healers, health workers		Me, Ch, Rh, Fe, Flu, Im, Ij	
Silva *et al*. 2008	Paraíba	Elderly settlers	Le, Fl	Fe, Flu, Cu, He	In
Beiski *et al*. 2012	Mato Grosso	Settlers		I) Me II) Fe	I) In E II) In I
Medeiros & Albuquerque 2012	Pernambuco	Dr. Serpa's recipes	Fl, Fr, Ba, Ro	Pu	
Vendruscolo & Mentz 2006	Santa Catarina	Settlers	Le	Co	De
De Luca *et al*. 2014	Paraná	Settlers	Le, Fl, Ba, Ro	Di, Ca, Ar, Mu	In, De
Maia *et al*. 2021	Paraíba	Health workers, healers	Le, Fl, Ro	Fe, Flu, He, Co, Hy, If	In, De
Scarpa *et al*. 2016	Product catalog	Criollos de valles subandinos	I) Lo, Le, Fl II) Fl	I) An, At, Am II) Dp.	
Del Vitto *et al*. 1997	San Luis	Pharmacists, herbalists, fairground vendors		Dp, Dg, Du	
Rondina *et al*. 2008	Analgesic sp.		I) Pa II) Le	I) St II) As	II) De
Goleniowski *et al*. 2006	Córdoba	Healers, herbalists, consumers	Fl	Dp, Dg, Du	
Martínez 2008	Córdoba	Settlers	Fl	I) St II) Su, St	II) Co De
Martínez & Lujan 2011	Córdoba	Settlers		Inj, Tr, Wo	Wa
Luján *et al*. 2017	Córdoba	Healers, herbalists	I) Le II) Fl	I) As II) Si, Ne	
Luján & Martínez 2019	Córdoba	Healers	Le, St	Ad	
Keller & Romero 2006	Misiones	Small farmers		Am, At, Co	
Keller 2010	Misiones	Guaraníes	Fr	Am	Ru
Zamudio & Hilgert 2011	Misiones	Argeninean, brazilian, paraguayans		I) Af, flu, Br, Ast, II) y III) At, St, br, flu, Ne IV) Flu V) Co, Ast	I) H II) Haa III) Hl IV) Ha V) Hc
Kujawska *et al*. 2012	Misiones	Farmers,polish settlers		Ne	Hf
Kujawska & Hilgert 2014	Misiones.	Polish migrants	Le, St	If	In
Kujawska & Schmeda-Hirschmann 2022	Misiones.	Paraguayan migrants and their descendants	I) Fl II) Fl III) Le IV) Fr	I) At II) At III) Bc IV) Hz	I) In II) In Cp III) In bu IV) Wa
Mansfeld 2023			I) Fr II) Le	II) Re	I) J

Organ: Ba: Bark; Fl: Flower; Fr: fruit; Le: leaf; Lo: Log; Pa: plant apex; Ro: root; St: Stem. **Therapeutic action/disease**: Ad: antidiabetes; Am: anti-measles; An: Anti-inflammatory; Ar: articular diseases; As: Antispasmodic; Ast: asthma; At: antitussive; Bc: Blood cleansing; Br: bronchitis; Ca: Cardiovascular disease; Ch: Chickenpox; Co: cold; Cu: cough; Di: Diseases of the respiratory systems; Dg: Digestive; Dp: Diaphoretic; Du: Diuretic; Fe: Fever; Flu: flu; He: headache; Hy: hypertension ; Hz: Combat Herpes zoster; If: infections; Im: Improves circulation; Inj: injuries; Ij: inflamed joints; Ld: lung diseases; Me: Measles; Mu: musculoskeletal diseases; Ne: pneumonia; Pu: purgative; Rh: Rheumatism; Re: Remedy; Si: Sinusitis; St: Sore throats; St: stroke; Su: Sunburn ; Tr: trauma; Wo: wounds. **Preparation method**: Co De: cold decoction; De: Decoction; H: Honey + *S. autralis*; Ha: H + aipo; Haa: H + ambay + agrion; Hc: H + cane brandy; Hf: H + fennel; Hl: H + lemon + sage + ambay + mamón macho + agrión; In buy: Infusion with baby urine; In Cp: Infusion with *Cecropia pachystachya* leaves; In: Infusion; In E: Infusion, external use; In I: Infusion, Internal use; J: Jams; Ru: rub the fruit on the skin rashes; Wa: washings.

