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Antioxidant and Biological Activity of *Cissus sicyoides* and *Rosmarinus officinalis* Extracts

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Abstract

This chapter will describe the antioxidant and biological activity of *Cissus sicyoides* and *Rosmarinus officinalis* leaf extracts, which represent an important natural source of antioxidants. These plants contain several bioactive compounds with high antioxidant activity, such as phenolic compounds, which are compounds that prevent or delay oxidative stress, acting as free radical scavengers (FRSs), and thus reduce the onset of cardiovascular disease, cancer, diabetes, epilepsy, stroke, among other diseases. The supercritical fluid extraction (SFE) has been studied to obtain antioxidant compounds from natural sources, without the drawbacks associated with conventional extraction processes, such as the use of organic solvents, which present toxicity and contaminate the extracts, is proposed.

Keywords: *C. sicyoides*, *R. officinalis*, antioxidant activity, biological activity, supercritical extraction

1. Introduction

The Amazonian biodiversity presents a great source of foods and medicinal plants rich in antioxidant compounds whose study and conscious exploration contribute to the region sustainable development [1, 2]. The plants have a great importance due to their medicinal and nutritional properties. About 70–90% of the world population prefers the use of medicinal plants or plant extracts to treat common diseases [3, 4]. Plants have been extensively studied in recent years for their antioxidant activity. The main classes of plant chemicals are phenolic compounds, tocopherols, carotenoids, and alkaloids. Among these compounds, phenolic compounds are the most important. They prevent or delay oxidative stress, acting as free radical scavengers (FRSs), and thus reduce the onset of different chronic diseases [5–8].

Antioxidants are a set of substances that can delay or inhibit oxidation reactions and act as a defense mechanism to neutralize the harmful effects of oxidation in biological systems and foods [6, 9, 10]. Oxidative stress is considered a state of imbalance where excessive amounts of reactive oxygen and nitrogen species (ROS/RNS, for example, superoxide anion, hydrogen peroxide, hydroxyl radical, peroxynitrite) exceed the capacity of endogenous antioxidants (uric acid, superoxide dismutase, catalase, glutathione peroxidase), leading to the oxidation of a biomacromolecule variety such as enzymes, proteins, DNA, and lipids. Exogenous antioxidants (phenolic compounds, carotenoids, tocopherols, and ascorbates) are consumed in the diet mainly of fruits, leaves, seeds, vegetables, and cereals, they have the function of increasing or protecting the antioxidant defense in biological systems and, therefore, they are important for endogenous oxidative stability [11–13].

It is conflicting that oxygen and nitrogen, considered essential for biological processes, are also cofactors for toxic and degenerative processes. In this sense, the antioxidant compounds act through different chemical mechanisms in order to minimize or maintain redox balance in vivo [9, 14]. There are several mechanisms by which oxidation can be inhibited. In general, the mechanisms involved include FRSs, ester bond enzymatic hydrolysis, transition metal ion sequestration, and enzyme-catalyzed peroxide reduction. The last three mechanisms mentioned do not cease reactive species action, but prevent the formation of molecules capable of promoting free radical chain reactions [15].

There is a growing interest in new sources of natural antioxidant compounds due to synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) in the food industry being severely restricted, since they may show carcinogenic effects on living organisms [16–18]. In this sense, the scientific community and consumers are looking for new bioactive compounds of natural origin that can be used to develop new treatments against diseases. In addition, they may be employed in the food industry as functional food ingredients.

Cissus sicyoides L., which belongs to the Vitaceae family, is also known as vegetal insulin, anil-trepador, bejuco-caro, cipó-pucá, or puci. It is considered a plant from the Neotropical region and is usually found in the Amazon region [19, 20]. According to studies on *C. sicyoides* extract composition, the presence of bioactive compounds with high antioxidant activity as carotenoids and phenolic compounds (flavonoid, resveratrol, coumarins, and tannins) was found [21–23]. Therefore, it is a plant traditionally used by Brazilian popular medicine to treat rheumatism, epilepsy, stroke, abscesses, arthritis, and diabetes [23, 24].

Rosmarinus officinalis is an aromatic plant of the Lamiaceae family, native to the Mediterranean region. Today, it has been grown in many parts of the world and is known as rosemary [25, 26]. It has been recognized as one of the plants with great antioxidant activity. Among the most effective antioxidant constituents, cyclic diterpene diphenols, carnosic acid, rosmarinic acid, and carnosol have been identified. *R. officinalis* extracts have been used in the treatment and/or prevention of diseases such as cancer, Alzheimer's disease, urinary and gastrointestinal infections, diabetes, ischemia, and atherosclerosis [17, 25–32]. *R. officinalis* extract has been commercially exploited as a natural antioxidant [5, 16].

Supercritical fluid extraction (SFE) has already been studied to obtain bioactive compounds from natural sources. Salazar et al. and Carvalho et al. showed that the application of SFE technology is successful in obtaining extracts from *C. sicyoides* and *R. officinalis*, respectively, with high antioxidant capacity [24, 33]. SFE is based on the use of solvents with temperatures and pressures above their critical points, which have a high solvency power. One of the most commonly used solvents in SFE is carbon dioxide (CO₂) since its critical points are moderate, nontoxic,

non-flammable, affordable, chemically inert, and apolar and has an ideal behavior for thermosensitive compound extraction. In SFE it is possible to obtain totally solvent-free extracts without compound thermal degradation due to the low operating temperatures applied [34–37].

