Antioxidant activity of the commercial *Casearia sylvestris* Swartz extract

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**ABSTRACT**

This research aimed to evaluate the total antioxidant capacity of commercial *Casearia sylvestris* extract. Two different methods (Folin-Ciocalteau and Fast Blue) were used to quantify the content of total phenolic compounds. The ABTS⁺, Beta Carotene/Linoleic Acid, Phosphomolybdenum Complex, DPPH, and FRAP assays were used to evaluate the total antioxidant activity. The HPLC technique was used to identify and quantify bioactive compounds in the plant extract. Total phenolic compound levels were quantified in both assays used, with the Fast Blue method being able to quantify higher levels of these substances (526.18 ± 41.35). Twelve bioactive compounds were identified and quantified by HPLC. The major phenolic compound found in *C. sylvestris* extract was caffeic acid. This research indicates that the commercial *C. sylvestris* extract has strong bioactive potential.

**Keywords:** Medicinal plant. Bioactive compounds. Total antioxidant capacity.

**RESUMO**

Esta pesquisa teve como objetivo avaliar a capacidade antioxidante total do extrato comercial de *Casearia sylvestris*. Dois métodos diferentes (*Folin-Ciocalteau* e Fast Blue) foram usados para quantificar o teor de compostos fenólicos totais. Os ensaios ABTS⁺, Betacaroteno/Ácido Linoleico, Complexo de Fosfomolibdênio, DPPH e FRAP foram utilizados para avaliar a atividade antioxidante total. A técnica do HPLC foi utilizada para identificar e quantificar compostos bioativos no extrato vegetal. Os teores de compostos fenólicos totais foram quantificados em ambos os ensaios utilizados, sendo que o método Fast Blue foi capaz de quantificar teores mais elevados dessas substâncias (526,18 ± 41,35). Doze compostos bioativos foram identificados e quantificados por HPLC. O principal composto fenólico encontrado no extrato de *C. sylvestris* foi o ácido cfeático. O presente estudo indica que o extrato comercial de *C. sylvestris* apresenta alto potencial bioativo.

**Palavras-chave:** Planta medicinal. Compostos bioativos. Capacidade antioxidante total.
INTRODUCTION

Oxidative stress (OS) is one of the leading causes of chronic disease. OS is caused by the overproduction of reactive oxygen species (ROS), promoting oxidative damage to biomolecules or changes in antioxidant activity (ECEIZA et al., 2022). His sudden increase can cause chronic diseases such as atherosclerosis, Alzheimer’s, Crohn’s, ulcerative colitis, and leukemia, among others (TESTA et al., 2018; ZHEVAK et al., 2020). Bioactive compounds from plant extracts have been developed to inhibit this condition due to their biological activity and pharmacological effects (SINGH et al., 2022).

Natural products have a key role in the development of new drugs, as the use of medicinal plants has expanded across the globe. Thus, medicinal plants are endowed with a range of bioactive compounds that will play a therapeutic potential and can be used over synthetic drugs or associated with these conventional drugs (ELKORDY et al., 2021; JUGRAN et al., 2021; NAZARIAN-SAMANI et al., 2018).

In this regard, *Casearia sylvestris* Swartz (Salicaceae) is a medicinal plant in Latin America. In Brazil, it is known as “guaçatonga”, “erva-de-lagarto”, and “cafezinho-do-mato” (CLAUDINO et al., 2013; SIQUEIRA et al., 2021). This plant is rich in bioactive compounds such as monoterpenes, sesquiterpenes, diterpenes, ellagic acid derivatives, and flavonoids (CARVALHO et al., 2018; FERREIRA et al., 2011; PEREIRA et al., 2017).

*C. sylvestris* leaf extracts have been studied due to potential use in the treatment of diarrhea, trauma, skin lesions, ulcerations, chest cold, influenza, fever, herpes, snake bite, topical leprosy, traumas, ulcer, used as an appetite suppressant, weight loss products, topical anesthetic, antiseptic, osteoarthritis, and cicatrizant in skin diseases (ANTINARELLI et al., 2015; PASCOALINO et al., 2020; XIA et al., 2015). Due to its pharmacological potential, this work aimed to determine the antioxidant capacity of the commercial extract of *C. sylvestris*.

MATERIALS AND METHODS

*Obtaining extract*

The extracts of the leaves (tincture) of *C. sylvestris* were purchased in a pharmacy of manipulation in the city of Lavras-MG, 30 mL of the hydroalcoholic extract with an alcohol content of 54 ºGL for internal use, presenting greenish brown to dark brown color.
and 10% of the plant derivative. Subsequently, it was transported to the Laboratory of Postharvest of Fruits and Vegetables from the Federal University of Lavras, Lavras - Minas Gerais. It was stored in amber glass flasks at -18°C until the analytical determinations were determined in three repetitions and triplicate.

