South African Journal of Botany 111 (2017) 336–340

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Antioxidant and allelopathic activities of *Smilax brasiliensis* Sprengel (Smilacaceae)

J.C. Fonseca, M.A. Barbosa, I.C.A. Silva, J.M. Duarte-Almeida, A.H.F. Castro, L.A.R. dos Santos Lima *

Universidade Federal de São João Del-Rei, Campus Centro-Oeste, 35501-296 Divinópolis, MG, Brazil

ARTICLE INFO

Article history:
Received 18 November 2016
Received in revised form 15 March 2017
Accepted 13 April 2017
Available online xxxx

Edited by L Sebastiani

Keywords:
Allelopathy
Antioxidant
Phenolic compounds
Rutin
Smilax

ABSTRACT

Ethanol extract and fractions obtained from leaves of *Smilax brasiliensis* Sprengel were examined in order to determine their total phenolic and flavonoid contents, as well as antioxidant and allelopathic activities. Analysis by thin-layer chromatography suggested the presence of rutin in the ethanol extract and in the ethyl acetate and hydroethanol fractions. In addition, chlorogenic acid was present in the ethanol extract and in the dichloromethane, ethyl acetate and hydroethanol fractions. The antioxidant activity was significantly more pronounced for the ethanol extract and fractions than that of the commercial antioxidant 2,6-di-tert-butyl-4-methylphenol (BHT). The allelopathic effect against the seeds of *Allium cepa* showed promising results, predominantly with effects in growth inhibition of hypocotyls and radicles at the lower concentration tested (125 μg mL^{-1}). The results of this study suggest that the extract and fractions obtained from *S. brasiliensis* could be used as natural antioxidants and herbicides.

1. Introduction

Currently, the interest in obtaining natural antioxidants has grown rapidly, mainly due to the etiology of several degenerative and aging-related diseases. Cancer, cardio- and cerebro-vascular diseases have been attributed to oxidative stress and subsequent free radical damage to lipids, proteins and nucleic acids (Matkowski, 2008; Choi and Lee, 2009). Furthermore, synthetic antioxidants may have toxic, carcinogenic and abnormal effects on humans (Baydar et al., 2007). Antioxidants prevent damage by interfering with free radical propagation cascades before they attack biological targets in cells (Matkowski, 2008). Enzymes (such as superoxide dismutase) and other molecules, such as ascorbic acid (AA), uric acid, glutathione, tocopherols, carotenoids and phenols, have been suggested to be natural sources of antioxidants. In recent years, there has been increased interest in the use of natural phenolics as antioxidants and functional food due to their safety and prevalence in nature (Matkowski, 2008; Tomás-Barberán and Andrés-Lacueva, 2012; Wu et al., 2014).

Several plants also exhibit allelopathic potential (Scrivanti, 2010; Scognamiglio et al., 2012; Mabrouk et al., 2013; Rowshan et al., 2014) because they biosynthesize a great variety of substances generically named allelochemicals that, when released into the environment, can positively or negatively affect the life of other coexisting plants and animals (Bouwmeester et al., 2003). The important allelochemicals include alkaloids, terpenoids, flavonoids, steroids, tannins, and phenolic compounds (Fiorentino et al., 2008; Scrivanti, 2010; Areco et al., 2014). The allelochemicals affect plant growth and development and can be employed successfully against pathogens for weed reduction and for enhancement of yield in crops (Xuan et al., 2005).

Smilax L. is the only genus of Smilacaceae, with 310 species distributed throughout all continents in temperate, subtropical and especially tropical regions (Andreata, 2009). *Smilax* species are known in Brazil as 'salsaparrilha' or 'japecanga'. The roots and rhizomes are used in traditional medicine as a stimulant, an anti-hypertensive agent, sudorific, an anti-syphilitic agent, diuretic and tonic as well as for cutaneous affections and rheumatism (Jiang and Xu, 2003; Breitbach et al., 2013). Recently, studies performed with different *Smilax* species have demonstrated cytoprotective effects against oxidative stress in pulmonary cells and in the liver (Rajesh and Perumal, 2014); antioxidant and antifungal potential (Morais et al., 2014); hypoglycaemic and hypotensive activities (Amaro et al., 2014); and cytotoxic activities (Liang