Supercritical CO₂ (Sc-CO₂) has a limitation in dissolving polar molecules. However, this disadvantage can be solved by polar solvent addition, called modifiers or cosolvents, which modify the supercritical fluid polarity and, consequently, improve polar fraction extraction rich in bioactive substances, such as phenolic compounds related to high antioxidant activity [37, 38]. The aim of this chapter is to describe the antioxidant and biological activity of *Cissus sicyoides* and *Rosmarinus officinalis* leaf extracts that represent an important natural source of antioxidants. In addition to providing an overview of the SFE that is currently presented as a modern and environmentally safe extraction technology for antioxidant compound extraction.

2. *Cissus sicyoides*

2.1 Botanical description

Cissus sicyoides L. or *Cissus verticillata* L., which belongs to the Vitaceae family, is also known as vegetal insulin, cipó-pucá, or puci. It is considered a plant of the Neotropical region and is usually found in the Amazon region. It is a climbing plant, which can reach up to 6 m in length, and presents fleshy articulated branch, alternating leaf of ovate format, pale or yellowish-green flowers, and round fruit, with variations of color from violet to black (**Figure 1**) [19, 20].

2.2 Chemical composition

The bioactive compounds present in the leaf and stem are represented by carotenoids (α -carotene and β -carotene) [39] and phenolic compounds such as

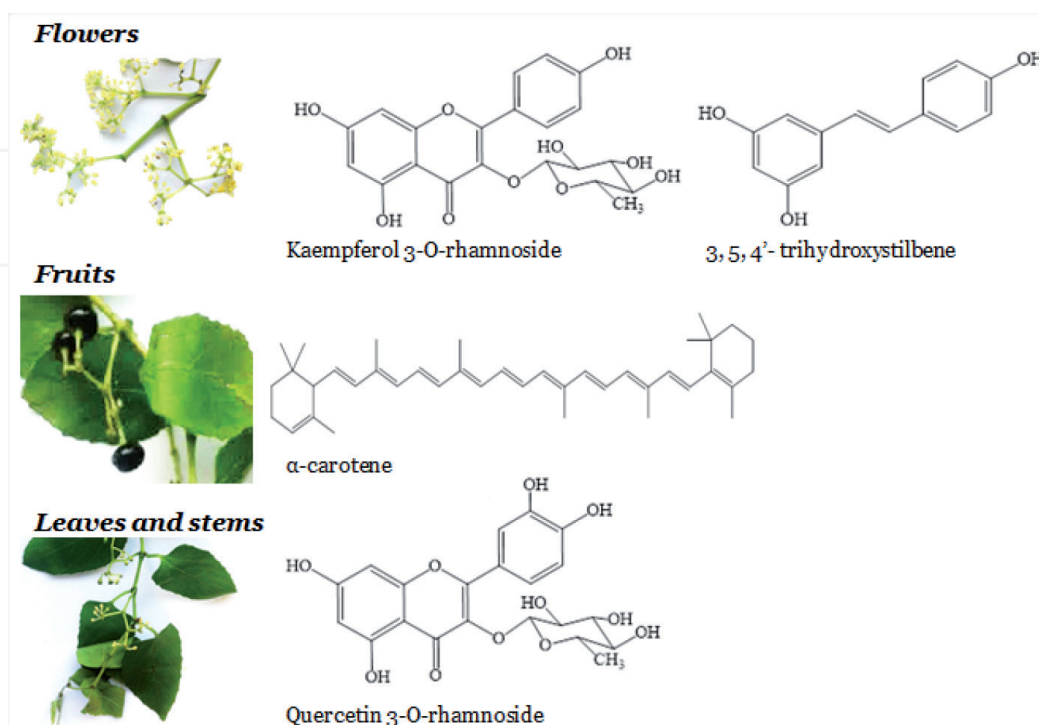


Figure 1.
 Description of *C. sicyoides* parts and the main chemical structure of the antioxidant compounds.

flavonoids (quercetin 3-O-rhamnoside and kaempferol 3-O-rhamnoside) [21]. But also, three new flavonoid glycosides were found, denominate cissosides I, II, and III (kaempferol 3-O- α -L-(5''-O-acetyl)-arabinofuranosyl-7-O- α -L-rhamnopyranoside, quercetin 3-O- α -L-arabinofuranosyl-7-O- α -L-rhamnopyranoside, and quercetin 3-O- α -L-(5''-O-acetyl)-arabinofuranosyl-7-O- α -L-rhamnopyranoside) [22].

Recently, three different flavonoids were identified (quercetin-3-O-hexoside, quercetin-3-O-deoxyhexoside, and kaempferol-3-O-deoxyhexoside) [23]. In addition, resveratrol (3,5,4'-trihydroxystilbene) [40] and a new benzofuran-type stilbene (cissusin) [22], tannins, coumarins (glycoside 5,6,7,8-tetrahydroxycoumarin-5 β -xylopyranoside and sabandin), and steroids (β -sitosterol and 3 β -O- β -D-glucopyranosyl sitosterol) were found [21]. The presence of essential oils was also detected [41]. In the supercritical extracts of *C. sicyoides* phytochemical screening obtained from leaves and stems by high-performance thin-layer chromatography (HPTLC), the presence of terpenes, phenolic compounds, and flavonoids was evidenced [24]. In the fruit composition analysis, three anthocyanins were found (delphinidin-3-rutinoside, cyanidin-3-rhamnosyl-arabinoside, and delphinidin-3-rhamnoside) [42]. Therefore, the fruit of this plant may have potential use as a food coloring. **Figure 1** shows the chemical structures of the main antioxidant compounds found in *C. sicyoides*.