**Determination of total phenolic compound (TPC) content**

Using two different methodologies, it was used a microplate reader (EZ Read 2000, Biochrom®) to determine the levels of total phenolic compounds (TPC). The first method was the Folin-Ciocalteau (WATERHOUSE, 2002), aliquots of 30 μL of extract were added to the wells of a 96-well, flat-bottom microplate, along with 150 μL of Folin-Ciocalteau reagent 10% and 120 μL of sodium carbonate 4%, and left to react for 120 min in the absence of light and the reading was performed at 750 nm. The Fast Blue methodology was performed as proposed by Medina (2011) with some modifications, in which aliquots of 250 μL of the extract were added to wells of a 96-well, flat-bottom microplate, along with 25 μL of Fast Blue 0.1% reagent and 25 μL of 5% sodium hydroxide, and left to react for 90 min in the absence of light and reading was performed at 420 nm. In both methodologies, a standard curve of gallic acid was considered for the calculations, and the results were expressed as mg gallic acid equivalent (GAE) per 100 mL of extract.

**Antioxidant activity determination**

The antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (BRAND-WILLIAMS et al., 1995), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS+) (RE et al., 1999), of the phosphomolybdenum complex (PRIETO et al., 1999), of iron reduction (FRAP) (RUFINO et al., 2010) and the β-carotene/linoleic acid system (MARCO, 1968). The DPPH and ABTS+ results were expressed as % reduction, that of phosphomolybdenum in mg ascorbic acid equivalent per mL of extract (mg AAE.100 mL⁻¹), that of iron reduction in μM ferrous sulfate per mL of the extract (μM ferrous sulfate.mL⁻¹), and that of β-carotene/linoleic acid in % protection.
Determination of bioactive compounds by high-performance liquid chromatography (HPLC)

The determination of bioactive compounds was performed on a Shimadzu model high-performance liquid chromatograph (HPLC-DAD/UV-Vis) (Shimadzu Corporation, Kyoto, Japan) equipped with four high-pressure pumps (LC-20AT), photodiode array detector (SPD-M20A), degasser (model DGU-20A5), controller (CBM-20A), oven (CTO-20AC), and injector (SIL-20A). Separations were performed using a Shimadzu Shim-pack ODS GVP-C18 column (4.6 × 250 mm, 5 mm) connected to a Shimadzu ODS GVP-C18 pre-column (4.6 × 10 mm, 5 μm). The mobile phase was conducted with 2% (v.v-1) acetic acid in deionized water and mobile phase B at 70:28:2 (v.v-1) methanol/water/acetic acid at a flow rate of 1.0 mL.min⁻¹ with a gradient elution program: 20% B (0-5 minutes), 40% B (25 minutes), 45% B (43 minutes), 80% (50 minutes), 0% B (55-65 minutes) and a run time of 65 minutes. The injection volume was 20 μL, and the analyses were conducted at 35°C. The bioactive compounds were identified at the wavelength of 280 nm by comparison of the retention times of the peaks with the available standards.

RESULTS AND DISCUSSION

The results obtained for total phenolic compounds (TPC) and antioxidant activity (AA) are shown in Table 1.

<table>
<thead>
<tr>
<th>Determinations</th>
<th>C. sylvestris extract</th>
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<tbody>
<tr>
<td>TPC (mg EAG 100 mL⁻¹)</td>
<td>270.54 ± 7.40</td>
</tr>
<tr>
<td>Folin-Ciocalteau</td>
<td>526.18 ± 41.35</td>
</tr>
<tr>
<td>Fast Blue</td>
<td>96.51± 0.38</td>
</tr>
<tr>
<td>ABTS⁺ (% Radical Reduction)</td>
<td>34.92 ± 6.44</td>
</tr>
<tr>
<td>β-Carotene/Linoleic acid (% Protection)</td>
<td>3265.32 ± 189.00</td>
</tr>
<tr>
<td>Phosphomolybdenum complex (mg AAE mL⁻¹)</td>
<td>82.03 ± 0.88</td>
</tr>
<tr>
<td>DPPH⁻ (% Radical Reduction)</td>
<td>35.55 ± 1.13</td>
</tr>
</tbody>
</table>

The TPC’s were determined by two methods, Fast Blue and Folin-Ciocalteau, and the Fast Blue method obtained a content approximately two times higher (526.18 ± 41.35). This variation in results may be associated with the interferences to which the
Folin-Ciocalteau method is subject. According to Azeem et al. (2020) non-phenolic antioxidants, reducing substances such as glucose and ascorbic acid, amino acids (tyrosine and tryptophan), and proteins containing these amino acids are examples of interferents that also form blue coloration with Folin-Ciocalteu reagent. Fast Blue methodology uses a reaction mechanism that is restricted to reactions between the diazonium salt and reactive aromatic compounds, such as phenolic compounds (GARCIA-HERRERA et al., 2022).