Abbreviations: TLC, thin-layer chromatography; BHT, 2,6-di-tert-butyl-4-methylphenol; EE, ethanol extract; HEX, hexane fraction; DCM, dichloromethane fraction; AC, ethyl acetate fraction; HE, hydroethanol fraction; EtOH, ethanol; AA, ascorbic acid; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; GAE, gallic acid equivalents; QE, quercetin equivalents; MES, 2-(*N*-morpholino) ethanesulfonic acid; TPC, total phenolic content; TFC, total flavonoid content.

* Corresponding author at: Universidade Federal de São João Del-Rei, Campus Centro-Oeste Dona Lindu, Rua Sebastião Gonçalves Coelho 400, Chanadour, Divinópolis 35501-296, MG, Brazil.

E-mail addresses: julianacostafonseca@hotmail.com (J.C. Fonseca), myriambarbosa@gmail.com (M.A. Barbosa), izacaputo@hotmail.com (I.C.A. Silva), maudall@ufsj.edu.br (J.M. Duarte-Almeida), acastro@ufsj.edu.br (A.H.F. Castro), luarsantos@ufsj.edu.br (L.A.R. dos Santos Lima).

http://dx.doi.org/10.1016/j.sajb.2017.04.003
0254-6299/

et al., 2016; Wan et al., 2016). The main components found and shared by most species of the genus are the steroidal saponins, phytosterols, triterpenoids, flavonoids and phenolic acids (Cáceres et al., 2012; Breitbach et al., 2013; Petrica et al., 2014; Wu et al., 2014; Liang et al., 2016).

Despite different biological activities and bioactive compounds that are well documented for some *Smilax* species, thus far, there have been no data concerning *Smilax brasiliensis*. The goals of this study were to determine the total phenolic and flavonoid contents and to demonstrate their antioxidant and allelopathic potential.

2. Materials and methods

2.1. Plant material and extraction

Leaves of *S. brasiliensis* Sprengel were collected in Ijaci, South Minas Gerais State, Brazil (21°13′46″S and 44°55′65″W, average altitude 908 m above sea level) in March 2014. The plant material was identified by Dr. Regina Helena Potsch Andreata, and a voucher specimen (57078) was deposited into the Herbário PAMG of the Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG) in Belo Horizonte, Minas Gerais, Brazil.

Extraction of the dried and powdered leaves (115.13 g) by percolation (ethanol, 7 L, 72 h) provided the ethanol extract (EE) (23.40 g). Part of this extract (15.60 g) was dissolved in ethanol/water (1:1) and successively extracted with hexane, dichloromethane and ethyl acetate, resulting in 4.76, 2.68, 1.65 and 5.72 g of hexane (HEX), dichloromethane (DCM), ethyl acetate (AC) and hydroethanol (HE) fractions (Morais et al., 2015). The extract and fractions were screened qualitatively for the presence of different classes of natural products, such as alkaloids, steroids, triterpenoids, coumarins and flavonoids, by thin-layer chromatography (TLC) (Wagner and Bladt, 2001). The analysis was performed on Merck silica gel 60 F_{254} aluminium plates using rutin, quercetin, and chlorogenic, caffeic and gallic acids (Sigma, St. Louis, USA) as standards. Other tests described by Matos (1997) were carried out to determine the presence of tannins and saponins.

2.2. Total phenolic content

The total phenolic content was estimated using the Folin–Ciocalteau test in the EE and fractions (Singleton and Rossi, 1965), with modifications. The Folin–Ciocalteau aqueous solution (2.250 μL; 1:4 *v*/v) was added to the standard solution or samples (250 μL) and, subsequently, to a sodium carbonate solution (250 μL). After vigorous shaking, these solutions were kept at rest for 30 min at room temperature. The absorbance was determined by spectrophotometry at 750 nm (Thermo Scientific Genesys 10S, USA) after 30 min of incubation at room temperature with a blank sample as well as a standard solution and samples. Gallic acid was used as a reference compound, and the total phenolic contents were expressed as micrograms of gallic acid equivalents (GAE) per milligram of extract or fraction. All assays were performed in triplicate.