2.3 Antioxidant and biological activity

In *C. sicyoides* polyphenolic profile, we can find flavonoids: the quercetin and kaempferol as the majority and its various isomers. The mechanism of quercetin antioxidant action has been associated mainly with the reduction of ROS/RNS, which is a compound that prevents or retards oxidative stress, which enables the prevention of various chronic diseases [6, 43]. In the study conducted by Crespo et al., treatment with quercetin and kaempferol prevented the production of ROS such as peroxides, superoxide anion, and nitric oxide. These results confirmed the differential protective effect of these flavonoids in the diet against oxidative stress induced by pro-inflammatory stimuli in parenchymal liver cells [44]. Resveratrol plays an important antioxidant role in reducing hydroxyl radicals, superoxide, and metal-induced radicals, as well as showing antioxidant abilities in ROS-producing cells. Also, it has a protective effect against lipid peroxidation in cell membranes and DNA damage caused by ROS/RNS [45]. Benzofuran is a potent radical scavenger capable of inhibiting lipid peroxidation, its FRS capacity being greater than α -tocopherol [46].

Figure 2 shows the results of the qualitative analysis of antioxidant activity by HPTLC of *C. sicyoides* extracts obtained by supercritical extraction (essays 1–15), hexane extract (HE), and ethanolic extract (EE) obtained with conventional extraction by Soxhlet. The plaque was derivatized with DPPH (2,2-diphenyl-1-picrylhydrazyl), and it was possible to detect the presence of yellow spots on the plaque purple bottom resulting from the reduction of the DPPH \bullet ; in the presence of antioxidant substances, 2,2-diphenyl-picryl-hydrazine is reduced, losing its purple coloration. The study results confirmed the presence of chemical constituent characteristic of this plant, with antioxidant activity. In the same study, a quantitative determination of the antioxidant activity by the DPPH method was carried out. It was demonstrated that with the extractive methodologies (SFE and Soxhlet) used it was possible to extract with low EC₅₀ values, related to a high antioxidant activity; for EE, the value of EC₅₀ (325.67 g of extract/g of DPPH) is similar to the value of EC₅₀ (404.81 g of extract/g of DPPH) obtained with SFE [24].

In the in vitro antioxidant activity determination by the ABTS method of the *C. sicyoides* aqueous extract obtained by decoction, it was evidenced that the extract has an antioxidant activity of IC₅₀ = 13.0 \pm 0.2 μ g/ml. These results indicate that

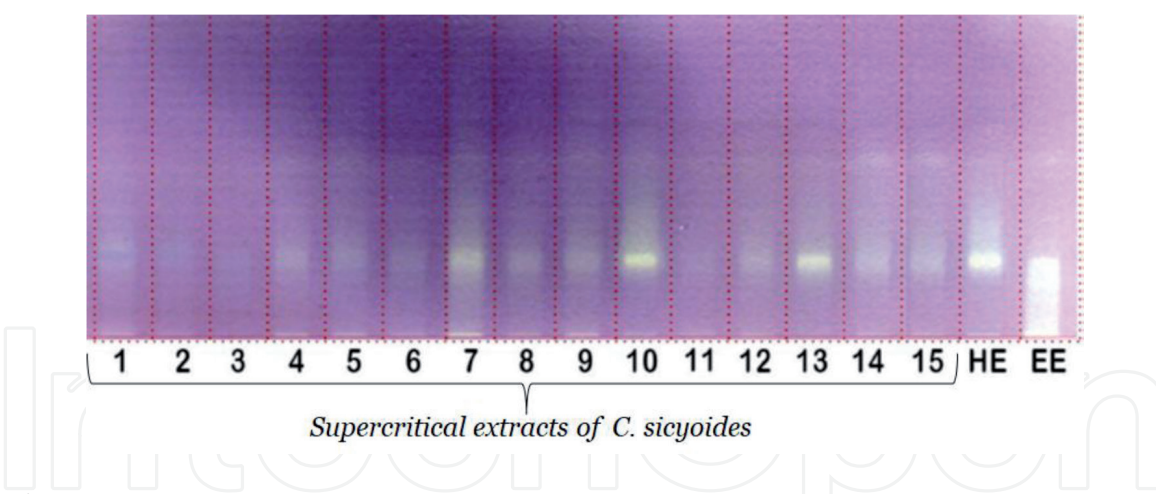


Figure 2.
 Qualitative analysis of antioxidant activity by HPTLC of *C. sicyoides* extracts obtained by supercritical extraction and conventional extraction (Soxhlet) [29].

the extract is a potential source of natural antioxidant and may be useful in the prevention of diseases associated with oxidative stress [47]. Thus, the antioxidant activity results of *C. sicyoides* extracts are related to the extraction methods and to the solvent used.

Due to antioxidant properties, *C. sicyoides* has been used by folk medicine to treat rheumatism, epilepsy, stroke, abscesses, arthritis, and diabetes; it has also been used to treat respiratory diseases. Some biological activities are attributed to the plant as anti-inflammatory, antirheumatic, antiepileptic, antihypertensive, antimicrobial, antipyretic, antioxidant, antiallergic, anticancer, and antidiabetic activities [23, 40, 48–50].

Several studies point to the application of *C. sicyoides* in the treatment of various diseases, in order to demonstrate the cytotoxic activity of the *C. sicyoides* aqueous extract obtained by decoction against cells human epidermoid carcinoma no. 2 (HEp-2 cells), showing complete inhibition of cell division after 24 h of treatment [51]. Also, the antitumor activity of *C. sicyoides* hydroalcoholic extract obtained by maceration, in animals at doses of 300 and 600 mg/kg in weight, was investigated, being demonstrated that the extract showed an inhibition of tumor activity in sarcoma-180 of 49 and 62% and Ehrlich carcinoma of 69 and 84% [52].