In Argentina, the oldest record is the use of the log, leaves and flowers of *S. australis* by the Creoles of sub-Andean Valleys in the province of Jujuy (Scarpa *et al*. 2016). The use of this species was also recorded by Del Vitto *et al*. (1997) in the province of San Luis and by Rondina *et al*. (2008) among species from Argentina with possible analgesic activity. Several interviews with sellers, specialists in home medicine, healers and consumers were carried out in the province of Misiones and were published by several authors (Goleniowski *et al*. 2006; Martínez 2008; Martínez & Luján 2011; Luján *et al*. 2017; Luján & Martínez 2019). Martínez and Luján (2011) are worth mentioning because they state this species is used to wash wounds and lesions in animals to prevent infections. Other authors (Keller & Romero 2006; Keller 2010; Zamudio & Hilgert 2011; Kujawska *et al*. 2012; Kujawska & Hilgert 2014; Kujawska & Schmeda-Hirschmann 2022) conducted interviews in the province of Misiones. All of them highlighted the importance of data collection there, because it is an area with high immigration impact, both from neighboring countries such as Paraguay and Brazil, and from Poland, and they coexist with Creoles and Guarani communities. In particular, the Guarani community uses the fruits of *S. australis* to rub on measles rashes on the skin (Keller 2010), while Paraguayan migrants employ the same fruits Herpes zoster by washing (Kujawska & Schmeda-Hirschmann 2022). According to the Mansfeld's World Database of Agricultural and Horticultural Crops (Mansfeld's 2023), the *S. australis* is mainly cultivated close to settlings in Argentina. Its berries are used in jam preparations, and its leaves are used as medicine. The ethnobotanical studies carried out in Argentina are summarized in Table 3, where the geographical site for plant collection, the organs used and how they are consumed for each condition are highlighted.

The review of Milliken and Gardens (1997) presented the Latin American plant species used to combat fever and malaria. They mentioned the use of *S. australis* leaves to combat fever and the positive results of in vitro tests against *Plasmodium falciparum*, the parasite that causes malaria. On the other hand, Bennett and Prance (2000) carried out a survey of introduced plants used by the indigenous people of northern South America. In the survey, only two plants were brought from South America, one being *S. australis*.

Finally, a recent review on the most studied species, the *Sambucus nigra*, was published (Corrado *et al*. 2023), and the range of uses and effects described for *S. australis* is comparable to those mentioned for *S. nigra*, which could be attributed to its rich phytochemical composition.

Conclusion

There is extensive knowledge regarding the geographical distribution, systematics and phylogeny, morphological and anatomical characteristics, chemical composition, biological activity and ethnobotany of *Sambucus australis* that contributes to its valorization as a wild food plant with edible and medicinal properties, and to its conservation and sustainable resource use. However, little is it known about its chemical composition and biological activity. Also, the knowledge about fruit morphophysiology and about its role as functional food is still incipient, being this topic relevant for when this species is included in the Argentinean Food Code. Some strengths for considering *S. australis* a Horticultural Crop can be mentioned, as it spontaneously grows in an extensive area of South America. This fact will favor its domestication and cultivation in sites with different soils and climates, including marginal areas. Its remarkable biological activity can be summarized as anti-inflammatory, antioxidant, allelopathic, antiproliferative, antigenotoxic, antiparasitic, hypoglycemic, lipid-lowering, antibactericidal and synergizing among others, due to its biochemical composition and mineral richness. That is why it is an ancient and traditional wild species whose different organs are widely used for medicines and, to a lesser extent, as a natural herbicide and for making processed fruit products like jams. Its beautiful flowers, which also have highlighted properties, could be considered not only a novel food product, but also an ornamental production, as for other *Sambucus* species. In conclusion, this background information shows that the *S. australis* is a good candidate to be considered a horticultural crop, and for health, food and non-food uses, which are also favored by the wide use of European and American cultivated *Sambucus* species. However, the fact that all the mentioned products are made from wild plants is a severe limitation because this species domestication is much incipient just as the development of new markets for the growing demand for functional products. This context highlights the need for new knowledge and a breeding program for promoting current and potential uses. All these purposes could increase consumer demand through multi-disciplinary activities and projects.

Acknowledgments

The authors thank the Parque Nacional Ciervo de los Pantanos, the Reserva Natural Costanera Sur and the Reserva Punta Lara for allowing the study of *Sambucus australis*. This review was carried out within the framework of the projects PIP CONICET 11220200102292CO and PICTO MINCYT-UM 0003.

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Conflicts of Interest

The authors declare no conflict of interest

Authors' Contribution

Sosa AV: Conceptualization, Investigation, Writing – original draft, Software, Formal analysis. Arena ME: Conceptualization, Resources, Writing – review & editing. Radice S.: Conceptualization, Resources, Writing – review & editing.