2.3. Total flavonoid content

The total flavonoid content was estimated according to the Dowd method, with modifications (Morais et al., 2014). Exactly 2 mL of 2% aluminium trichloride ($AlCl_3$) in ethanol was mixed with the same volume of the extract or fraction solution (1.0 mg mL^{-1}). The absorbance was read at 415 nm using a spectrophotometer (Thermo Scientific Genesys 10S, USA) after 30 min, with a blank sample consisting of a 2-mL extract or fraction solution with 2 mL methanol without $AlCl_3$. Quercetin was used as a reference compound to produce a standard curve, and total flavonoid contents were expressed as micrograms of quercetin equivalents (QE) per milligram of extract or fraction. All assays were performed in triplicate.

2.4. DPPH radical scavenging assay

The radical scavenging abilities of the extract and fractions were based on reactions with a 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and were compared to standards 2,6-di-tert-butyl-4-methylphenol (BHT) and AA. The determination of antioxidant activity using the DPPH method was adapted for use with microplates (Araújo et al., 2013). A DPPH solution (0.002% *w*/*v*) was prepared in 80% methanol. Exactly 75 μL of the samples or standards (1, 10, 100, 250 and 500 μg mL^{-1}) were added to the wells in a 96-well flat-bottom plate containing 150 μL of DPPH solution. The plate was then covered and left in the dark at room temperature (25 °C). After 30 min, the absorbance at 517 nm was measured with a spectrophotometer (Biotek Power Wave XS2, USA), and 80% methanol was used for baseline correction. Scavenging ability was expressed as the inhibition percentage and was calculated using Burda and Oleszek's (2001) equation:

$$\text{Scavenging ability}(\%) = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control} \times 100} \times 100,$$

where Abs control is the absorbance of the DPPH radical in 80% methanol and Abs sample is the absorbance of samples and standards in 80% methanol + DPPH. The antioxidant activity of each of the samples was expressed as IC_{50}, which is defined as the concentration (in μg mL^{-1}) of samples required to inhibit the formation of DPPH radicals by 50%. IC_{50} values were calculated using the Probit analysis (Finney, 1980). All assays were performed in triplicate.

2.5. Allelopathic assay

The allelopathic activity of the extract and fractions was evaluated through the effects on the growth of onion (*Allium cepa* L. cultivar Red Creole, Topseed Garden, Brazil) and lettuce (*Lactuca sativa* L. var. repolhuda, Feltrin, Brazil) seeds. Each of the dried samples was dissolved with deionized water, and their pH values were buffered with 10 mM 2-(*N*-morpholino) ethanesulfonic acid (MES), adjusted to 6.0–6.2 with a sodium hydroxide (NaOH) solution. Concentrations lower than 200 μg mL^{-1} were obtained by a dilution series. Growth studies were conducted in 100-mm Petri dishes containing a 9.0-cm sheet of Whatman No. 1 filter paper as a support. Then, 25 lettuce or onion seeds were placed into each dish with 7 mL of the test (500, 250 and 125 μg mL^{-1}) or control solution (deionized water with MES). All tests were performed in triplicate and were repeated at least once. The dishes were covered with Parafilm to reduce evaporation and were then incubated in the dark at 25 °C in a controlled-environment growth chamber (Fanem 346-MD, Brazil) for 7 days. After this time, the lengths of the radicle and hypocotyl were measured. During the measurement process, the dishes were kept at 4 °C to avoid subsequent growth (Freitas et al., 2015).

The effects on the growth can be calculated using the following formula (Pinto et al., 2013):

$$\%\text{of the growth} = \frac{\text{Ma} - \text{Mc}}{\text{Mc}} \times 100,$$

where Ma is the mean value of the seeds with samples tested and Mc is the mean value for the control (seeds grown without addition of samples tested). Thus, zero represents the control, positive values represent stimulation of the studied parameter and negative values represent inhibition.

2.6. Statistical analysis

Student's t-test was utilized to evaluate the statistical difference between the control group and the group exposed to EE and fractions of *Smilax campestris*. The analyses were performed using GraphPad Prism