Regarding the plant anti-inflammatory activity, it has been demonstrated that the oral administration of 300 and 500 mg/kg of the *C. sicyoides* stem's aqueous extract obtained by decoction in mice with induced edema has a potent anti-inflammatory activity, and administration of the extract produced an approximately 50% reduction of the induced edema [53]. Resveratrol was indicated as one of the constituents responsible for the anti-inflammatory and antiallergic properties presented by *C. sicyoides* alcoholic extract obtained by maceration [40]. However, modern ethnopharmacological use reports that the *C. sicyoides* hydroalcoholic extract by percolation has anti-inflammatory and antidiarrheal actions, due to the abundant presence of flavonol-O-glycoside derivatives of quercetin and kaempferol, which are mainly responsible for the plant pharmacological effects [23].

In addition, pharmacological effects were detected in the treatment and/or prevention of dysfunctions such as hypertension and vasoconstriction of arteries, veins, and capillaries with aqueous extract of *C. sicyoides*. These compounds act at the membrane level, increasing the calcium entry through the membrane as well as acting on the internal calcium deposits [54].

In the evaluation of the antidiabetic potential of *C. sicyoides*, the effects of leaf tea from the plant were studied; the *in vivo* experimental model chosen proved to be an appropriate treatment, reducing blood and urine glucose levels [48]. Later, it

was demonstrated that treatment of diabetic rats with *C. sicyoides* aqueous extract obtained by decoction, for 7 days (100 and 200 mg/kg), reduced blood glucose levels by 22 and 25%, respectively [49]. However, *C. sicyoides* leaf tea was used to investigate the plant therapeutic efficacy in volunteers who are diabetic and intolerant to glucose. A single dose of tea (1 g of dried leaf powder in 150 ml of water) was used for a period of 7 days. It was observed in people intolerant to glucose that the tea had antidiabetic activity [55].

C. sicyoides has antibacterial activity, showing inhibitory capacity against bacteria that cause food poisoning [56], which causes acute effects in the gastrointestinal tract and, in some cases, a high severity that patients come to death (*Bacillus cereus*, *Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus aureus*, and *Escherichia coli*). In addition, the antifungal activity of plant leaf and stem alcoholic extracts was demonstrated, inhibiting the growth of fungi *Cladosporium sphaerospermum* and *Cladosporium cladosporioides* [57].

Recently, Salazar et al. carried out the biological activity determination of *C. sicyoides* supercritical extract; an in vivo test using a focal cerebral ischemia model was performed, and the extract had shown to have a neuroprotective and anti-inflammatory effect, justifying the use in traditional folk medicine for central nervous system diseases. These effects were associated to the presence of phenolic compounds in the extract [24]. Therefore, the results of these studies justify the traditional use of *C. sicyoides*, pointing to the plant extract potential benefit as a possible alternative medicine in disease treatment.

3. *Rosmarinus officinalis*

3.1 Botanical description

Rosmarinus officinalis is an aromatic plant of the Lamiaceae family, native to the Mediterranean region, and is also cultivated in Central Asia, India, Southeast Asia, South Africa, Australia, the United States, and Brazil. Today, it has been grown in many parts of the world and is commonly known as rosemary. The plant is a bush that reaches from 0.50 to 1.50 m in height, with very pungent aroma leaves and blue, violet, and white flowers (**Figure 3**) [25, 26, 58].

3.2 Chemical composition

R. officinalis chemical composition varies greatly, due to some factors that directly influence the quality, amount of oil, and extract produced. However, it is possible to verify, through the literature, that its main chemical constituents are flavones, diterpenes, steroids, and triterpenes [17, 26, 27, 31]. The phenolic compounds present in *R. officinalis* were grouped into three classes: (i) phenolic acids (vanillic, caffeic, ferulic, and rosmarinic acids), (ii) diterpenes (carnosol, rosmadial, carnosic acid, methyl carbonate, rosmanol, epirosmanol, epiisorosmanol, epirosmanol methyl ether, and epiisorosmanol ethyl ether), and (iii) flavonoids (hesperetin, apigenin, genkwanin, 4'-methoxytecto-chrysin, cirsimaritin, scutellarein, 4'',5,7,8-tetrahydroxyflavone, homoplantagin, and 6-hydroxyluteolin 7-glucoside) [27]. Recently, a *R. officinalis* chromatographic analysis was carried out, which revealed two large groups: oxygenated monoterpenes and hydrocarbonated monoterpenes. The main constituents of these groups were 1,8-cineole followed by camphor, borneol, and α - and β -pinene. The oxygenated and hydrocarbonated sesquiterpenes were composed of caryophyllene and caryophyllene oxide [26]. In the supercritical extracts of *R. officinalis* leaves, chemical analysis confirmed the