Following Vasco et al. (2008), the content of phenolic compounds can be classified into three categories: low (<100 mg GAE.100 g⁻¹), intermediate (100-500 mg GAE.100 g⁻¹), and high level (>500 mg GAE.100 g⁻¹). According to the data obtained in the present study, C. sylvestris extract falls into the third category and is therefore considered to have a high level of phenolic compounds. The antioxidant activity of C. sylvestris extract was evaluated by five different methods. For the evaluation of the antioxidant activity, it is recommended to use two or more different techniques since there is no standard method to evaluate this mechanism due to the complexity in the different raw materials. Furthermore, each method may present a distinct mechanism of action that may identify the mechanism of antioxidant action of the tested material (ALAM; BRISTI; RAFIQUZZAMAN, 2013; GULCIN, 2020).

For all the tested methods, the studied extract showed antioxidant activity. Specifically, for the ABTS⁺ and DPPH⁻ radical scavenging methods, C. sylvestris extract could scavenge almost all the radicals present in the medium (96.51 ± 0.38 and 82.03 ± 0.88, respectively), which indicates its high antioxidant action. According to Hassimotto et al. (2005) the antioxidant capacity of the β-carotene/linoleic acid system is classified as: i) high levels (>70% protection), ii) intermediate (40-70% protection) and iii) low (<40% protection). Thus, C. sylvestris extract showed low antioxidant capacity by this method. Several assays for evaluating antioxidant activity and its principles have been reported previously, endorsing that the ethanolic extract of C. sylvestris presents antioxidant capacity. However, there is a scarcity of studies that have demonstrated such results in the literature. Only the study by Espinosa et al. (2015) performed the antioxidant activity using the DPPH⁻ (% inhibition) assay, comparing such assay with two control methods, butylhydroxytoluene (BHT) (synthetic antioxidant) and quercetin (natural antioxidant). The results expressed a relevant antioxidant capacity of the ethanolic extract, where the lowest dose tested showed significant antioxidant activity. Moreover,
the concentrations of BHT 0.005 and 0.01 mg.mL⁻¹, and quercetin (0.005 mg.mL⁻¹) showed lower DPPH radical reduction potential in all tested concentrations of *C. sylvestris* extract (ESPINOSA et al., 2015). The study of Souza e Moya (2015), reported that the aqueous extract of the bark of *C. sylvestris*, also exhibited phenolic compounds (1.90 ± 0.1), and antioxidant activity (DPPH EC50 = 0.0867 ± 0.04; FRAP = 0.848 ± 0.02).

In the present study, bioactive compounds were determined using HPLC, and 11 chemical substances were identified (Table 2) in the sample. It was possible to detect eight phenolic acids (Caffeic Acid, Chlorogenic Acid, Ferulic Acid, M-Coumaric Acid, O-Coumaric Acid, P-Coumaric Acid, Rosmarinic Acid, and Syringic Acid), one stilbene (Resveratrol), one phenolic aldehyde (Vanillin) and one flavanol (Catechin). The presence of these compounds justifies the antioxidant action found employing the previously mentioned methods. *C. sylvestris* extract is, therefore, a rich source of bioactive compounds with antioxidant action.

### Table 2 - Mean values ± deviations relative to phenolic profiling with 12 compounds quantified.

<table>
<thead>
<tr>
<th>Bioactive compounds</th>
<th><em>C. sylvestris</em> extract (mg. 100 mL⁻¹)</th>
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<tbody>
<tr>
<td>Caffeic Acid</td>
<td>41.48 ± 2.95</td>
</tr>
<tr>
<td>Chlorogenic Acid</td>
<td>8.42 ± 3.10</td>
</tr>
<tr>
<td>Ferulic Acid</td>
<td>9.10 ± 1.38</td>
</tr>
<tr>
<td>M-Coumaric Acid</td>
<td>0.88 ± 0.14</td>
</tr>
<tr>
<td>O-Coumaric Acid</td>
<td>0.57 ± 0.10</td>
</tr>
<tr>
<td>P-Coumaric Acid</td>
<td>8.52 ± 3.10</td>
</tr>
<tr>
<td>Rosmarinic Acid</td>
<td>0.62 ± 0.47</td>
</tr>
<tr>
<td>Syringic Acid</td>
<td>10.08 ± 1.27</td>
</tr>
<tr>
<td>Catechin</td>
<td>1.03 ± 0.04</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>Vanillin</td>
<td>1.66 ± 0.25</td>
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</table>

### CONCLUSION

The present study demonstrated that the commercial *C. sylvestris* extract showed higher TPC content according to Fast Blue method. Regarding the determination of the total antioxidant capacity, five different methodologies were used, proving that the extract presented antioxidant activity in all assays tested.
ACKNOWLEDGMENTS

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