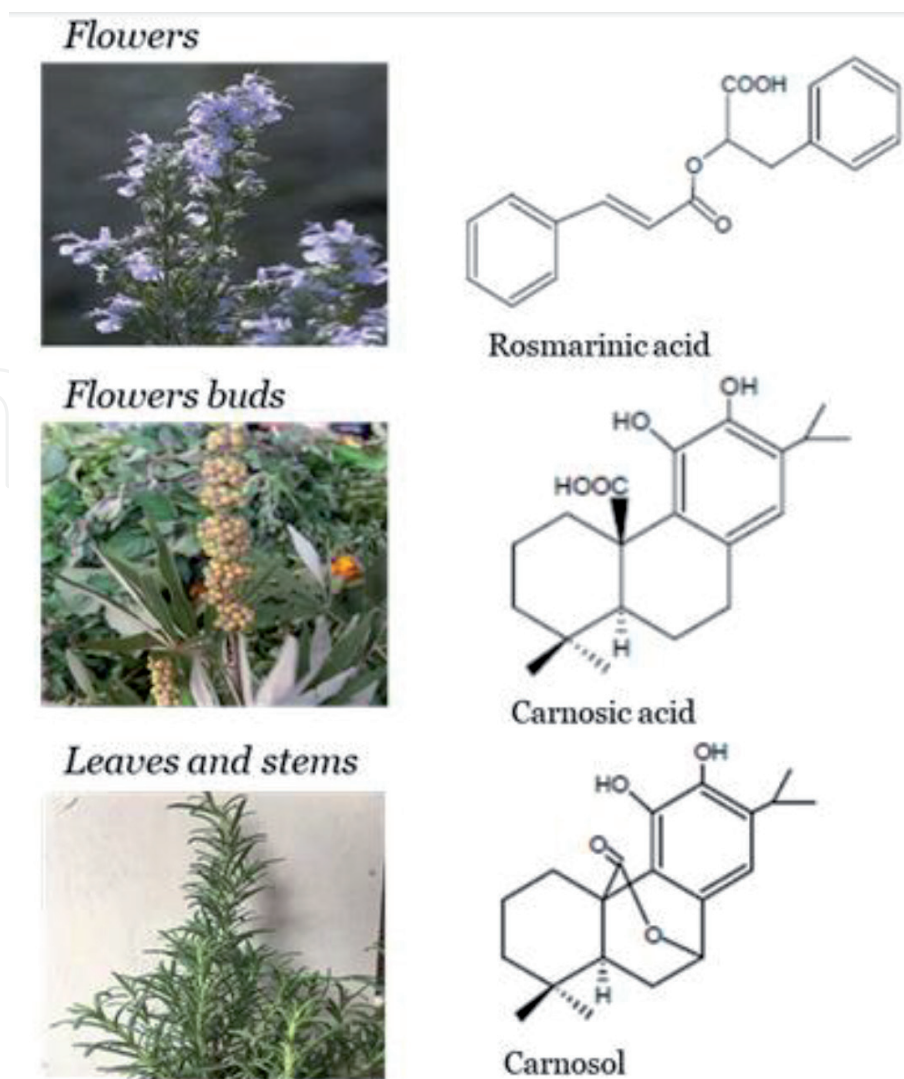


Figure 3.
 Description of *R. officinalis* parts and the main chemical structure of the antioxidant compounds.

presence of 1,8-cineole, camphor, carnosic acid, and rosmarinic acid [17, 33, 59]. **Figure 3** shows the chemical structures of the main antioxidant compounds found in *R. officinalis*.

3.3 Antioxidant and biological activity

R. officinalis has been recognized as one of the plants with high antioxidant activity [28–30]. Its antioxidant effect is due to the phenolic compounds present in the leaves and stems [60]. Among the most effective antioxidant constituents, cyclic diterpene diphenols, carnosic acid, and carnosol were identified. In addition, its extract contains epirosmanol, rosmanol, metilcarnosato, isorosmanol, and other caffeic acid derivatives [61, 62].

The action mechanism of these compounds has been widely discussed in several studies. The carnosic acid and carnosol act as potent sequestrants of peroxy radicals and are responsible for 90% of the antioxidant properties, where both are inhibitors of lipid peroxidation in liposomal and microsomal systems, besides being good sequestrants of hydroxyl radicals. Specifically, carnosic acid removes hydrogen peroxide but may also act as a substrate for its ability to increase or maintain the superoxide dismutase and glutathione peroxidase activities. The most important elements in the *R. officinalis* structure are the diterpenes containing the aromatic ring (C₁₁–C₁₂) in the catechol group, with the conjugation of three basic rings. The

catechol group is responsible for eliminating the radical electrons formed as an oxidation result. Lactone carnosol, rosmarinic acid, and hesperetin were cited in the literature as important FRSs [31, 63–65]. Rosmarinic acid has two aromatic rings, each with two OH groups that are capable of donating hydrogen and chelating metals [66]. Caffeic acid derivatives may act as metal ion chelators (Fe^{2+}), thus reducing the formation of ROS [67].

The antioxidant of *R. officinalis* extracts obtained in SFE was confirmed. Carvalho et al. analyzed the plant antioxidant activity through a coupled reaction of β -carotene and linoleic acid; the results indicated that the extracts obtained at high pressures and low temperature (300 bar/40°C) exhibited the highest antioxidant activities, in comparison to extracts obtained in low pressures (150 bar/30°C). In any case, antioxidant activities were always above the control used (β -carotene and linolenic acid) as shown in **Figure 4**. The authors state that the antioxidant action remained approximately constant for the 3-h reaction for all extracts tested. The major compounds detected in the extracts were camphor (0.6% d.b.) and 1,8-cineol (0.043% d.b.). The extract obtained by hydrodistillation showed the highest yield of camphor (1.22% d.b.) and 1,8 cineol (0.23% d.b.) compared to other extraction methods (SFE and Soxhlet) [33].

Thus, the antioxidant capacity of *R. officinalis* leaf and stem extract obtained with supercritical CO_2 was also evaluated by the ORAC method. The extract showed a high antioxidant capacity (1.9 mol Trolox/mg) similar to that of BHT and vitamin E (2.8–3.0 mol Trolox/mg). In addition, the extract presented a high percentage of lipid oxidation inhibition (88%) of fatty acids present in an analyzed cosmetic foundation. The extract volatile fraction was characterized by compounds such as camphor, 1,8-cineol, and trans- β -caryophyllene present in relative amounts of less than 25% [59].

Due to antioxidant properties, *R. officinalis* has been used in food preservation and in disease treatment. In food preservation, components such as rosmanol and carnosol prevent oxidation and microbial contamination and also can be up to four times more effective than BHA and equal to BHT as an antioxidant [27, 68, 69]. Different studies have demonstrated the potent activity of *R. officinalis* in inhibiting the formation of hydroperoxides, reducing carotenoid color loss, and retarding lipid oxidation in corn [78] and hazelnut oils [70].

The *R. officinalis* extract has been successfully commercially exploited as a natural antioxidant, for its use as synthetic antioxidants such as BHA, BHT, and TBHQ, in the food industry and is severely restricted as they may have carcinogenic effects on living organisms [5, 16, 17]. In this way, *R. officinalis* extract may be useful to

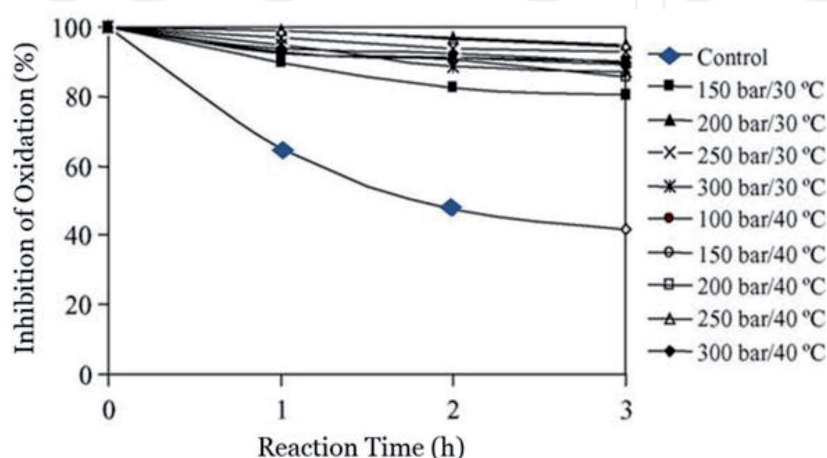


Figure 4. Antioxidant activity of *R. officinalis* extracts obtained with supercritical CO_2 [38].

replace or even decrease the synthetic antioxidants in foods. As preservatives, the extracts offer several technological advantages and benefits to consumers [71].

Health problems derived from lipid oxidation have attracted consumers' and researchers' attention, since numerous diseases are linked to dietary and biological lipid oxidation products. Therefore, *R. officinalis* extracts have been related to several biological activities, such as anticancer, antidiuretic, anti-inflammatory, antibacterial, antidiabetic, antiangiogenic, antioxidant, and hepatoprotective [28, 71–74], besides allowing the use of the plant in the treatment and/or prevention of Alzheimer's disease, urinary and gastrointestinal infections, diabetes, aging, ischemia, and atherosclerosis [25, 32].

Among the most important groups of compounds isolated from the plant, phenolic diterpenes account most of their biological activity. These compounds have been indicated in recent years as inhibitors of neuronal cell-induced death by a variety of agents both in vitro and in vivo, confirming the therapeutic potential of these compounds for Alzheimer's disease, due to the compounds multifunctional nature in the neuronal protection mediated by the plant antioxidant activity [32].

Several studies show that *R. officinalis* has pharmacological activity for chemoprevention and cancer therapy. In the extract antiproliferative activity evaluation against human ovarian cancer cells, it was corroborated that the extract inhibited the proliferation of cancer cell lines, affecting the cell cycle in multiple phases. In addition, it induced apoptosis by modifying the multiple gene expression that regulates apoptosis. Thus, the extract can be considered as an adjuvant to chemotherapy [62]. Also, the antiangiogenic effect of the carnosic acid present in *R. officinalis* extract was corroborated in angiogenesis models using human umbilical vein endothelial cells in relation to the tube formation in the reconstituted basement membrane, chemotaxis, and proliferation. Carnosic acid from the extract may be useful in preventing disorders due to angiogenesis, and its antiangiogenic effect may contribute to a neuroprotective effect [72].

The actions of *R. officinalis* leaf ethanolic extract obtained with Soxhlet extraction were tested in glucose homeostasis and antioxidant defense in rabbits. Serum levels, glucose levels and insulin levels were studied in diabetic rabbits (alloxan was used to induce diabetes); it was shown that at a dose of 200 mg/kg was possible to reduce the blood glucose level and to increase the serum insulin concentration. In addition, during 1 week of animal treatment with the extract, it was demonstrated that it had an ability to inhibit lipid peroxidation and to activate the antioxidant enzymes. Due to its potent antioxidant properties, the plant extract has a remarkable antidiabetogenic effect [75]. Recently, the antidiabetic and antihypercholesterolemic action of flavonoid-rich fractions of *R. officinalis* (fractions obtained with n-butanol and diethyl ether) in diabetic mice induced by streptozotocin was evaluated. Both fractions showed a decrease in the glucose level at a dose of 400 mg/kg, especially the fraction obtained with diethyl ether; plasma glucose levels decreased up to 60.38%. The pancreas histopathological study showed that both fractions regenerated the pancreatic β cells and increased the mass of islets. *R. officinalis* fractions exhibited a potent antidiabetic effect, while the fraction obtained with n-butanol showed a high anti-hypercholesterolemic activity [76].

The anti-inflammatory activity of *R. officinalis* supercritical extracts was studied. Absorptions of extract fractions were tested on monolayers of Caco-2 cells (2–12 h of incubation). Human macrophages were treated with basolateral fractions, and TNF- α , IL-1 β , IL-6, and IL-10 secretions were measured by ELISA. Fractions obtained after 8 and 12 h in absorption experiments caused a considerable reduction in the excretion of pro-inflammatory cytokines. This reduction in cytokine secretion levels was associated with the amounts of carnosol and carnosic acid. Thus, the *R. officinalis* supercritical extract can be used in formulations to inflammatory disease prevention [77].

In relation to the antibacterial activity, *R. officinalis* essential oils obtained by hydrodistillation exhibited antibacterial activity against *Escherichia coli*, *Salmonella typhi*, *S. enteritidis*, and *Shigella sonnei*; this activity was associated with the oil ability to reduce DPPH radical formation ($CI_{50} = 3.82 \mu\text{g/ml}$) [61]. However, the antibacterial and antifungal activities of *R. officinalis* leaf extracts obtained by SFE extraction were confirmed, and the extracts showed antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and antifungals against *Candida albicans*. Obtaining *R. officinalis* extracts by SFE has been shown to be a promising extraction with respect to its incorporation into various foods, cosmetics, and pharmaceuticals products that a natural aroma, color, and antioxidant/antimicrobial additive are desired. These properties are also necessary for the food industry in order to find possible alternatives to synthetic preservatives [17].

4. Supercritical fluid extraction (SFE) of antioxidant compounds from plant matrices

When a new extract from a natural source is tested, the most important aspects to take into account are the extraction method and the type of solvent used, as this will affect the antioxidant properties. Several extraction methods for the selective extraction from plant matrices such as *R. officinalis* were identified in the scientific literature [71].

Thus, the bioactive compound extraction has been considered one of the most important steps in the approach of obtaining or recovering bioactive compounds. Conventional extractions have been the most used technology for these compound recovery. It is based on the extraction power of different solvents and the application of high temperatures, promoting mass transfer. However, there are drawbacks associated with conventional extraction processes such as the use of large amounts of organic solvents, toxic to human health and the environment, extraction time, and the use of high temperatures that can degrade the thermosensitive compounds [6, 8]. They motivated the search for environmentally safe extraction techniques such as microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE), pressurized liquid extraction (PLE), and supercritical fluid extraction (SFE) [36, 78]. SFE has already been studied to obtain antioxidant compounds from natural sources [24, 33, 77, 79]. **Table 1** presents the antioxidant activity values of different plant extracts obtained with SFE, involving the plants under study (*C. sicyoides* and *R. officinalis*).

4.1 SFE procedure

A solvent is considered a supercritical fluid when the pressure and temperature of the system are above its critical point. This point is defined as the highest temperature and pressure at which a substance can exist in equilibrium between the liquid and vapor phases. Above its critical temperature (T_c) and critical pressure (P_c), the supercritical fluid can be considered as an expanded liquid or as a compressed gas, whose density (ρ) is relatively high and consequently has a high solvency power. This effect gives the solvent a certain degree of selectivity, in addition to allowing easy separation of the solvent from the solute, which can be achieved by a simple system depressurizing, resulting in products totally solvent-free and without thermal degradation of the compounds of interest, due to low operating temperatures [35, 38].

Plants	Extraction conditions	Solvents	Method of determination	Antioxidant capacity	Biological Activity	Refs.
<i>C. sicyoides</i>	40°C/400 bar	CO ₂ + 10% of ethanol	DPPH	404.81 ± 2.78 EC ₅₀ : g of extract/g of DPPH	Neuroprotective and anti-inflammatory effect	[29]
<i>R. officinalis</i>	40°C/300 bar	CO ₂	DPPH	12.85 ± 0.10 IC ₅₀ : µg.ml ⁻¹	Antioxidant, antibacterial, and antifungal	[21]
	100°C/350 bar	CO ₂	DPPH	0.23 ± 0.01 IC ₅₀ : mg/ml	Antioxidant	[84]
	50°C/300 bar	CO ₂	ORAC	1.9 ± 0.10 µmol Trolox/mg extract	Antioxidant	[59]
<i>Mangifera indica</i> L.	55°C/100 bar	CO ₂ + 20% of ethanol	DPPH	2.13 ± 0.24 EC ₅₀ : DPPH µg/µg dry extract	Antioxidant	[85]
<i>Eugenia uniflora</i> L.	60°C/400 bar	CO ₂ + ethanol	DPPH	>200 EC ₅₀ (µg/ml)	Antioxidant	[86]
<i>Raphanus sativus</i> L.	35°C/400 bar	CO ₂	DPPH	359 mg TE/100 g dry extract	Antioxidant and anti-inflammatory	[37]
<i>Piper nigrum</i> L	40°C/300 bar	CO ₂	DPPH	103.28 EC ₅₀ : of µg.ml ⁻¹	Antioxidant	[87]

Table 1.
Presentation of the antioxidant activity values of different plant extracts obtained with SFE, involving the plants under study (*C. sicyoides* and *R. officinalis*).

One of the most commonly used solvents in SFE is carbon dioxide (CO₂) because its critical points are moderate (T_c = 31.1°C, P_c = 73.8 bar, and critical density (ρ_c) = 0.468 g/cm³), nontoxic, non-flammable, affordable, chemically inert, and apolar and has an ideal behavior for thermosensitive compound extraction [34, 37]. In addition to the supercritical CO₂ (Sc-CO₂), there are other substances that are also used as supercritical fluids, as shown in **Table 2**.

Due to its low polarity, Sc-CO₂ presents a limitation to dissolve polar molecules. However, this disadvantage can be solved by the addition of polar solvents, called modifiers or cosolvents, which modify the supercritical fluid polarity and, consequently, improve the extraction of polar fractions rich in bioactive substances, such as phenol compounds related to high antioxidant activity [37, 38]. Methanol is the solvent most used as a modifier for various plant matrices, but it is toxic and

Fluid	T _c (°C)	P _c (bar)	ρ _c (g/cm ³)
Nitrous oxide (N ₂ O)	36.5	71.0	0.457
Ethane (C ₂ H ₆)	32.2	48.8	0.203
Propane (C ₃ H ₈)	96.7	42.5	0.220
Propylene (C ₃ H ₆)	91.9	46.2	0.230
Benzene (C ₆ H ₆)	289.0	48.9	0.302
Toluene (C ₇ H ₈)	318.6	41.1	0.290
Ammonia (NH ₃)	132.5	112.8	0.240
Water (H ₂ O)	374.2	220.5	0.272

Table 2.
Critical properties of some substances used as solvents in supercritical extraction processes.

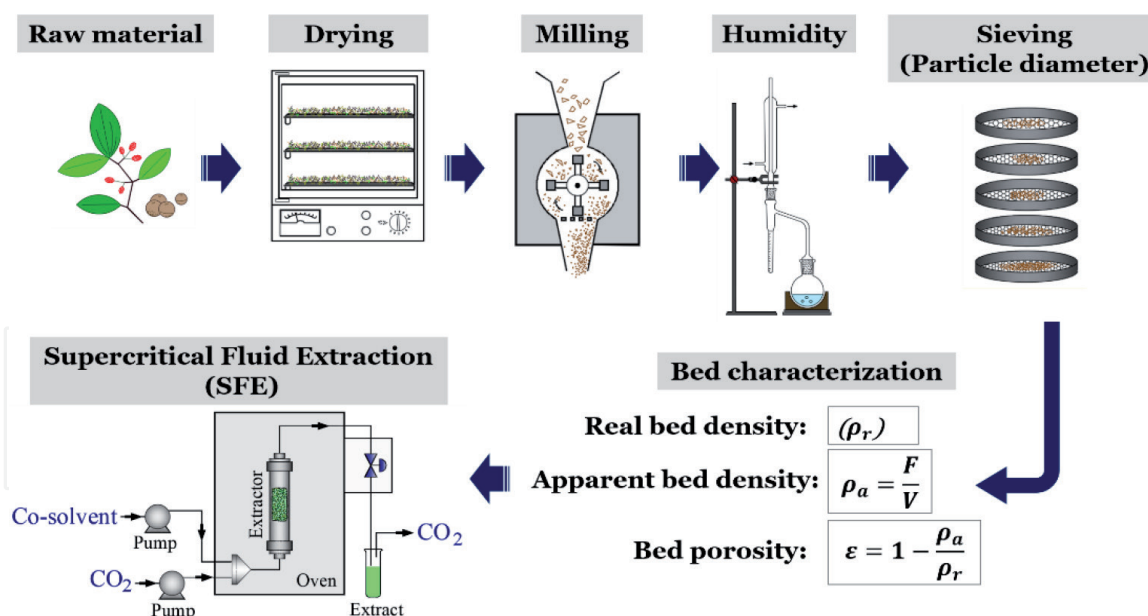


Figure 5.
Scheme of the SFE procedure of plant matrices.

different from ethanol, which is an environmentally safe solvent being a good choice for SFE processes, and can be used in the extraction of natural products [80, 81]. Water is also a very attractive cosolvent for natural product extraction due to its high polarity, which considerably increases the polarity of Sc-CO₂ [79].

For antioxidant compound extraction and recovery by SFE, several vegetable matrices were used, such as seeds, fruits, leaves, flowers, rhizomes, roots, fruit peels, and tree branches. The SFE process consists basically in the extraction of soluble compounds present in the solid matrix by a supercritical solvent and then separates these compounds from the solvent after depressurizing the system. In order to achieve an efficient and adequate extraction, several factors must be taken into account, having a careful control of the operating conditions and process step optimization [35, 36, 82].

Initially, the raw material must pass through a pretreatment stage before being fed into the fixed bed extractor; this procedure is performed to prepare the solid particles, allowing a greater efficiency to be achieved in the extraction process [83]. As shown in **Figure 5**, after the raw material is collected, one of the first stages of its pretreatment is the solid matrix moisture reduction, for example, drying leaves in an oven with air circulation. Generally, the plant matrix moisture should not exceed 14% (wet basis). Another important step is the moisture content determination by the distillation method of the Jacobs immiscible solvent, with the purpose of knowing if the quantity of water in the sample is adequate for the supercritical extraction process. The sieving stage is applied to standardize and determine the average particle size of the solid particles. The real and apparent density and bed porosity determination is also very important as they affect the particles packaging in the extraction vessel and consequently the solvent flow and the mass and heat transfer processes [35, 82].

After a suitable pretreatment, the solid matrix is placed in an extraction vessel forming a fixed bed. Depending on the compounds of interest, the supercritical solvent (Sc-CO₂) or solvent + cosolvent is fed by the solvent pump and/or cosolvent into the extraction vessel, where it continuously flows through the fixed bed and dissolves the extractable components from the solid matrix. The mixture of solutes that is removed from the solid matrix is called extract. In the separation step, the mixture formed by the solvent extraction + extract leaves the vessel and feeds the separator (collection flask) where the mixture is separated by rapid reduction of

pressure (ambient pressure). The extract precipitates in the separator, and the solvent is removed from the system [38, 83].

5. Conclusion

The identification of new natural antioxidant compounds is of great interest to the food, pharmaceutical, and cosmetic industry in order to find possible alternatives to synthetic antioxidants. In this way, plants such as *C. sicyoides* and *R. officinalis* have been extensively studied for their antioxidant activity. The *C. sicyoides* extract obtained by SFE has a neuroprotective and anti-inflammatory effect; these effects are associated with the presence of phenolic compounds and the high antioxidant activity in the extract. *R. officinalis* extract is antibacterial, antifungal, anti-inflammatory, and effective, associated with the presence of carnosic acid, carnosol, rosmarinic acids, and hesperetin. It has been corroborated that these plants contain chemical compounds that exhibit the capacity of FRSs and reduce the onset of different diseases. Finally, obtaining extracts from plant matrices using environmentally safe extraction technology such as SFE represents a great opportunity to obtain bioactive compounds.

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Conflict of interest

The authors have no conflict of interest to declare and are responsible for the content and writing of the manuscript.

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